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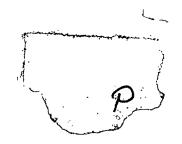
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Change on School Leaving

British universities are at last being given their chance to comment on the proposals for a new sixth form curriculum and pattern of examinations which the Schools Council has been preparing in the past year. The Standing Conference on University Entrance has already set in train arrangements for sounding opinion, and its chairman, Sir Robert Aitken, has issued a public denial of reports at the recent Headmasters' Conference that there is at present no means by which a dialogue between the schools and universities on the subject of university entrance can be maintained. Understandably, Sir Robert thinks that the Standing Conference on University Entrance will do the job and he would be within his rights to claim that the Standing Conference was set up precisely so that it could be the negotiating body between the schools and the universities. What remains to be seen is how successfully it can work in the extremely difficult field in which it will be compelled to operate in the months ahead. If it can somehow find a means of winning the agreement of the universities on such controversial proposals as those now to be considered, it will have done wonders. But it is also reasonable to ask whether it would not in the long run be better if the basis of the Standing Conference were somehow broadened. As things are, it is extremely difficult to rouse the interests of university teachers in proposals for reform which do not affect them personally and painfully.

The broad outlines of the Schools Council's proposals are now quite clear. There is to be a pattern in which entrants to university will be selected on the basis of examinations in two subjects at advanced level by methods comparable with those now in use. It is also proposed that students leaving school should follow a number of "elective" courses which may or may not be linked with their chief preoccupations and which will be examined separately, often by the schools themselves. Finally, there is to be a series of courses called "common courses" in the schools intended to provide a broad education for many students but not intended as subjects for examination. If the Schools Council can win the agreement of the universities, it would like to launch an experiment in the schools which could hardly begin before 1969. With luck and good management, it is just possible that the first university entrants under this scheme would make their appearance in 1975 or thereabouts.

Several questions arise immediately. In the first place, there is nothing in the competence of the Schools Council to decide how the universities will make use of the proposed scheme for selecting students. One possibility, of course, is that universities might settle simply for a consideration of the formally examined subjects at Advanced level, which would allow students in the schools to spend more time on unconnected studies but which would also increase the present somewhat arbitrary dependence of would-be undergraduates on the numerical values awarded to them in

the examination. Another possibility, more likely at the beginning, is that a great many university departments would scrutinize not merely the Advanced level examination results but those in the elective subjects as well. If things went badly, the result of that could be that the pressures on the sixth form curriculum by the requirements of university entrance would be substantially unchanged, and that students would be compelled, as now, to choose courses of study at universities before they are old enough to do so wisely. In other words, even if the Schools Council wins approval for its new venture, there is always a danger that the potential advantages in the schools will be perverted by the unwillingness of university departments to change their present habits. And certainly, of course, there is every reason why university chemists or doctors, for example, should insist that it would only be possible to accept the spirit as well as the letter of what the Schools Council is proposing if there were also changes in the pattern of what happens at universities-longer courses, perhaps.

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It is also unfortunately possible and even likely that the spirit of the proposals will be overwhelmed by the way in which the schools react to them. The most serious objection to the present pattern of education in the schools is that it is too easy for students and particularly bright students to follow narrow courses of study. Although some enlightened establishments may seize on the proposed curriculum as a means of providing a more liberal education, schools which continue to be obsessed with success in winning places at universities may continue to provide a narrow education. In other words, what the Schools Council is proposing is not a means of making sure that modern education in British schools is made more liberal, but merely a starting point from which the schools may be bullied or even shamed into a more independent frame of mind.

In the circumstances it is only natural that the proposals will be attacked from two opposing points of view. There will be many in the universities who say that a less rigid entrance system to the universities will make the quality of university education deteriorate, but this is not merely unfashionable but unimaginative as well. It is impossible to believe that universities could not adapt themselves quickly to the kind of scheme which the Schools Council has devised, particularly if one of the benefits were to be recruitment of more able and more liberally educated students from the schools. It will be a great shame if arguments like these prevail.

The more serious objection to the proposals is that they do not go far enough, and it will certainly be hard for anyone to suppress the question of whether it is feasible to wait until 1975 for the radical revision of the school curriculum which has been an urgent need for several decades. But the nature of the proposed reforms also leaves much to be desired. In particular, they do not offer the urgently necessary assurance that school

education will become more liberal and the careers of undergraduates at the universities much less closely predetermined. This is why the universities will do well, in the discussions which lie ahead, to ask whether the Schools Council's proposals could at this stage be replaced by a much more radical and rewarding scheme. For one thing, it would seem essential that a real broadening of the curriculum should somehow be attained, and an obvious way of doing this is to encourage a pattern in which university entrants follow more obligatory courses and not fewer. But it is also important to explore the consequences for the universities of the present difficulty of recruiting a diversity

of students into specialized departments at the universities. Are four years essential after all? Or is it possible that a shorter long vacation would solve a lot of problems? Because these and other questions are inevitably bound up with a thorough discussion of what the Schools Council is proposing, it is inevitable that the Standing Conference on University Entrance will have to fight a great many battles in the months ahead. Its success will depend not just on winning agreement of some kind or another but on the vigour with which it can push the universities in the direction of more radical reforms, however unpleasant and unfashionable they may seem.

What Professor Flowers Should Do

THE appointment of Professor B. H. Flowers as chairman of the Science Research Council from the beginning of this month is an important development if only because it provides an opportunity for the council to find a permanent pattern of operations. The council as it is at present is still clearly the rump of the old Department of Scientific and Industrial Research, principally because of its involvement in the support of university research and postgraduate education. But the past decade has been one long sequence of upheavals. First there was the Jephcott Committee, which changed the relationship between the council of part-timers and the full-time staff. Then came the dismemberment of the department in the aftermath of the Prend Report and the election of the Labour Government in 1964. At various times in this short period, the department has been financially responsible to the Lord President of the Council and to the Department of Education and Science. At the beginning it was a more or less free-living organism, but now its activities are controlled to some extent at least by the Council for Scientific Policy. In the circumstances it is not surprising that the Science Research Council has failed so far to create as clear an image as it should have. It is also relevant that in the United States the National Science Foundation is also having difficulty in persuading scientists that it can be counted on as an effective intermediary between the government and research. The most obvious difficulty is the sheer diversity of the work on which both organizations are engaged.

This, fortunately, is a pointer to an immediate need in the planning of what the Science Research Council should attempt in the months immediately ahead. For one thing, there is a need somehow to create a feeling among professional scientists that the Science Research Council is on their side and not, for example, that of the Treasury. There is no shortage of causes by means of which demonstrations like

What Price for Electricity?

THE British electricity generating industry probably cannot help creating the impression that it speaks with two voices on prices. Only a few weeks ago,

these could be arranged. The way in which the employment of the council's own staff at laboratories such as Daresbury is plagued by troubles about pensions (see page 5) is an obvious place at which to start. Behind the scenes, no doubt, the Science Research Council has been as vigorous as anybody in doing battle with the Treasury on this issue. But there is no reason why it should not fight this kind of battle more openly. It would certainly thereby command the confidence of scientists more easily. It might also win more often.

These, however, are comparatively minor issues. In the long run the effectiveness of the Science Research Council as a focus for the financing of fundamental research in Britain will depend on the capacity to anticipate the changing needs of various kinds of research. It must learn to take the initiative and not wait on events as it often seems to have to do. The most obvious way of winning a reputation in this field would be to re-create within the council some of the planning functions which used to flourish in the days of the Department of Scientific and Industrial Research and then to strengthen them enormously. In other words the Science Research Council would do well to create some means of knowing in advance just where the new developments in academic science are likely to need more generous support. It has made a start in this direction in the past few years by setting up machinery for exploring new avenues of research in biology. It is also enormously to be admired for the way in which it has converted astronomy in Britain into a flourishing field of research. But these are necessarily isolated and even sporadic initiatives. What is needed now is some means of taking a grip on the whole development of academic science and on a good deal of policy on higher education in science as well. Here again, it is no accident that the National Science Foundation in the United States has set its heart on such a development, so far without conspicuous success.

there was a storm because the Government had sanctioned increases in retail prices for electricity working out, on the average, at 15 per cent or so.

But earlier this week the Chairman of the Electricity Council, Sir Ronald Edwards, took the occasion of the publication of its annual report and that of the Central Electricity Generating Board (House of Commons Papers 625 and 626, HMSO, 16s. 6d, and 10s. 6d. respectively) as a chance to draw attention to the way in which nuclear power stations now promise to be substantially cheaper than the conventional variety. The detailed proof that an advanced gas cooled reactor can produce electricity at 0.48d. per kilowatt hour, compared with 0.58d. for a conventional plant in exactly similar circumstances, will no doubt put an end to the confusion there has been in the past year on just this issue. Lord Robens, speaking on behalf of the coal industry, has been particularly diligent in clouding this issue. But does it therefore follow that the electricity industry will promptly give up the construction of other kinds of power stations than those based on uranium, and does it follow that the price of electricity will eventually be reduced? The simple answer to the first question is that it would be unwise for the Central Electricity Generating Board to take hard and fast decisions at this point. Left to itself, of course, it is unlikely that it would build coal burning power stations. But there is always the possibility that natural gas could be a cheaper source of fuel, particularly where off-peak supplies of power are necessary. any case, there is still room in the British electricity system for small packages of power produced from oil. Although there has been a further small improvement in the average load factor, with the result that in 1966-67 the 34,000 megawatts of generating capacity were in use for 52 per cent of the time, there are still very large variations in the demand for electricity from one time of the year to another. The smallest demand on the system is still less than 10,000 megawatts on many occasions, and this is a measure of how little room there is in the system for the building of nuclear power stations which must, by their nature, operate at a high load factor if they are to do so economically. This, however, does not imply that there is any cause for the electricity board—and the Atomic Energy Authority—to mute their celebrations of the way in which advanced gas cooled reactors seem to have provided them with new opportunities. And it is perhaps churlish to remember that only five years ago, when the first advanced gas cooled reactor prototype was being built, the electricity people were sceptical and even resentful of the development.

It does not unfortunately follow that the planning of the future pattern of power generation will be conducted by the simple rules of pure reason. By all accounts, the Ministry of Power is about to produce a new White Paper on fuel policy for the 1970s—Mr Richard Marsh said as much when he spoke at Scarborough earlier this week. The difficulty is that in the present economic climate, in which resistances to change are reinforced by what seems to be adversity, there is a serious danger that too much concern for keeping collieries in being will set a precedent of protection that will last well beyond the immediate

crisis into the decade ahead. Mr Marsh was saying as recently as July that he was determined in the seventies to see full-blooded competition in terms of price between alternative kinds of fuel. But he has had an uncomfortable time since then. Will he still be able to write a White Paper based on the assumption of equal competition, and will he go as far as saying that it is not for the Ministry of Power but for the electricity boards to decide how to manufacture electricity most cheaply? These are the tests by which his policies should be judged.

The confusion about price is likely to persist, particularly now that the cost of generating electricity is a declining percentage of the cost of operating the industry as a whole. So much is clear from the annual report now published. The cost of transmission is by contrast an increasing burden. The generation of electricity, on the average, worked out in 1966-67 at 0.792d. per kilowatt hour, which is less than half the total expenditure of the industry in supplying a unit of electricity—1.767d. In circumstances like these, it is evident that savings of the order of 20 per cent on the cost of generation at base load power stations will not make comparable savings in the cost of providing electricity to customers. Indeed, the industry will be lucky if the coming into service of nuclear power stations enables it to keep the cost of generation And there is very little doubt that the Minister of Power could do more than any engineer to reduce the price of electricity in the immediate future. He could, for example, take steps to remove the anomaly by means of which the electricity industry pays a tax on fuel oil which is not levied on the gas producers. And there is obviously great scope for a more thorough examination of the principle on which the electricity industry is required to earn each year a surplus of more than 12 per cent of the capital employed.

But does not public subvention for the development of nuclear power stations amount to a kind of subsidy for electricity? This is also a question which the Minister of Power and the electricity boards should face. Theoretically, the industry will pay a royalty on each advanced gas cooled reactor which it builds particularly so as to recompense the Atomic Energy Authority for the developments already carried out. But what if the advanced gas cooled reactor had been a failure? Then there would have been no royalties to pay. In other words, it does appear that the separate development of new kinds of reactors by the Atomic Energy Authority is a means by which the electricity industry is shielded from the risks of new developments. So should the development of new reactors be handed over to the Central Electricity Generating Board? This, no doubt, would be a course of action that would simplify the problems of the Ministry of Technology which cannot much longer postpone decisions on the future of the Atomic Energy Authority. But a marriage of this kind between the two authorities would undermine the chances that British reactors will be sold elsewhere. The ideal is, rather, some partnership between the two organizations by means of which the risks of new developments could be more accurately apportioned. If they were private companies the solution would be simple—one would buy a stake in the other. Is it entirely outrageous therefore to suggest that there should be some common membership between the Central Electricity Generating Board

and the Atomic Energy Authority? In the long run that would be an effective means of making sure that new reactor developments were likely to find a ready customer. In the immediate future it would help to ensure that the present unnecessary, let alone wasteful, duplication of research laboratories by the two organizations could be somehow done away with.

Prices for Drugs

THE trade association for the British pharmaceutical industry has reacted vigorously to the publication last week of the Report of the Government Committee which has been studying the relationship between the pharmaceutical industry and the National Health Service (Cmnd 3410, HMSO, 17s. 6d.). The principal recommendations of the Sainsbury Committee are that there should be established an independent medicines commission with power to license the introduction of new drugs, to regulate their use, to help doctors decide which medicines should best be employed and to control advertising of drugs. The report also says that the Ministry of Health should be empowered to obtain from manufacturers detailed statements of the cost of individual drugs and that it should be able to determine prices paid for drugs supplied to the National Health Service on the basis of a reasonable return on the capital employed. In its reply to the report, the Association of the British Pharmaceutical Industry says that some of the recommendations of the Sainsbury Committee would "effectively amount to state control" of the industry. The association takes the line that the Sainsbury Committee may have been misled in arriving at its views on present pricing policies in the industry by the profits earned by a small number of companies.

However this dispute may be resolved, the Sainsbury Committee will have done a considerable service by providing a detailed picture of the state of the British pharmaceutical industry. The total output of the industry amounts to roughly £250 million a year, and between 55 and 68 companies account for more than 95 per cent of the total value of materials supplied to the National Health Service. Of the medicines supplied on prescriptions in 1966, 27 per cent came from British companies, nearly a half (by value) from companies controlled from the United States and the rest from companies controlled from the mainland of Europe. Exports run at about £30 million a year net. The value of drugs supplied to the National Health Service has risen over the years, largely because of the increasing proportion of proprietary medicines now used in the National Health Service, from £43.6 million in 1949 to £173.5 million in 1965. The views of the Sainsbury Committee on drug prices are based on an analysis of the profitability of 27 important companies each selling more than £1 million worth of drugs to the National Health Service each year. Seventeen of these companies earn profits of more than 20 per cent of the capital employed and 8 of these had rates of profit exceeding 30 per cent. Not all companies, however, were equally prosperous. Four of them earned profits in the three years 1963-65 which work out at less than 10 per cent of their capital employed. In its comment on these figures, the committee suggests that drugs supplied to the National Health Service in the early sixties may have been priced excessively "to the extent

of several million pounds". It rejects the view that high profitability is necessary if companies are to be encouraged to undertake research on a sufficient scale by saying that "firms which have sustained a research programme involving a substantial number of research personnel have emerged with encouraging regularity with products which have remained dominant in their field of therapy for a considerable time".

In the industry as a whole, the amount spent on research directly related to National Health Service products in 1965 was £16 million, or 9.7 per cent of the total sales of National Health Service drugs. Altogether, fundamental research aimed at the production of entirely new products seems to have accounted for between 56 and 58 per cent of the total research bill. By comparison, process development took much smaller sums of money—13.5 per cent. Because of the way in which many of the companies which operate in Britain make use of research carried out elsewhere, it appears that more than £3 million each year should be added to the value of the work done in British laboratories. The Sainsbury Committee suggests that some of the work classified by companies as fundamental research may not always deserve that name, but nevertheless considers that the figures available do not support assertions that the industry neglects long-term work. The committee does, however, consider that there could be closer links between the companies and the universities. All this implies that the pattern of research in the British pharmaceutical industry is unlikely to be seriously disturbed by the appearance of the Sainsbury report.

Double or Nothing

THE launching of a new communications satellite over the Pacific last week should make the advocates of a fifth transatlantic telephone cable rush to put in a good word for cables. But what is it? Reliability, perhaps, or longevity or familiarity; the same arguments would do for the horse. The fact is that new cables can only add to existing capacity, while satellites can almost multiply it. Cables allow only pointto-point communication; satellites can allow many to talk at once. Even from a political point of view, they are more forward looking. Telephone calls from one underdeveloped country to another usually have to be routed through one of the old centres of power-New York, London, Tokyo or Paris. But with satellites, just as distance means nothing (once it is 22,300 miles up), neither do national or natural boundaries.

Much has been made by those who hanker after cables—among whom are included the conventional communications carriers in the most articulate countries—of the failure of the first INTELSAT II satellite to achieve its proper orbit last autumn. But even that

functioned for about eight hours a day and its successor a few months later made available, all around the clock, all types of telecommunications between the Pacific Coast of the United States and the Far East. The Pacific satellites have done something more: brought down cable rates. The trans-Pacific charges set by Comsat, the United States Communications Satellite Corporation, were so much lower than those set by the cable carriers that the Federal Communications Commission ordered these to cut their prices or let Comsat take over their business with the American defence department. This is why the American Telephone and Telegraph Company is now preparing another cut in trans-Atlantic rates, presumably to match the satellite competition.

The economics of satellites are uncertain. The first sent over the Pacific was, in a sense, a waste of money (although a failure rate of one in five launchings has been budgeted for). It is no longer in use. But Early Bird has been working a full year longer than expected on the Atlantic run; estimates for the lifetime of satellites have been deliberately pessimistic. The chances that they will be cheaper than anyone thought are probably better than that they will be more expensive.

The recent launch put the fourth INTELSAT II into the sky. (One is over the mid-Atlantic, where it can make up for the inability of Early Bird to provide links with the southern hemisphere as well as simultaneous television and telephone transmission.) The newest has been sent into synchronous equatorial orbit over the Pacific at approximately 175 degrees East. All are about twice the weight of Early Bird, weigh about 192 pounds and are 56 inches in diameter and 26.5 inches high, not counting antennae. Next year, however, the stout string and sealing wax stage should be over for good.

The INTELSAT III series of satellites should by then have been launched, all six of them. Built by TRW Incorporated for a basic price of \$32 million, these will have three times the capacity of the INTEL-SAT II satellites and should ensure continuous operation, even if one or even two go dead. By 1969, moreover, the total number of ground stations around the globe is expected to be 40—including one in Spain, which is also being mentioned as a possible terminus for the new telephone cable.

Victory on Pensions

The Institution of Professional Civil Servants seems to have won a tangible if modest victory in its dealings with the Department of Education and Science on behalf of scientists working for the research councils who may find themselves being transferred to the British Civil Service. The issue is the ease with which people moving into or out of the Civil Service can retain their pension rights in full. Hitherto transfers of this kind, an increasingly common hazard now that public laboratories of all kinds are beginning to respond to the public clamour for mobility, have been governed by the rule that entitlement to pension is within the discretion of the Civil Service. Although the research councils and the Civil Service operate pensions schemes which are closely similar, and which are in particular non-contributory, it has been possible for the Civil Service to take an awkward line in its dealings with transfers. Sometimes, for example, the question has been raised of whether people leaving a Civil Service laboratory for a job with one of the research councils have had no assurance that the full value of their pensions rights would be transferred. seems now to have happened is that the Institution of Professional Civil Servants has won the agreement of Mr Patrick Gordon-Walker, Secretary of State at the Department of Education and Science, that transferability would in future be automatic for movements between the Civil Service and the research councils. Transferability between the Civil Service and the research councils on the one hand and the rest of the world on the other continues to be impeded by lack of transfer rights, although it is hard to see how the Fulton Commission on the Civil Service can fail to recommend the abolition of this anomaly.

News of the dispute between the Institution of Professional Civil Servants and the Science Research Council about the pensions rights of those employed at the Daresbury Laboratory is less cheerful. A writ against the council by the institution is due to be heard in the courts later this year. The issue is exceedingly complicated, chiefly because of the reorganization of government science under the Science and Technology Act of 1965. The act was the legal instrument which established the Science Research Council and which transferred to it the activities of the National Institute for Research in Nuclear Science, then the operator of the Rutherford Laboratory at Harwell and the body responsible for building the Daresbury Laboratory. Because the Rutherford Laboratory had grown out of the Atomic Energy Authority's laboratory at Harwell, it had been agreed that employees at the Rutherford Laboratory should retain, if they chose, membership of the contributory pensions scheme operated by the AEA. The need to perpetuate this arrangement under the new legislation was apparently recognized only at a late stage of the passage of the bill through Parliament, and an amending clause was introduced in February 1955. This clause laid down that the right to opt for AEA pensions should apply not merely to those already "employed by the National Institute for Research in Nuclear Science" but also to those subsequently taken on "to work on activities taken over ... from the National Institute for Research in Nuclear Science", but Lord Snow told the House of Lords at the time that there was no intention that this provision should apply to Daresbury as well as the Rutherford Laboratory. The Institution of Professional Civil Servants has taken issue with the British Government's view that the Act will permit such an Whatever the courts decide, it is interpretation. hard to think that these arrangements will survive the acid test of feasibility, let alone the report of the Fulton Commission now only half a year away.

Aldabra Expedition Returns

THE first phase of the Royal Society expedition to Aldabra, the atoll in the Indian Ocean where the Ministry of Defence proposes to build an air staging post and the BBC would like a transmitter, is now complete. Seven scientists have returned after spending five weeks on the atoll, making preliminary investigations of numbers and distribution of animals and plants, both on the atoll and in the lagoon. They have come back convinced that Aldabrashould be preserved,

for it is the last place in the Indian Ocean where island life can be studied in isolation.

The expedition landed at the west island, where there has been a settlement since 1888, and from there made excursions to the south and north, and, together with parties from the Ministries of Defence and Public Building and Works, to the east end of the atoll. The aim was for each member to become acquainted with as many habitats as possible. The eastern end proved to be the area of greatest ecological interest. It has the greatest concentration of giant land tortoises, Testudo gigantea. There are apparently more than 10,000 of these tortoises on Aldabra; elsewhere they are only found in the Galapagos Islands. The eastern end has also the greatest number of frigate birds, as many as a thousand birds could be seen hanging above one of the fresh water pools which attract them. The main breeding area of these birds is in the middle island where hundreds of thousands were seen nesting.

This survey work is being continued by four scientists and two technicians of the Royal Society party who remained on Aldabra to carry out the second phase of the expedition. If the Ministry of Defence project is not implemented, a long term thorough ecological survey will be planned; if the project goes ahead the expedition will make the fullest possible ecological survey before the ecology of the atoll is changed by the construction work.

Aldabra has remained untouched because it is so inhospitable to man; the terrain is difficult and apart from rain there is very little drinking water. proposed developments will be extensive, and the whole ecology will suffer. When construction begins the weeds which are associated with settlements, and which are now contained by the native vegetation, will spread through Aldabra. There will be a flow of foreign weeds and invertebrates into the islands, and the feral mammals now confined to the south and west islands will spread throughout the atoll. When the cats find the flightless rails these birds will not last very long. The danger is not, however, of large scale extinction; the RAF would make efforts to preserve the giant tortoise and flightless rail, which in any case can be preserved in zoos. The inevitable consequence of construction work will be the destruction of the integrity of an unspoiled island ecosystem. This is the last remaining ecosystem of its kind in the Indian or Pacific Oceans, and that for the Royal Society is reason enough for preserving it.

Money for Chemists

The Chemical Society is appealing to its members for money. This is not because of careless accountancy—in fact the society seems to have been scrupulously careful—but is one result of a move which the society plans to make to the rooms occupied until recently by the Royal Society in Burlington House. The Royal Society itself has moved to Carlton House Terrace, and most of the rooms left empty have been offered to the Chemical Society rent free by the British Government. The snag is that the move will be expensive—the society estimates that it will need £300,000 to adapt and furnish the new rooms, build a new publications warehouse at Letchworth and share the cost of a new lecture theatre in Savile Row. By what it describes as prudent husbanding of its re-

sources, the society has saved £125,000, and is now appealing to its members, to the chemical industry and to charitable trusts for the extra £175,000.

Chemists who feel that this is an expensive move should reflect that the new rooms will enable the society to group together staff previously distributed all over London. It will also provide a council room and a much larger library. They might also be reminded that it has cost the Royal Society £863,000 for its move to Carlton House Terrace. £250,000 was supplied by the Government, and an equal sum by the Nuffield Foundation, but much of the remainder is still outstanding. Ominously for the Chemical Society's hopes, an appeal to fellows of the Royal Society has not so far produced everything the organizers hoped for. And gratitude to the Government for its generosity in supplying accommodation should perhaps be tempered by the thought that once there were splendid hopes for a home for scientific societies in a science centre on the South Bank. It is a long time since that idea was last heard of.

Temptations of Science

THE British preoccupation with the need to persuade young people into science and engineering, but particularly the latter, was continued last week by the Research and Development Society, which devoted the second of its one-day symposia to the subject. Adults. at least, are prepared to take the subject seriously, and 150 turned up at Imperial College to listen to a panel of speakers which included many of those whose names are now associated with the definition of various aspects of this problem—Professor Michael Swann, for example, and Mr G. S. Bosworth, Director of Personnel at the English Electric Company. Mr Bosworth was strong in his statement that "technology is not a second-rate science". To pretend otherwise is now, of course the most sure way in which a man can lose his public reputation. But Mr Bosworth also said that engineers are too often diverted from the objectives by irrelevant details in atomic theory, and that industry often mistreats those whom it recruits so that they hurry back to the "world of the PhD". Mr D. A. Head, from the aero-engine division of Rolls Royce, took the line that, however the difficulties of attracting scientists into industry have arisen, only industry can handle the problems thus created, which means that companies must put recruits into the right jobs and make sure that they know about their prospects of promotion.

The symposium seemed to agree about the shortcomings of education in British schools and universities. For one thing, there was a general complaint about early specialization, one of the consequences of which is the alleged reluctance of narrowly educated people in industry to change from one kind of job to another. On the whole, those attending the conference took the view that specialization should be postponed until 18. There was also a general feeling that science courses at universities should be broadened so as to provide students with an appreciation of other kinds of studies and of social problems of various kinds. One speaker went so far as to say that the British educational system is designed to produce "cultured gentlemen", with the result that trained scientists consider that collaboration is a kind of cheating, that engineering is inferior and that the profit motive is even worse.

Yet teamwork, technology and business sense are essential for the survival of the British economy.

But what if you cannot even bring the horse to the water, let alone persuade him to drink properly? The symposium depressed itself with speculation about the reasons why young people may now be reluctant to go in for science and technology. Again there seemed to be general agreement that the young are infected with a "new fatalism" and have no vision of a golden future to which science and technology may contribute and no inkling of the enthusiasm which professional scientists have for their work. It was all good gloomy stuff. One of these days something may be done about it.

Instant Teachers

How long does it take to turn a student of the humanities into a science teacher? Three years is the answer given by Nottingham Regional College of Technology. In its new Department of Education, Nottingham is basing its acceptance of candidates for its three year Certificate of Education course in science teaching on general ability and interest in science teaching as well as academic qualifications in science. Thus if a candidate can demonstrate his ability in other subjects and his interest in science teaching, the college will accept him even if he does not know one end of a thermometer from the other.

Because of the shortage in applications from science candidates to teacher training colleges and universities, the department has decided not to compete for pure scientists but to offer Applied Science and a second opportunity to those who opted for the humanities instead of science at school. As the courses are designed for the Nuffield programme of science teaching, where a feel for the subject is much more important than facts, it is hoped that general ability will make up for any lack of specific knowledge.

The courses will tend towards either Biological Sciences or Physical Sciences and an Engineering course is under discussion. The students of the physical sciences will spend four terms working on American courses, which start from first principles for beginners but allow scientists to be rapidly directed into more profitable work. The teaching scheme is based on individual study rather than lecture programmes, and tutors will make much use of written material, acting as directors of study and tailoring the work to each student's needs. Students will also be able to take short courses from a range which will include Computer Science, Chemical Technology, Building Science, Food Science, Materials Science and Mechanical Engineering. These short courses should enable the students to base their teaching on practical applications when they go into the schools.

Nottingham Regional College of Technology is one of five technical colleges—the others are John Dalton College, Manchester, Sunderland Technical College, Barking Regional College of Technology and North Western Polytechnic-which are now running courses for the Certificate of Education. Previously these three year courses could only be taken at Colleges of Education. The Principal Lecturer, in charge of the science courses at Nottingham, is Dr Michael Bassey, who will be remembered for his trenchant criticisms of school science syllabuses at the British Association meeting in 1961.

More Cancer Research

A NEW research block consisting of laboratories for Tumour Immunology and Radiobiology was opened at Sutton, Surrey, on September 27. The unit is part of the Chester Beatty Research Institute, and the £150,000 it cost has come from the Napier Trust, the British Empire Cancer Campaign, the Medical Research Council and other donations. Block X, as it is called, has been operational since June, only 17 months after the idea for such a unit was suggested. Because the unit is alongside the Sutton branch of the Royal Marsden Hospital, it is hoped that any positive results that are obtained will be speedily applied.

Work has been going on for some time on various forms of immunotherapy, and in particular the treatment of primary sarcomata by injection of either immunized lymphocytes or of immunized cancer cells, that is, cells taken from the tumour, after removal by surgery, and rendered "sterile" by irradiation. Some of the impetus for the new research unit has come from research described earlier this year by Alexander, Delorme, Hamilton and Hall (Nature, 213, 569). Evidence has been found in animals of host resistance to tumours, and it is hoped that by boosting this reaction by the injection of immune lymphocytes it will be possible to provide some control. This form of treatment would be particularly important after surgery.

Under some conditions this treatment has proved successful in experimental animals, but it is emphasized by the staff of the new unit that experience of this treatment is too limited for any definite conclusions to be drawn. The mechanism of the cytotoxic action of

lymphocytes is also being studied.

American workers S. H. Nadler and G. E. Moore have also carried out transplant experiments in which tumours from two patients were transplanted to each other and after 10-14 days lymphocytes from each patient were transfused back to the other to fight the original growth. In several cases remission of the cancer for considerable lengths of time has occurred. This method of treatment is also being considered for investigation at the new unit.

Refining Steel

ELECTRO-SLAG Refining Technology, the British Iron and Steel Research Association's first development unit which was formed to exploit the electro-slag refining process, has fully justified its existence in its 15 months of operation on a commercial basis. It has recorded widespread use of its hire and contract facilities and has been responsible for a number of technical developments. Five plant manufacturers have entered into agreements with ESRT, and have installed or are installing fourteen plants with ingot producing capacities ranging from 8 to 36 in. The unit has also supplied 250 ingots to some twenty-five customers, covering a range of thirty-five alloys. ingots have enabled customers to evaluate the electroslag refining process. 224 ingots have been made on a miniature plant in thirty-eight individual contract evaluation programmes for fourteen clients. result of these studies, techniques have been developed for production melting of many alloys both in Britain

Electro-slag refining is a secondary refining process

for producing quality controlled steels and alloys in fairly high tonnages. It is economically competitive with other processes at present used, as the capital outlay is fairly low and the running cost is the same as if not cheaper than the other processes.

Shocks in Store

THE bid by the General Electric Company for Associated Electrical Industries seems to have taken AEI by surprise. AEI is taking a week to think about the bid, worth £120 million in stock and cash, before committing itself to a view. GEC has offered five GEC shares and 80s. cash for every eight AEI shares, which effectively puts a value of 52s. 1d. on the AEI shares against a market price before the bid of 43s. 6d. By October 3, AEI shares stood at 56s. 4.5d., as high as they have been this year, indicating a feeling among investors that AEI will offer at least some opposition to the takeover. The terms, AEI is likely to claim, are based on a poor performance by AEI last year, and a forecast of profits of about £9.2 million this year. It is likely to add that better times are on the way, but shareholders have been told this many times before. GEC profits are far more buoyant, running at about twice those of AEI, although GEC is a much smaller group.

Both companies employ large research departments. AEI has a large central research laboratory at Rugby, which carries out basic research for the whole company as well as contract research work for government and other companies. AEI Power Group has a laboratory at Trafford Park in Manchester which includes among its interests scientific instruments, traction and magnetohydrodynamic power generation. The Telecommunications Group runs two laboratories, one at Blackheath and one at Harlow, and the Cables Group has a laboratory at Gravesend concerned with insulating materials, and one at Woolwich studying high voltage phenomena. The joint AEI-Thorn semiconductor company also runs a research department.

GEC's main laboratory is the Hirst Research Centre in North Wembley, named after Sir Hugo Hirst, the founder of the company. This has divisions serving all branches of the business—lamps and lighting, telecommunications, electronics, valves and semiconductors. It also includes three divisions doing more fundamental work, in engineering, metallurgy and electronics materials, and a division which supplies chemical and technical services. In addition, GEC Electronics runs two applied electronics laboratories, at Stanmore and Portsmouth, and there is another telecommunications laboratory at Coventry. The Osram lamp company has two laboratories, and other companies associated with GEC also run their own development laboratories.

If the takeover goes through, some rationalization of this mass of laboratories seems inevitable. This is particularly likely in electronics, where the new group would hold 40 per cent of the market. GEC has a joint semiconductor company with Mullard, while AEI shares its electronics interests with Thorn; if the deal is successful, GEC hopes to merge these interests and save money on research. It is not yet clear how the deal would affect AEI's nuclear power interests—it owns 10 per cent of the shares of the Nuclear Power Group, Ltd. GEC's association with

nuclear power ended some years ago, and was not a happy one. GEC was involved in the building of an unsuccessful power station in Japan, and still has problems to solve at the Hunterston A station which it built. AEI's nuclear power interests have certainly been more profitable than this, but whether this would encourage GEC to go back into nuclear power as part of the Nuclear Power Group, or pull out altogether, is still not clear.

Laboratory of All Trades

SEWAGE is unattractive but inescapable. At the Water Pollution Research Laboratory, the staff continues to study it with every evidence of enthusiasm, and this year is spending £360,000 both on the treatment of sewage and on its effects when allowed to escape. A scheme in collaboration with the Hydraulics Research Station and a firm of consulting engineers is intended to find the ideal position for sewage outflow in the Thames Estuary. In parallel, the laboratory is studying the effect of pollution on fish, together with the related problems of the variation and measurement of oxygen concentration in streams and rivers.

The laboratory has been hard at work on the two chief methods of biological treatment—the activated sludge process and biological filtration. One interesting development is the use of plastic filters in biological filtration, and a system has been devised for controlling automatically the intensity of aeration so as to maintain oxygen at an optimum concentration. A new treatment of sewage, by chemical oxidation at high temperatures and pressures, is undergoing preliminary investigation, and as a means of reaching high pressures it has been suggested that the sewage be poured into very deep holes in the ground. The laboratory is optimistic about the change from hard to soft detergents; this, it says, has had the effect of markedly reducing the amount of foam in rivers. The manufacturers and the laboratory are hoping to find even softer detergents, and are conducting an experimentat a small sewage works serving a village community specially supplied with experimental detergent by the manufacturers.

Pollutants are also studied at the Warren Spring Laboratory, but in this case air is the dispersion medium. The laboratory provided background information for the Clean Air Act and is at present completing a five-year national survey of smoke and sulphur dioxide. Smoke concentration in the air has been falling, and although total emission of sulphur dioxide has risen slightly, the overall concentration of the gas has fallen.

Recovery of metals from scrap, studies of process control and separation of dry minerals by air fluidization form an important part of the work of the mineral science and technology section and the chemical engineering section. Both work in consultation with industry. The chemical engineering section has considerable interest in catalysts. A pilot plant producing acetone by dehydrogenation of isopropanol has been linked to a computer for process control studies, but this system has limited application. Control by simple logic systems of batch processes such as distillation of binary mixtures is being studied and is likely to be more useful. The laboratory employs a staff of 440, and is expanding slightly year by year, with annual expenditure standing currently at £1 million.

An Industry in Hard Times

By our Special Correspondent

The British motor industry has had a hard year, and the winter is still ahead.

Although more than a year has passed since the crisis measures of July 20, 1966, the British motor industry has not forgotten them. The combination of a freeze on wages and pressure on credit which Mr Wilson prescribed gave the industry the most uncomfortable autumn and winter it can remember. All the manufacturers suffered, but the worst hit was the British Motor Corporation, which declared 12,000 men redundant—and lost another 3,500 who decided that they would be better off elsewhere. BMC made a loss of £7.5 million in the first six months of the financial year, and the second half report, still to come, will show only a small profit. There is little doubt that the other motor companies did badly over the same period, but they may have been saved some embarrassment by not having to produce a financial statement quite so soon.

The crisis at BMC has left a legacy. It has been traditional for BMC to take the largest share of the British market—usually around 40 per cent—but now its share is down to 32 per cent. Even this is not as bad as the 29 per cent to which it was reduced at the height of the crisis. People are now openly doubting whether BMC will be able to regain its dominance in the market without a new range of models. The Mini, now eight years old and continuing in virtually its original form, is too small as a basic unit. The 1100 model, the next in the range, is too expensive and the 1800 range has not been a success. By an unhappy blunder, BMC hardly competes in the 1.5 litre range, where Ford finds all its profits. In the bigger sizes, BMC has a positive embarrassment of models since it took over the Jaguar company, but all of them are beginning to look dated.

To some extent, BMC's troubles can be traced to an unwillingness to take full advantage of its size. Although the merger between the Nuffield organization and Austin took place 15 years ago, only in the last two or three years have the demarcation lines between the two organizations disappeared. Worse still, BMC still sees it as its duty to perpetuate the names of Riley and Wolseley—and others—by decorating perfectly honest Austins with the trappings of the more luxurious makes, walnut facias and all. It is surprising that supporters of the old marques are satisfied with such unconvincing imitations. Next year BMC will make a tentative step in the right direction by abolishing the distinction between Austin and Morris in the commercial vehicle field, but progress towards unification is slow.

The multiplicity of models is matched by a geographical diversity of factories. The whole British Motor Holdings group, including BMC, Jaguar and Pressed Steel-Fisher, controls 26 factories. There are at least two reasons for this; on the one hand, mergers have provided factories everywhere in Britain and, on the other, the group has been persuaded by the government to open new factories in depressed areas—Bathgate in West Lothian, and Llanelly in Wales. The results of this are unfortunate. To take one example, exhaust systems for the group are made at Morris Radiators plant in Oxford at the rate of 30,000

a week. But the actual silencers are made in Llanelly and taken across country by road for assembly into complete systems. After assembly, the systems are once again packed up and distributed by road to Longbridge and Cowley. Car doors are made in Llanelly, and transported to the main factories, again by road. This certainly introduces extra costs which a fully integrated plant avoids.

There are more optimistic signs. At Longbridge, BMC is building an integrated plant for the manufacture of engines. When finished, the new plant will be able to handle 5,000 engines a week on a highly automated assembly line controlled by computers. This development, which is to cost £16 million, will produce its first engines by the end of this year if there are no further delays—it is already four months behind schedule. Near Oxford, BMC has built a new spares warehouse as part of the service department. This project has cost £6 million, and should provide BMC with one of the most efficient spares services in the world

These developments, and others, will be financed out of profits if BMC can start making them again. Much will depend on the economic climate. The other British motor companies will get only a small measure of satisfaction from BMC's discomfiture, principally because they are in almost as unhappy a position themselves. Ford's profit margins, once the wonder of other manufacturers, have been winnowed away over the years until they are little better than the rest. Only the Cortina in Ford's range is making money. Rootes, meanwhile, have found the experiment of going to Scotland to build their Imp range is not a success, and are currently ringing the changes on a basic model which is selling very slowly indeed. Vauxhall is troubled by labour stoppages and by the fear that its American owner, General Motors, favours a European marketing policy based on Opel, its German subsidiary, rather than on Vauxhall. Only Leyland-Triumph seems to have the models and the management to take advantage of the situation, and a government inspired merger between Leyland and BMC is beginning to seem increasingly possible. Certainly it is known that both the Ministry of Technology and the Industrial Reorganization Corporation have been trying to push in that direction, so far without success.

Perhaps this is why BMC is often heard plaintively to say that it is no more than the government's economic football, a phrase once reserved for the private steel companies. This is hardly fair—nobody has yet suggested that BMC should be nationalized, though it could conveniently be done without even a change of name. It is certainly true, however, that government pressure has damaged the industry by forcing it to expand in areas away from the centres of distribution. For all the fine phrases about the motor industry in Scotland—even repeated this week by an American, the new chief executive of Rootes—there is little doubt within the industry that the Scottish experiment has been a failure.

NEWS AND VIEWS

Is Ethology Respectable?

PROFESSOR W. H. THORPE makes a brave claim on behalf of ethology in his remarkable review of two books on animal behaviour (this issue, page 17) and everybody will respect him for it. And he is right, of course, to plead for a more serious recognition of the potentiality of a serious pursuit of studies in ethology. The field has been too neglected for too long. A part of the trouble is lack of money, for studies of this kind can be expensive and biologists have been slow to follow the physicists in forming a sufficiently ambitious conception of the scale on which studies of this kind should be conducted if they are to be profitable. And then, of course, it is often more convenient to stay at home in some laboratory than stump the Serengetti or some other outlandish place in search of suitable experimental conditions. It is, however, probable that the most serious of the problems to have beset the ethologists are intellectual in character. present, at least, the concepts are necessarily ill defined. if only because their formation entails the choosing of names for complicated patterns of behaviour which can only be defined descriptively. In the circumstances it is no wonder that the ethologists have often been hard pressed to distinguish between what they do and what used to be known as natural history. In this, they have not always been successful.

The problems of the ethologists are typified by the continuing dispute about the part played by Dr K. Lorenz in the foundation of the subject. To ethologists, Lorenz is a kind of Freud. Professor Thorpe, for example, after tracing the origins of the subject back to Pavlov and Sherrington, goes on to say that the subject was "fragmentary and uncoordinated until Konrad Lorenz, inspired primarily by Heinroth, succeeded in welding these concepts together so as to form a thorough-going theory of animal behaviour". It is only fair to admit that Lorenz is not always held in such high regard. Only a few months ago Sir Solly Zuckerman was taking Lorenz to task for sloppiness —and worse—in the book On Aggression (see Nature, 212, 563; 1966), and it is plainly especially difficult for ethologists to turn the other cheek to the charge of inaccurate observation. But it is equally hard for them to know what to make of the charge against Lorenz that his formation of concepts may have been clouded by a kind of wishful thinking. Those who claim that Lorenz is sometimes willing to play to the gallery of the animal lovers may have more than suspicion on their side. But may it not be that the critics and the devotees are both correct? If the ethologists find Lorenz's concepts helpful, does it matter if they are not based on the most rigorous arguments and the most unshakable experimental evidence? It would then be necessary to condemn all the other distinguished people who have been led to splendid theories by shaky arguments. There is a sense in which the proof of the pudding is in the eating. This is how Lorenz—and the ethologists as a whole—will eventually have to make their way.

This is perhaps the connexion in which it is most sobering to see it put in black and white that ethology so compels a comparative attitude of mind that it has already broadened the horizons of psychology and is about to do the same for social psychology, psychopathology and perhaps even sociology as well. In some sense, of course, this must be true, but Professor Thorpe's avowal is evidently not intended as a trivial self-justifying truth, so that the question is to know to what extent ethology will transform the social sciences, and in what ways. For one thing, there are some obvious pitfalls to be avoided. It does not, for example, follow that because rats kept densely packed in cages develop signs of stress, that people who live in cities are more likely to become neurotic than those who happen to live in the countryside. (To say this does not of course imply that seriously minded ethologists would make such an unsubstantiated comparison.) One obvious difficulty is that people seem to be exceedingly complicated, either because they are or because human observers tend to be quite well informed about their behaviour. Another is that there seems to be no obvious way of bringing about a reconciliation between the ethologists and the body of people who have done most self-consciously to describe and understand the intricacies of human behaviour—the psycho-analysts and especially the Freudians. Whatever the rights and wrongs of that dispute, it is wrong just to ignore these men and women. None of this implies in any sense that a proper study of what is called ethology would fail to illuminate in several ways important human problems, and it is entirely right and proper that the enthusiasts should think there is a great deal to be done. Dispassionate observers will be forgiven for putting some eggs in other baskets.

Chronology of Magnetic Reversals

THERE is a certain monumental quality about the geomagnetic survey of the lavas of Eastern Iceland reported on page 25 of this issue. Although the long succession of overlapping lava flows in Iceland has played an important part in the development of rock magnetism in the past fifteen years, the study which has now been carried out is at once more detailed and more comprehensive than most others. Altogether there are some 900 separate lava flows lying on top of each other and these have been sampled at a score of places by cores drilled to the underlying basalt. The direction of magnetization of more than 2,000 samples representative of individual lava flows has been determined. Perhaps the most valuable of the prizes to be won by this activity is an extension of the chronology of the sequence of magnetic field reversals from a few million years to an interval of time which spans the past twenty million years. Iceland is exceptionally well suited to such an enterprise because of its close geological relationship with the mid-Atlantic Ridge.

So far as can be told, chronology for the sequence of reversals of the Earth's magnetic field obtained from Eastern Iceland agrees well with other studies which span a shorter interval of time. Precision in this kind of work is possible only when direct measurements can be made of the age of rocks found to be polarized in a direction opposite to that of the contemporary magnetic field of the Earth. Potassium-argon dating has been of crucial importance in this respect. A year ago, Doell and Dalrymple were able, for example, to fix the age of a sequence of volcanic rocks in New Mexico laid down between 700,000 and one million years ago, which placed the most recent reversal of the Earth's magnetic field at 700,000 years ago. The same measurements would suggest that that period of reversed magnetism was comparatively short lived-100,000 years or so. It seems as if the youngest of the lavas in the sequence from Eastern Iceland, corresponding as it does to a period of normal magnetization, coincides either with the period of normal magnetization just this side of a million years ago or that immediately before it. It is, however, more striking that the long sequence of sixty changes of polarity now identified in Iceland agrees both in character androughly-in chronology with the sequence of magnetic reversals which appears to be fossilized in the igneous rocks spreading outwards from the ocean ridges. On the assumption that the rate of spreading of the ocean floor is a centimetre a year or thereabouts, the rocks 100 kilometres each side of an ocean ridge span a total of ten million years. As Dagley and his collaborators point out, it is a pleasing feature of their work that 32 reversals of the Earth's magnetic field ago, they find a long period of normal magnetization which coincides with such a one already reported from studies of the ocean floor near the East Pacific Rise. Plainly there is at least a possibility that further work along these lines, and the patient correlation of measurements with rocks which can be dated easily and those whose ages must be inferred from assumptions about the rate of spreading of the ocean floor, will yield such a detailed picture of the pattern of reversals in the past 20 million years for the Earth's magnetism to be a rapid and an important means of dating all kinds of the Earth's physical phenomena. This, no doubt, is a goal which will be vigorously pursued.

One particularly interesting feature of the results of the survey in Iceland is that there appear to have been 73 lava flows whose directions of magnetization were anomalous and that at least 55 of these are unambiguously intermediate—magnetized neither in the normal direction nor the opposite direction. The fact that such a high proportion—five per cent or so—of the lava flows examined turn out to have anomalous polarity is itself a proof of how conspicuous a part of the history of the last twenty million years have been these processes of field reversal. With such a large number of examples with which to work, it seems fair to point out, as Dagley and his collaborators do, that this may be evidence for a comparatively gradual process of reversal. Moreover, it may indicate that in the course of a reversal, there is a swinging round of the direction of the Earth's magnetic field and not simply a diminution of its intensity and then an increase in the opposite direction. Whether evidence of the kind which has now been collected in Iceland will in itself be sufficient to decide unambiguously between several possibilities in this field remains to be seen, but there can be no doubt that a long sequence of measurements like those which have now been obtained will be of the greatest value in suggesting which hypotheses it will be most profitable to test.

What Controls Chromosomes?

THE chromosomes of wheat, like those of *Drosophila*, have been an extraordinarily fruitful field of study. The reconstruction of the evolution of modern cereals in prehistory from the cytogenetical evidence is, of course, a classic; the Neolithic peoples of the Mediterranean were not geneticists, but it is clear that they were plant breeders with a vengeance. But the chromosomes of

wheat have also been an important object study in the understanding of the functioning of chromosomes as such. So much is clear, yet again, from the report by Riley and Chapman (page 60, this issue) of the role of a particular chromosome of common wheat (*Triticum aestivum*) in the genetic control of the behaviour of chromosomes during the meiosis. The point at issue is

that of knowing precisely how it is arranged that, in all divisions which halve the number of chromosomes as a prelude to sexual reproduction, there should be a regular pairing and segregation of homologous chromosomes. What Chapman and Riley have done is to show how one chromosome can set an orderly stamp on the behaviour of others.

The usefulness of common wheat in work of this kind depends on the ease with which it is possible to obtain plants with sets of chromosomes which differ in controlled and known ways from the normal set of forty-two. This, no doubt, is itself a consequence of the way in which the set of forty-two is derived, in the evolution of modern wheat, from two successive hybridizations of closely related species of more primitive plants, the result of which has been to lump together three sets of seven pairs of chromosomes. In practice, the different sets of chromosomes—the three genomes are conventionally named A, B and D—are distinctive enough to ensure that the polyploid behaves as a diploid. In other words, one chromosome will always pair with its homologue from the same genome and not with the homoeologous chromosomes from the other two genomes.

Riley and Chapman are particularly concerned, in this most recent work, with that pair of chromosomes in the genome B which is known as 5B. These chromosomes are markedly asymmetrical, with the long arm much longer than the short. It has been known for some years that this pair of chromosomes is necessary for normal pairing or synapsis during meiosis. Without 5B, homoeologous pairing is rife—with only one of the pair of chromosomes in a plant, homologous pairing becomes the rule. Extra 5B chromosomes make no difference, but extra pieces of the chromosomes consisting of the long arms alone bring about abnormalities of pairing. So much is known. By growing plants with an excess or sometimes even a deficiency of the long arm, with and without the short, Riley and Chapman have shown that the long and short arms have antagonistic effects on the orderliness of pairing at meiosis. They follow Feldman in the view that the 5B chromosome functions by influencing the spatial distribution of chromosomes before synapsis, and in particular that the long arm ensures that the members of pairs of homologues are closer to each other than to the corresponding homoeologous chromosomes. But

Water into Steel

EVERYBODY knows that steel makers are among the most prolific users of water, but it now seems that they are also enormously variable in this respect. A survey of the steel industry in the United States which has been carried out by the United States Geological Survey shows that some steel companies use very large amounts of water and that others are, by comparison, much more sparing in the use they make of it. The biggest variation in the use of water seems to arise from differences between steel manufacturing plants in the practice of re-using water. The results of this survey, based on the inspection of 16 ore production plants and

an excess of the long arm will make all distances too great, so that the efficiency of pairing is impaired. Only the short arm of 5B can counteract the effects of an excess of the long arm, again by influencing the distribution and orientation of all the chromosomes.

By themselves, these findings will be an important addition to what is already known about the influence of particular chromosomes in common wheat. Previous studies have, for example, shown that the long arm of the 5D chromosome is necessary if homologous chromosomes are to pair normally at low temperatures. The long arm of the 5A chromosome has a similar though less pronounced effect, and the third chromosome from each of the three genomes is also necessary for regular pairing. Given the ease with which it is apparently now possible to add or subtract pieces of particular chromosomes to or from the genetic constitution of wheat, there is every prospect of a much fuller understanding of how each portion plays its part. It is more difficult to see precisely how these and other results will be interpreted in terms of molecular biology. If the long and short arms of the 5B chromosome are somehow involved in positioning of the chromosomes of wheat as a prelude to cell division, how precisely is this effected?

Is there some molecule manufactured by 5B which can attach itself or otherwise influence all the other chromosomes? Are there—an unlikely possibility—long range forces which do the job? Or is there some underlying structure in the medium within which the chromosomes lie which can be influenced by 5B? As yet these and other possibilities remain but speculations

Wheat cytogenetics also has its practical side. It has, for example, been possible to introduce resistance from closely related species by interfering with the activity of the long arm of 5B so that hybrids can undergo normal meiosis, when genes for resistance may be incorporated into the wheat by the recombination of genetic material which occurs after pairing. What has now been discovered about the short arm of 5B may be exploited to increase homoeologous pairing. The practical consequences may be much delayed. New strains take decades to reach the farms. But if the immediate interest of wheat cytogenetics is theoretical, its more distant influence in agriculture may be more significant.

29 manufacturing installations during 1957 and 1958, are described in Water-Supply Paper 1330-H of the US Geological Survey (to be had from the US Government Printing Office). It is not entirely clear why it should have taken the survey the best part of a decade to produce this analysis.

Estimates of water consumption in steel manufacture in the past have been exceedingly variable among themselves. More than a decade ago, however, the Senate Select Committee on Water Resources is said by the Geological Survey to have reported that the manufacture of a ton of steel consumes 31,842 US gallons of

water. The average consumption of the plants covered in the survey works out at 34,000 gallons per ton of ingot steel. The production of iron ore from mines in the United States involved the consumption of 5,900 gallons for each ton of concentrate.

Most of the water used in steel manufacture is used for cooling, which is why great savings are possible. Among the integrated steel plants (which include blast furnaces, steel making furnaces and possibly processing plants as well) the intake of water seems to have ranged from 1,340 gallons per ton to 66,300 gallons a ton, with a median of 24,300 gallons a ton. Variations in the amounts of water actually employed in the manufacture of a ton of steel seem to have ranged from 11,200 gallons to 100,000 gallons, with a median of 33,200 gallons (and an average of 39,500 gallons). But actual consumption—the difference between intake and output—works out at merely 1.8 per cent of the intake. The most prolific users of water among the integrated steel plants used irrevocably 2,010 gallons of water for each ton of steel, compared with the average of 491 gallons per ton. More than half of this water (263 gallons per ton) was lost by evaporation from cooling systems, and 150 gallons of water per ton of steel was used to keep the boilers supplied.

Collagen Discussion Group

ROUGHLY 60 people took part in the second meeting of the Collagen Discussion Group which took place in Manchester on September 25. There were three communications under the general heading "The Nature of Soluble and Insoluble Collagens", in the first of which Professor D. S. Jackson outlined the methods for the extraction of soluble tropocollagens from tissues and showed why the various types obtained depended on the conditions.

Dr F. S. Steven dealt with the insoluble collagen remaining after removal of the soluble varieties. This material can be rendered dispersible in acetic acid by treatment with crude bacterial α -amylase to an extent which varies with species and type of tissues. By raising the pH of the acetic acid dispersion, a fibrous mass called "polymeric collagen" is precipitated. EDTA and other metal chelating agents are as effective as α -amylase, and both types of pretreatment produce polymeric collagens identical under the electron microscope and with respect to aminoacid composition (apart from ornithine content), but EDTA gives a product containing rather more hexose and hexosamine than that derived from α -amylase pre-treatment.

It was suggested that polymeric collagen consists of tropocollagen molecules polymerized by intermolecular covalent cross-linkages, not necessarily similar in chemical structure to the established interchain cross-linkages of tropocollagen. In mature connected tissues, the polymeric collagen fibrils are probably bound together by an intercibrilon substance to form the collagen fibre. A polymeric cibrilon could be released from the fibre by the enzymes of the crude bacterial α-amylase preparation or by chelating agents.

Professor Jackson summarized in his contribution existing knowledge aimed at visualizing the processes in the formation and deposition of collagen fibres in living tissue. The first stage would involve cross-linking of tropocollagen molecules to form microfibrils. These might then be further associated by interaction with mucoproteins to form fibrils of large diameter. Interaction of these with mucopolysaccharides would complete the process. The noncollagenous components might also determine the structure and function of the different connected tissues.

The contribution by Dr R. Reed and Mr S. J. Kennelly dealt with a comparatively new technique for preparing specimens in such a way that alterations due to mechanical distortion and chemical action are minimized. The specimen from the skin is rapidly cooled to the temperature of liquid nitrogen and then cut to give a plain surface. Evaporation of the ice crystals leaves an "etched" surface, a replica of which is prepared for electron microscopy. This method was used to study changes after treatment of the skin with soluble collagen extractants and to study prolonged treatment with alkali which converts the insoluble to the so-called "eu-collagen" which is then soluble in acidic solutions. Alkali was shown to disorganize the collagen fibres and eventually convert them to a mass of fine filaments of widely varying lengths, while in specimens treated with acid they were dispersed and shortened. This method of freeze etching showed promise in this type of study.

More about Haemoglobin

from our Molecular Biology Correspondent

EVER since the determination of the crystal structure of the haemoglobin molecule, a final explanation of its mechanism has appeared to be just over the next hill. Some interesting new experimental data are now described in four papers by Guidotti (*J. Biol. Chem.*, **242**, 3673, 5919: 1967). He has studied the dissociationassociation equilibrium between sub-units in solution in deoxyhaemoglobin and liganded haemoglobins and on the basis of these results has shown that the association state of partially liganded haemoglobin (of which the most important example is partly oxygenated haemoglobin) is compatible only with the existence of mixed tetramers of the type $(\alpha\beta)(\alpha*\beta*)$ where the asterisk denotes a liganded chain. This idea has been previously expressed by the Benesches and their associates, and it is satisfactory that Guidotti's results provide such strong support. The conclusion is general and will apply to any situation in which hybrid tetramers consisting of two different dimers can be formed.

This central hypothesis leads to a complete description of the oxygenation mechanism in favour of which Guidotti has assembled a considerable body of evidence. The dimer $(\alpha\beta)$ is envisaged as the effective functional unit of the molecule in that α and β will both exist in the same reactivity state. Oxygenation will then begin with an essentially simultaneous reaction of the two haems of one as dimer. Thus at any one time, a solution of haemoglobin in the presence of oxygen will contain five species, three tetrameric $(\alpha_2\beta_2, \alpha_2^*\beta_2^*)$ and $\alpha\beta\alpha^*\beta^*$ and two dimeric $(\alpha\beta)$ and $\alpha^*\beta^*$. The relative $\alpha\beta\alpha^*\beta^*$) and two dimeric ($\alpha\beta$ and $\alpha^*\beta^*$). concentrations of the dimers and tetramers will depend on the total protein concentration, and an analytical version of the Hill equation is developed in terms of concentration, association constants for dimerization of the deoxy- and oxy- forms and intrinsic binding constants for oxygen. Not only does this equation describe the observed oxygen uptake curves under normal conditions, but it also predicts the hitherto inexplicable high value of the Hill constant (reflecting high haem-haem interaction) under conditions where the haemoglobin is dissociated. All the parameters in the equation can be evaluated by independent experiments, and the fit must therefore be seen as a strong argument in favour of the hypothesis.

The scheme demands differences in conformation of any chain when it combines with the ligand, and from dissociation behaviour and reactivity of sulphydryl groups Guidotti shows that there appear to be conformational differences between the deoxygenated dimer $\alpha\beta$, the oxygenated dimer $\alpha^*\beta^*$, and the former in its combination with the latter, i.e., as $\alpha\beta\alpha^*\beta^*$. Guidotti expresses the hope that X-ray analysis of oxy- and deoxyhaemoglobins at high resolution will reveal conformational differences, no matter how small, within the chains.

So far this hope has not been too well borne out. The latest X-ray study from Perutz's laboratory (Muirhead et al., J. Mol. Biol., 28, 117; 1967) reports the structure of human deoxyhaemoglobin at 5.5 Å resolution. Detailed comparison is available only with horse oxyhaemoglobin, but it is clear that in terms of tertiary structure the two proteins are closely similar in all respects, including the relationship between the haem group and the peptide chain. The change in the relative orientation of the sub-units, which amounts to a considerable rotation, has been precisely characterized, and changes in the contacts between sub-units are noted. At only two points, one in the a and the other in the β chain, are slight conformational differences observed, though these are interpreted with caution. How such small differences of conformation might be capable of accounting for the very considerable chemical differences observed by Guidotti remains in doubt. The possibility that these reactions are governed by ionization differences which do not significantly affect the conformation must be considered. Guidotti's mechanism of ligand binding at this stage, however, still carries considerable conviction.

Chloroplast DNA

from our Cell Biology Correspondent

EVER since the discovery that mitochondria and chloroplasts contain DNA, the enzymes for DNA replication and transcription and distinct machinery for synthesizing protein, there has been speculation that these organelles evolved from symbiotic bacteria and that they have retained at least partial genetic autonomy. If this is so, it is obviously important to discover just how much genetic autonomy they have. Which proteins in the plastids are coded for by local DNA and which by nuclear DNA? In two papers in Biochem. Biophys. Res. Comm. (28, 598 and 604; 1967), Smillie and his collaborators report on the way in which the DNA in the chloroplasts of Euglena gracilis serves as a source of information.

Not surprisingly, given the evidence that plastid ribosomal RNA is distinct from cytoplasmic ribosomal RNA, Steele-Scott and Smillie find that ³²P-labelled ribosomal RNA from *Euglena* chloroplasts hybridizes with chloroplast DNA but that the total ribosomal RNA from cells grown in the dark, which lack well

developed chloroplasts, fails to hybridize. They interpret this as showing that the chloroplast DNA rather than the nuclear DNA codes for chloroplast ribosomal RNA. A rough estimate indicates that each chloroplast has of the order of 20–45 ribosomal RNA cistrons and presumably the chloroplast ribosomal proteins are also coded for by the DNA of the organelles.

To test whether photosynthetic enzymes either in the stroma or bound to lamellae are synthesized in the chloroplast and, therefore, by implication, whether they are coded for by the chloroplast DNA, Smillie et al. had to use a much more indirect approach. They reasoned that because protein synthesis in plastids and bacteria but not in plant cytoplasm is inhibited by chloramphenicol, whereas cycloheximide inhibits protein synthesis in plant cytoplasm but not in bacteria, enzymes made in chloroplasts should be inhibited by chloramphenical but not by cycloheximide. The inhibitors were fed to Euglena differentiating chloroplasts and the cell extracts were assayed for three bound proteins of the electron transfer system (ferredoxin-NADP-reductase, cytochrome-552 and cytochrome-561) and for two stromal enzymes (ribulose-1, 5-bisphosphate carboxylase and NADP-glyceraldehyde-3-phosphate dehydrogenase). Chloramphenicol, but not cycloheximide, inhibited the synthesis of the stromal enzymes, which must therefore be supposed to be synthesized in the chloroplasts. Synthesis of the electron transfer proteins was also inhibited by chloramphenical, and this implicates the chloroplast ribosomes as the site of synthesis. In this case, however, the result is less clear cut because cycloheximide also had some inhibitory effect. Smillie et al. suggest that this inhibition by cycloheximide may be caused by some secondary indirect effect of the inhibitor on the synthesis of some chloroplast protein in the cytoplasm.

Thus it appears that enzymes involved in photosynthetic reduction of carbon dioxide and electron transport in chloroplasts are made within the organelle and probably coded for by the chloroplast DNA. By contrast, soluble mitochondrial enzymes involved in the oxidation of carbon compounds to carbon dioxide (Roodyn et al., 1962; Clark-Waller and Linnare, 1967) and the mitochondrial cytochrome C (Beattie et al., 1966; Huang et al., 1966) are synthesized outside the mitochondria. These results suggest that a wider range of proteins is synthesized within chloroplasts than in mitochondria and therefore that chloroplasts have greater genetic autonomy than mitochondria.

Chromatography and Spectroscopy

from G. R. Primavesi

Almost from the beginning of gas chromatography, innumerable chromatographers and spectroscopists have collaborated in establishing the purity of standards and in identifying unknown components, but the first joint meeting of the Gas Chromatography and Infrared Discussion Groups of the Institute of Petroleum was held at Loughborough University of Technology on September 29.

A. R. Philpotts introduced the discussion with a quantitative estimate of the natural and technical limits of present techniques. Assuming that weak bands are just to be detected and that the whole of a sample can be put at the focus of an ordinary spectro-

meter, one micromole is about the smallest sample size. Various instrumental devices and tricks can sometimes improve this by one or two orders of magnitude and, in favourable cases, strong absorption bands can improve it considerably further, but such improvements tend to be costly and time-consuming. The small mass of component in a gas chromatographic peak makes trapping and transfer as a liquid extremely difficult. If the component is not trapped, the dilution is usually such that examination requires an impossibly long vapour cell. Multi-reflexion and light-pipe cells bring their own problems and need careful design to give any marked improvement. A combined infrared cell and trapping system was described by J. Tadayon. This is made entirely of silver chloride or bromide in which the component condensed by cooling is centrifuged into the cell and examined directly without special condensing optics. These devices are a real advance towards solving the transfer problem.

At the conference, P. A. Wilks described an attenuated total reflexion cell with a cold spot for trapping and examining liquid samples. There was considerable discussion on the effectiveness of this type of cell with the small quantities involved. He also described light-pipe gas cells which, however, require a considerable partial pressure of component to approach the postulated micromole minimum of sample. The use of a simple light-pipe cell in conjunction with a modified spectrometer and a standard gas chromatograph was described by H. A. Willis. A scanning cycle of 63 seconds makes it possible to examine nearly all peaks in a mixture in only one chromatographic run. Fairly high concentrations of components, with at least medium absorption bands, are, however, required.

A commercially available spectrometer was described by S. G. Perry using filters for dispersion with a 5 or 12.5 second scanning time. He concluded that this time was not really short enough and that the spectra obtained had important gaps and were usually too weak. The apparatus did, however, permit immediate comparison of a peak with a standard. D. Welti described a trapping method using a short length of packed column. This method is not new but has not been thoroughly explored. Welti eluted from the trap into special vapour cells and achieved a concentration of the component vapour greater than the original peak. He also described a technique, developed in Holland, which transfers material from a trapping column to a very small potassium bromide disk.

In the general discussion it appeared that nobody was satisfied with the present state of the art. The plea of the spectroscopists for increased concentration of sample may well be met by studying trapping techniques, although significantly increased mass may not be possible. The chromatographers want increased spectroscopic sensitivity (one, two or even five orders of magnitude), but this appears to be unattainable without some unexpected revolution in spectroscopic technique.

European Marine Biology

from a Correspondent

THE European Symposia on Marine Biology were begun in 1966 when what had been a series of German symposia was changed to one representing all European

countries. The object is to bring together, with as little formality as possible, workers in all branches of marine biology. Last year at Heligoland it became quite evident that there was a demand for such meetings, and the Committee for European Marine Biology Symposia (CEMBS) was set up to ensure continuity and to preserve the original character of the meetings. Each is held, by invitation, at a marine laboratory and may be sponsored by any organization. The committee tries to ensure that the venue changes from one year to the next. Once the allocation has been made, the choice of topics and all further organization is left to the sponsoring body. The new organization has been reorganized by the International Union of Biological Organizations. The president of the committee is Dr A. Barnes, Millport, Scotland.

The Second Symposium was held at the end of August at the Biological Station of the University of Bergen, Espegrend, and organized by Professor H. Brattström. The proceedings were opened by the rector of the university, Professor Hakon Mosby, himself a distinguished worker in physical oceanography. The formal papers were organized round a single broad topic—the importance of water movements in marine ecology. Possibly because the effects of water movements are so easily observed on the shore, a large number of the thirty-six papers were given over to littoral ecology. To begin with, Dr J. R. Lewis summarized the effect of water movements on the distribution of littoral organisms, and Dr T. Carstens dealt with the physics of the interaction of moving waves with solid objects. It soon became evident that the measurement of "exposure" presents major problems and there was vigorous discussion as to what can, and should, be measured and even whether the attempt is worthwhile. Even so, various attempts to measure water movement on both exposed coasts and in sheltered waters were described and an apparatus for simultaneously measuring several parameters of the littoral environment was demonstrated. Papers on the effect of water movement on the distribution and behaviour of a wide variety of organisms-bacteria, algae, and molluscs-were all discussed. The absence of physiologically orientated papers on the behaviour of marine organisms in relation to water flow seems to indicate that much more experimental work needs to be done in that field.

A second group of papers dealt with the relationship between water movements and the local and regional distribution of a variety of organismslargely planktonic but even including the giant squid. Some considered the factors in a restricted body of water, such as a tidal estuary on the western Baltic. while others dealt with wider areas-British and Canadian coastal waters, the Arabian Sea, Japan and Okhotsk Sea, the Adriatic and the Southern Oceans and were essentially biogeographical in character, the results being largely based on plankton collections taken during what may be termed "routine" surveys. Once again it seemed to many that definitive answers to many of the interesting and important problems raised as a result of the analysis of such data will only be obtained by a more experimental approach.

The Third European Symposium will be held at Arcachon, September 25–29, 1968, in the Institut de Biologie Marine of the University of Bordeaux

through the invitation of Professor R. Weill.

Industrial Research Associations in Britain

by C. J. ARLIDGE

Secretary, Committee of Directors of Research Associations The OECD has now published a study of industrial cooperative research in Britain (OECD, 17s. 6d.). The report has been prepared by Dr A. B. Hammond, Mr S. G. Attenborough and Mr G. A. Ingram.

Dr Hammond begins by pointing out that when the British Government laid aside £1 million in 1917 to finance the Department of Scientific and Industrial Research, it also said "The independence and initiative of the British manufacturer had contributed largely before 1917 to his success" but "after the war he will need all possible assistance in undertaking and developing research work as a means of enlarging his output and improving its quality. But if the help is to be effective it must increase the manufacturer's independence and initiative."

With the publication fifty years later of this comprehensive historical study by three members of the DSIR team most closely connected for many years with the evolution of a modern look in the British industrial research associations, it is possible to make a critical analysis. Was the legislation in 1917 well conceived? Is it still appropriate to a society more dependent on manufacturing industry than the 1917 legislators could possibly have imagined?

If the legislation was truly well conceived, then one must be puzzled by the relatively small growth in the total investment in the cooperative industrial research associations, from £1 million in 1917 to about £14 million today (£16 million if the non-grant aided research associations are included). To some extent, this question is answered by Appendix III of the report which lists the twenty-two industries which tried but failed to run their industrial research association laboratories as independent units. Eight of these were forced to merge with other more successful cousins (in two cases with the laboratories of nationalized industries). Only one has been continued as a cooperative research venture without grant aid. The rest were forced to close down temporarily or permanently.

The majority of research associations admit that their work is too little known. Active steps have been taken by at least a dozen of the laboratories to correct this omission, and wherever open publication of their work is practicable and permissible this is being encouraged. There is little doubt that this, and the staff career pattern activities described in the recently published OECD document, will enhance the opportunities for recruitment of larger numbers of well-qualified young people.

Of course there have been failures. It would be more surprising if there were not. But the cumulative advances in the use of the original legislation by different industries are incredibly extensive. From the adoption by the Production Engineering Research Association of a substantially sponsored research programme, a policy being adopted now by at least eight or nine other industrial associations, to the integrating and coordinating bank for research and information (rather than providing its own central research laboratory for the industry) instituted this year by the Construction Industry Research and Information Association, the pattern is changing so rapidly that Dr Hammond and his colleagues may consider themselves fortunate that their study was completed four years ago. If they started it now it is unlikely that they would ever have time to finish it.

If we subject the British industrial research associations

to a close examination and consider them in the light of European experience with cooperative research we find three encouraging signs:

- (i) Most British research associations have responded rapidly to the need to make sponsored research complementary to their cooperative research programme. To my knowledge no examination has been made of the reason for this rapid change in policy although it could be due to the considerable increase in American investment in Britain since the war, with the corresponding changes in industrial attitudes.
- (ii) On the international front, British research associations match their opposite numbers in France, Germany and Austria in the recognition that the best research is run on international lines.
- (iii) The industrial research associations whose work is demonstrably used to commercial advantage by their industries are also those with soundly based basic research programmes.

The last of these points seems to me to be the one in most urgent need of examination. The latest research and development statistics for 1964-65 show that the universities, which are believed to be too remote from industry for the effective utilization of their research, are now spending £4 million on applied research and £35 million on basic research; while the grant-aided research associations are spending a paltry £9 million on applied research and nothing at all on basic research. The zero in the basic research column is probably an error, but the relative proportions are interesting. I am tempted to suggest that £20 million for applied research in research associations and £4 million for basic research would be a good working minimum income for the present number of grant-aided industrial research associations. This could indeed be considered a good minimum figure from which to bargain with European industry in our attempts to join the Common Market.

One may ask, however, whether the time is not ripe for the introduction of fresh legislation which would put the laboratories of the industrial research associations on a similar footing to those of the universities—that is receiving Government revenue from the Treasury. Consider the case of the National Institute for Social and Economic Research. This Institute operates in a manner closely resembling the cooperative industrial research associations but receives a grant for its economic studies direct from the Treasury.

In order to assure the Treasury, however, that this form of investment in industrial research would be a sound one, it would certainly be necessary to constitute throughout the network of research associations a strategic policy of management integration with industry. Any scheme designed to cover this requirement would need to be comparable with those already in operation for the large science-based companies. In my view, once a scheme of this type had been established and linked to companies in membership of the research associations, grants direct from the Treasury to industrial research associations on a scale very much larger than that at present envisaged would be a very sound investment.

Zoology and Behavioural Sciences

by W. H. THORPE Why study animal behaviour? In what follows the author argues that this is—or should be—the foundation of a proper understanding and use of the social sciences. The argument is based on two books published recently—"Animal Behaviour" by Robert A. Hinde (McGraw-Hill, 84s.) and "Mechanisms of Animal Behaviour" by Peter Marler and William J. Hamilton (John Wiley, 113s.).

THE boundaries between the four behavioural sciences, physiology, psychology, ethology and sociology, are ill defined. It is true that the central aims of each science are different, so that the emphasis which each makes is characteristic. But as the separate sciences develop, the more they come to overlap both in techniques and in material. Sociology, the youngest of the four, is still in many ways in an embryonic state and tends to be closely restricted to its primary aim of elucidating the structure of human social systems. It is therefore very closely related on one side to anthropology, ethnology and economics, but as yet comparatively little developed in its relations with the first three behavioural sciences. Its connexions here are closest to psychology and ethology and more remotely to physiology. But if sociology is to develop as it should into a true science with an experimental basis, it must develop secure biological roots and become truly comparative. It would seem that it can best do this, at least to begin with, by means of ethology.

First Behaviour Studies

The physiological study of behaviour can be said to have begun with Thomas Willis, the Oxford anatomist who in the early seventeenth century first employed the concept of reflex in a scientific and practical rather than a philosophical manner. This started a chain of development which reached its first flowering with the nineteenth century Russian physiologists, among whom Pavlov may be included although in actual fact none of his work on the nervous system was published until the twentieth century. By itself, the concept of the reflex did not carry the workers of that period very far towards a useful analysis of behaviour, but with Pavlov's concept of conditioning all was changed. For the first time, the reflex theory seemed to be within reach of providing a reasonably satisfactory physiological explanation of animal behaviour. It is hard to exaggerate the importance of the complementary work of Pavlov and Sherrington in the first quarter of the present century in laying the foundations of the physiological approach to behavioural analysis.

The nineteenth century psychologists were solely concerned with human beings. They were so centred on the problems of the human mind, its feelings and emotions, that they could not provide the tools required for the scientific study of animal behaviour until they in turn, towards the end of the century, became imbued with associationism. Such nineteenth century psychological writing as there is on animal behaviour is monumentally

anecdotal and anthropomorphic, and this criticism applies also to at least one physiologist-Romanes. The limitations of the strict conditioned reflex approach to animal behaviour began to be apparent to the naturalists—the field zoologists-almost as soon as it developed; for about this time, zoologists were beginning to be more scientific and more objective about animal behaviour. really as a delayed result of Darwin and in particular of his Expression of the Emotion in Man and Animals (1872). But in the second decade of the present century it was not only the naturalists who became aware of the obvious deficiencies of the existing mechanist-physiological approach to the study of behaviour. Those among the physiclogists and psychologists who were studying the more highly developed perception of animals were equally sceptical, and so it was that Wertheimer1, working on the visual perception of movement, and later his disciples Wolfgang Köhler² and Koffka³, came to propound the concepts and theories which in due course produced the Gestalt psychology. This started a battle with the behaviourists which has waxed and waned as the two sides have changed their position slightly over fifty years, and is far from dead even now.

Coming of Ethology

The process whereby the field naturalists and zoologists came to organize themselves into a logically coherent scientific group was also, of course, slow. Some of the essential contents can be found, for example, in the writings of Lloyd Morgan and, later, Julian Huxley—and it is right to recall the forgotten mid-nineteenth century pioneer Douglas Spalding4. Essential contributions to modern ideas on animal behaviour can be found all appearing at about the same period in the writings of Wallace Craig⁵, H. S. Jennings⁶, Karl Lashley⁷ and C. O. Whitman⁸ in the United States. Equally, if not more, important was Oscar Heinroth's in Germany. But the output of all these workers, so far as theoretical consequences were concerned, was fragmentary and largely uncoordinated until Konrad Lorenz¹⁰⁻¹², inspired primarily by Heinroth, succeeded in welding his concepts together to form a thorough-going theory of animal behaviour. As a result, Lorenz is rightly considered the founder of the new movement. Some of his formulations are now regarded as outmoded and have been subject to strong criticism; but the fact remains that Lorenz was the first to provide a systematic and coherent survey of the field of animal behaviour which found a place for all the outstanding problems and without which the scientific study

of animal behaviour could not have been welded together into a distinct discipline as it has been with the adoption of the term "ethology".

This term has played an important part; it is peculiarly appropriate in that its use in the late eighteenth and early nineteenth centuries covered "the interpretation of character by the study of gesture" and also "the art of Thus it is remarkably apt for denoting the methods of the contemporary zoologist-naturalist student of animal behaviour, concerned as he is with interpreting, understanding and relating, both physiologically and psychologically, the movements, actions and action patterns of animals. Thus, about the same time, two large and complementary sections of the study of animals received new names—ecology and ethology—and thereby became better co-ordinated and greatly stimulated. Here again, of course, the fields overlap; though, just as in the larger divisions of science, the primary objectives are different. The ecologist studies the life and organization of animals primarily as an expression of the environmental influences, both animate and inanimate, to which they are exposed. The ethologist includes many of the same problems in his programme, but considers them chiefly as expressions of the nature and organization of the animal itself.

Understanding Behaviour

In one essential respect the writings of Lashley and Lorenz are very similar. Before them there had been much vague talk about the "internal drive" of the animal. Both Lashley and Lorenz realized independently that instead of trying to study this mysterious drive in its own right, and to utter vague generalities about tension, 'nervous energy" and so forth (which were in any case useless as explanatory concepts and of small value as guides for future research), it was better to describe and analyse the complex and stereotyped action systems themselves. These and their co-ordinating mechanisms were assumed to be the fundamental things, and the conviction was felt by both workers that a study of the mechanisms themselves might eventually throw further light on the problems of drive and motivation which are basic to the study of behaviour in animals or men.

Recently there has been an immense proliferation of ethological study and experiment and a stream of books on the subject. Among these many books are two which provide the occasion for this review, the significance of which cannot be fully appreciated except against a historical background. The first is Animal Behaviour: a synthesis of ethology and comparative psychology, by R. A. Hinde; the second is Mechanisms of Animal Behaviour, by P. Marler and W. J. Hamilton. Professor Hinde's book is more fundamental to the over-riding methodological and theoretical problems which are, in one way or another, the concern of all the behavioural sciences at present. It is an outstanding and highly original contribution to the task of synthesis. The book by Marler and Hamilton, while hardly competing in this particular respect, is, nevertheless, also a fine and timely achievement.

Hinde emphasizes the difference between the attempt to predict behaviour and the attempt to understand it. It is often possible to predict behaviour with fair accuracy without moving to a deeper or more fundamental level of analysis. In fact, prediction of this kind is, of course, what every animal trainer does. To understand the organization of behaviour, hypotheses must be judged not only at the behavioural level but also in terms of their compatibility with hypothesis at a deeper level—for example, a physiological level. Indeed, unless a theoretical system can be related to physiological data, it is unlikely to have wide validity even at the behavioural level.

This leads to a discussion of the virtues and vices of models, and Hinde points out that the virtue of a good

model is that it suggests further questions and tempts us to formulate hypotheses which are experimentally fertile. Although analysis down to a physiological level is always desirable, it is at present very often unattainable. But while "physiologizing" is a long term aim, it is also much more than that; it is a valuable source of information and inspiration to the student of behaviour. A fertile psychology cannot afford to ignore physiological data, which may suggest useful concepts; psychologists or ethologists who deny themselves such data are in danger of neglecting an important source of evidence. Models then, if well and carefully chosen, often facilitate bridge building between ethology and physiology.

Causes and Mechanisms

In his book Hinde makes much use of functional schemata to illustrate definite causal relationships. Though essentially quite abstract, they serve to indicate the job which the physiological machinery must be doing and provide the physiologist with an indication of what he must look for. This is, of course, exactly the objective at which the first formulations of Lorenz were aimed, and no greater justification of the method need be sought than the great success of the Lorenzian theories in energizing and stimulating behaviour study. But because such formulations are abstract, they continually need refinement and translation into the contemporary physiological and ethological terms. In this book, the essential problems brought into focus by the Lorenzian terminology are discussed and illuminated and often immeasurably advanced after three decades.

Often the development has been to break up what were originally considered to be quite simple explanatory ideas into a number of parts which then become amenable to experimental analysis. This is clearly seen in the term "drive", which used to be shared by psychologists and ethologists in previous years. Hinde has shown the dangers of clinging too long to the concept of the theoretical unitary drive system, and one of the great contributions of Hinde's own research and of his book is to show how developments in neuroendocrinology have now made possible an enormously more effective and detailed understanding of many cases which would previously have been merely dubbed as due to "an internal drive". The account of the general effect of the brain-stem reticular system on behaviour and its relation to the concept of general drive and the effects of sensory deprivation is particularly cogent.

The first main section of the book is a masterly summary and exposition of the whole field. The synthetic aspect of the book is especially apparent, in that its examples are drawn from the behaviour of animals in the field and in the laboratory, with physiological advances introduced wherever relevant.

The chapter on the control of movement is particularly valuable for its emphasis, in connexion with feedback control, upon the idea of the concept of target value "or equilibrium position"—terms which are to some extent synonymous with the German "Sollwert" (literally, the "should-be value"). In discussing the control of muscular action, the "Sollwert is regarded as achieved when the lengths of muscle and muscle-spindle bear a certain relation to each other"; and this concept is shown to be of fundamental importance to the whole understanding of those fixed action patterns, so interesting to ethologists because so characteristic of species, which used to be subsumed under the general title of "instinctive".

There is also much of interest in the other large section of the book entitled "The Development of Behaviour". One of the sections of principal importance is that dealing with the development of perceptual abilities, which raises the whole problem of perceptual learning, distance and depth perception, and the effect of lack of visual experience on species ranging from pigeons and rats to chimpanzees and human beings. Topics of this kind are very skilfully

used for building bridges between ethology and physiology on the one side and psychology on the other. Another section of particular concern to psychologists is that on "Displacement and Redirection", in which it is shown how several different types of mechanisms, from behavioural inhibition to "redirection", help to account for the puzzling and intriguing examples of behaviour hitherto often lumped together under the heading "displacement".

It is unfortunate that the work of Hinde and his collaborators13-15 on the behaviour of socially living groups of rhesus monkeys, under carefully controlled mild stress and deprivation, is too recent to have found much place in this book. This work shows how rhesus monkeys living in family groups can be used as experimental surrogates for human beings-especially for the study of such vital problems as the effect on subsequent behaviour of early stress and, specifically, the results of maternal deprivation. It is almost impossible to obtain satisfactory evidence of such effects with man, for the consequences take so long to develop and because the circumstances of life of the human subject are too complex for even reasonably scientific evaluation; and, of course, because it is impossible to carry out experimental analysis with human beings. The impacts of ethological studies of this kind on human paediatrics, social psychology and sociology are only just beginning and are potentially enormous. The book hints at what they may become and should serve to alert human psychologists and particularly those concerned with the development of sociology (which one hopes may become a real science in due course) to the absolute necessity that that science should be adequately and firmly rooted in biology.

Things to Come

Hinde's book is pre-eminent because he deals with all the fundamental theoretical questions of ethology with mature and contemporary understanding of all the disciplines involved—especially endocrinology and neurophysiology. The book is encyclopaedic yet kept within manageable proportions. It follows that it is highly condensed and hence not always easy reading. But among the present and forthcoming generation of research workers it will inevitably be the first choice as a guide and mentor—for it has no real competitor in any language.

The book by Marler and Hamilton is also by any standards first class. It does not show the same fundamental theoretical insight, nor does it attempt a similar critique of theoretical foundations and methodology. But it is, like Hinde's book, comparative, thorough and reliable. In short, many of the encomiums given to Hinde are also applicable to Marler. In some respects it is complementary to Hinde's book. Both range over the whole animal kingdom, but the majority of Hinde's examples are drawn from vertebrates whereas Marler and Hamilton's book is noteworthy for the fine series of examples and illustrations chosen from the invertebrate field of study. It is lavishly illustrated and beautifully produced.

What, then, is the future role of ethology in the last quarter of the twentieth century? Obviously there is still much for it to do in continuation of the integrative activity that it has already displayed during the last thirty years. It is characteristic of ethology that its approach is eclectic. That is to say, just because its subject is the whole animal, it is prepared to use whenever appropriate all types of analysis, all types of theoretical approaches, all types of observational and experimental techniques which it finds contribute toward the prime object—the total understanding of behaviour.

Before anyone can proceed with the scientific analysis of any phenomenon he must first classify it. If the phenomenon is a piece of behaviour, such as a righting reflex, we find that there are broadly, as Hinde shows, three forms of classification available to us—causal, functional and historical. The causal classification

presupposes the question: how does it work? The ultimate answer, therefore, tends to be in terms of physiological mechanisms—mechanisms of perception, neural or hormonal correlation and integration, and ultimately mechanisms involving biochemistry. Functional classification presupposes the question: what is this for? What is the adaptive or survival value of this behaviour? Such questions underlie the division into categories such as threat, courtship, parental behaviour and so on, and classifications of this type correspond to the use of words such as "legs" and "eyes" in morphology. As Hinde points out, functional categories are often used to include behaviour, for example. Historical classification comprises two quite distinct systems: specification according to source and classification according to methods of acquisition.

What Ethology Can Give

Ethologists, having approached the study of behaviour in the first place as zoologists, are particularly attracted by evolutionary explanations, and will start by looking upon similarities in patterns of behaviour such as the modes of cleaning or scratching in birds, similarities which are not obviously necessarily correlated with the specific structure of the forms studied, as expressions of an evolutionary relationship. The classification according to the method of acquisition is also historical, but is concerned more with history of the individual-is the behaviour learned or conditioned, and how? This is the approach that tends to be very characteristic of the psychologist, and much misunderstanding between psychologists and ethologists in the past has been due to failure to see that, though they may be discussing the same subject, the two are in fact asking different questions about it.

Ethology, then, has links on the causal side with physiclogy and, in so far as it asks historical questions, in the sense of being interested in the individual experience of the organism, with comparative psychology. Hinde has a useful passage on this key point. Although classificatory systems are basically independent of each other, "Possibilities of confusion constantly arise". Just because functional and causal categories often overlap, the temptation to confuse them may be severe. This is particularly dangerous when the functional categories are nothing more than descriptive generalizations. Reference to "self preservative" as opposed to "reproductive" instincts, for example, "both confuses functional and causal categories and implies something causally in common between, for example, feeding and preening". Similarly, although activities such as scratching, stretching and shaking can be described as "comfort movements", they have been shown to occur independently of each other. Provided these confusions are strictly avoided, and provided that we are always aware what kind of explanation we are seeking, then I believe the eclectic approach by the ethologist will continue to be of central importance in the future development of the behavioural sciences.

Conceptual muddles which have sometimes beset ethologists are, unfortunately, equally characteristic of other sciences, particularly in their early stages. The great contributions which ethology can make to the other behavioural sciences include just those contributions which zoology has, in the past, made to physiology, anatomy and embryology, which for historical reasons were for long purely concerned with the human being—namely the inculcation of the tremendous importance of being truly comparative. There are innumerable cases in all these disciplines of phenomena impossibly complex and difficult to understand when studied solely at the human level, but which immediately become amenable to attack when studied in some simpler, less highly organized or differently organized, organism. Where would neurophysiology be without the giant fibre? Where would

embryology be without the Echinus egg? Where would genetics be without Phaseolus and Drosophila? It is basically because of its evolutionary outlook that zoology was in a position to broaden the studies of the other disciplines in this way. As I see it, ethology is now providing just this service for the psychologist, saying, in effect, that what seems to be the longest way round may be the shortest way there. Predictions are notoriously risky in science; but I am prepared to forecast that ethology will provide the same kind of release and re-direction for social psychology, psychopathology and perhaps sociology in the not too distant future and will thereby show itself as an important unifying influence. In this process I believe that Hinde's researches and writing, especially this book, will have played a major part.

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Wind Erosion in East Yorkshire

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The marked erosion of agricultural land in East Yorkshire by the high winds in the first dry months of 1967 is linked with the formation of deposits by two former glacial lakes. There is, however, a danger that modern farming practice will tend to induce further erosion of this sort in this part of Britain.

In certain parts of East Yorkshire, the inhabitants have long been accustomed to localized wind erosion, in the form of sand- and dust-storms, known as "blowing". This produces temporary sheets of sand on metalled roads, dust in the houses, and creates distinctive solid banks along hedgerows, which act as sand traps. In 1967, however, the erosion which took place was far greater than usual. In what follows we shall define the area affected by blowing and attempt to infer some of the causes and effects on the countryside.

Because the erosion took place in February and March, it was essential quickly to visit the whole of that part of Yorkshire liable to wind erosion before the new growth of crops reduced and to a certain extent obscured the erosion and the associated sand deposits. This area is confined to the land covered by former glaciers and post-glacial lakes which have left glacial sands and gravels on or around the morainic deposits in the Vale of York, and lacustrine sands which were deposited on the margins of post-glacial lakes in the Vales of York and Pickering. Other areas liable to erosion are upland areas devastated by moorland fires¹, but these have been little affected in 1967.

After several hundred miles of driving it became apparent that not all the potential areas of erosion were in fact affected, and that erosion was mostly confined to two large areas.

The principal area is already well known for its tendency to blow, and lies on the east side of the Vale of York. During 1967 it has suffered patches of erosion in a belt from the Humber to the vicinity of Northallerton, an

area 50 miles long and 3-11 miles wide (see Fig. 1).

Blowing took place on "Great Sand Field", Market Weighton (SE.8740 and environs), with lesser areas near North Cave and Londesborough. Towards the north, the next affected area was Everingham Common and Allerthorpe Common, the latter extending towards Suttonupon-Derwent and Barmby Moor, in all more than 10 square miles. At Newton-on-Derwent, the small area of sand around the former village sand pit was blown. Farther west, many fields were eroded on Sandfield, North Duffield and Skipwith Common.

Around York the erosion was confined to the north and east. In New Field, the Common and the former warrens at Dunnington, erosion is estimated to have affected at least 800 acres. In nearly every nearby parish, isolated fields generated small sand blows-for example, near Strensall, High Catton, Skirpenbeck and Buttercrambe.

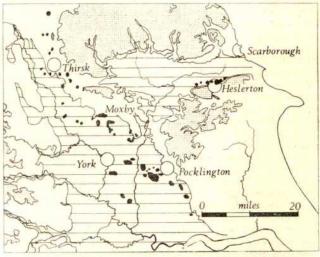


Fig. 1. Areas of blown sand, 1967, represented in black. Stippled areas are over 400 ft., and the horizontal shading covers the areas of glacial Lake Humber (around York) and Lake Pickering (west of Scarborough).

In the parishes of Claxton, Flaxton and Sand Hutton, more than 4 square miles were eroded on the former commons.

Around Sutton-on-the-Forest there were large patches of erosion in the former West Field, on East Moor near what was "The Common Sand Holes" and on Moxby Moor. Smaller areas were found at Tollerton, Myton Moor, Helperby Moor, Husthwaite, Sessay, Thirkleby (by Sand Hill House), Bagby, Carlton Miniott and Sandhutton. Another group of scattered sand blows was found east of Northallerton, as well as near Knayton and East Harsley.

The second and smaller area was located on the south side of the Vale of Pickering in a zone 7 miles long and 1 mile wide, and confined to the parishes of Heslerton, Sherburn, Potter Brompton, Ganton and Willerby.

The Geological Background

Fig. 1 shows the two glacial lakes, the one near Scarborough called Lake Pickering and the other in the Vale of York called Lake Humber. These lakes were formed by the trapping of glacial meltwater in the lowlands by a mass of ice occupying the North Sea basin. The map shows that most of the area which suffered from wind erosion in 1967 lies within the former lakes2. In the Vale of York, the lake stood at about 100 ft. o.D. and randomly deposited lacustrine sands and gravels on a glacial clay and sand gravel floor. These deposits were later partially overlaid, especially near the Humber, by warp clays. As a result, most of the areas liable to wind erosion are slightly raised areas of sand and gravel characterized by very light and fine grained sands, above the lower clays but below the edge of the lake. In the north, the glacial sands and gravels which lie beyond Lake Humber have provided a few limited concentrations of somewhat coarser sand, and these were duly eroded. Both types of sand and gravel are shown in some detail on the relevant Geological Survey sheets3.

Lake Pickering left behind a similar spread of sand and gravel, which in places are concentrated in beach like deposits. These are particularly evident in sand pits (as, for example, at Staxton Corner, TA.024794), where laminated sands can be clearly seen related to a water level at around 250 ft. o.d. The principal beach deposit and the principal area of erosion are on the south side of the Vale of Pickering at its eastern end, presumably because in this region there was more detritus in the water supplied by the Newton Dale and the Forge Valley overflow channels bringing meltwaters from the north.

In post-glacial times, the shoreline deposits were liable to blow, presumably before the natural vegetation blanketed them. Sand was blown eastwards in the vicinity of North and South Cave and Sancton over a distance of more than a mile on to Houghton Moor and Sancton Wold, with similar deposits on the southern side of the Humber.

Causes of the Erosion

January-April 1967 was relatively mild in East Yorkshire, with very little frost and snow. In January the rainfall was 39 per cent of the average, in February, 98 per cent, in March, 77 per cent, and in April, 61 per cent. In combination with temperatures above the average, these dried the surface, lowered the watertable and produced very dry topsoil conditions wherever the soil was exposed by ploughing. Although there were a few brief storms, the period was also characterized—especially in March—by the high frequency of gale force winds, predominantly from the west or south-west⁴.

Wherever the soil had a high fraction of sand, and especially if the sand was fine, it was whipped away to form sand dunes and sand sheets. Elderly people in the Moxby and the Heslerton areas had not experienced as much blowing in their lifetimes. In some cases, the excessive erosion cannot be readily accounted for by changes in physical conditions or in farming practice. In the Hesler-

ton area, however, a comparison of the present landscape with the 6 in. O.S. maps of the area reveals that numerous hedgelines have been grubbed out to permit large scale mechanized arable farming. This has undoubtedly led to increased exposure of the sandy areas to a greater "fetch" of wind, and to increased scouring of the topsoil. A similar origin has been attributed by local farmers to the blowing south of Thirsk and south of Selby.

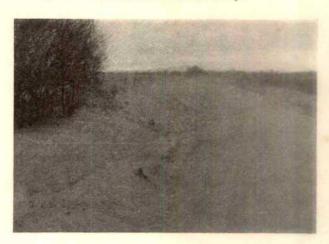


Fig. 2. Sand on the York to Helmsley Road, photographed during a

In the parish of Moxby, there were several areas with small dust-bowl scenes with dust blanketing visibility for more than a mile, but the most spectacular place was Moxby Moor. This is a low, sandy ridge with small hedged fields aligned east to west. One 7 acre field supplied so much airborne material that the York-Helmsley road was blocked at Easter (SE.583663), and the road was only afterwards kept open by stationing a road sweeping vehicle at the spot to sweep back and forth continuously on a 100 yard stretch of road for a week (see Fig. 2). Dunes well over 3 ft. high were visible for several weeks after the erosion had more or less ceased. Down wind from this, on a barley field in the lee of one hedge (Fig. 3), dunes up to 4 ft. high were formed over a distance of 275 yards and a width of more than 15 yards—an estimated minimum of 50,000 cubic feet. There are estimated minimum of 50,000 cubic feet. several derelict marl pits in the area, which suggests that marling was used on the local sands as an early remedy to blowing.

Farther south, small dust clouds were seen for weeks over fields at Full Sutton (SE.754560), at Buttercrambe (SE.715568) and at Claxton (SE.698602), where small

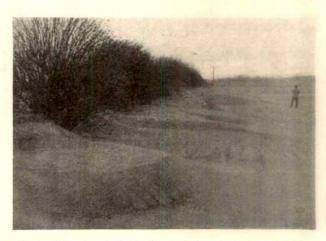


Fig. 3. Dunes forming in the lee of a hedge, photographed during a dust-storm.

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rippled sand sheets drifted in field corners and on to roads, but these will have very little long term effect. The large clouds of dust and sand on Sand Hutton Common (SE.6757, 6857) were more impressive; a grey peaty sand was swept into drainage ditches and onto growing winter wheat; in general, however, the fields are so big that the effect of the erosion is mostly a redistribution of the sand. Only a little is removed from the area.

In the Vale of Pickering, the post-glacial beach sands lie at the foot of the Chalk Wolds which are aligned east to west. The yellow brown sandy fields called "The Grits" in Sherburn are sharply distinct from the upland chalky white soils and the carrland peaty black soils beneath them. The agricultural pattern was formerly one of pasture with north-south pine windbreaks and a few arable fields distinguished by the high banks under their hedges indicative of decades of gradual erosion of the sands. The present trend on the sands is towards grain and bigger fields. In 1967, the wind was funnelled down the Vale of Pickering, and at Ganton and Potter Brompton (SE.966770) drifts of more than 3 ft. lined the fences, large patches of growing wheat were bruised and flattened, grass verges were blanketed and small sand sheets drifted across the A.64 road (see Fig. 4). There was no visible erosion at all on the clay soils of the area.

Presumably, the high winds which produced the blowing have affected areas of light sandy soil in other areas. Some

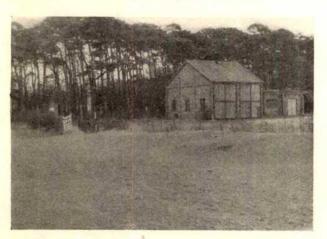


Fig. 4. Near Potter Brompton. Ripple covered dunes on the roadside, with a pine wind-break in the background, photographed during a dust-storm.

has been noticed south of Selby, near Burn and on Camblesforth Common (SE.630278 and vicinity) where it has become more significant in the last few years following the removal of some hedgerows.

Effects

Apart from the temporary dramatic manifestations of dust-storms reaching as high as the tops of electricity pylons, the dunes and the blocked roads and ditches, there are more lasting effects. It is clear from the names used in the area of study that the sandy areas were the parish commons and moors until they were enclosed about 1760-1830. That there has always been a little erosion each year is an accepted part of farm life, but the absence of windbreaks in some areas-for example, around Pocklington and Allerthorpe-coupled with the tendency to grub out hedges is bound to increase the erosion. The problem is aggravated when farmers, as witnessed in the York area, continued to disk their fields at the height of the gales. Perhaps the most significant effect is the loss of the topsoil, not as a mineral layer but as an expensively fertilized and artificially productive substance. Money is in fact being slowly blown away. In some areas the effect on crops already planted was noticeable. Fields around Pocklington planted with carrot seeds had patches of sand removed, seed and all. At Tollerton, sand-blow exposed seed barley before it had germinated and this survived, but winter wheat was battered and broken and even buried in some fields. Probably a return to marling, last practised on a large scale in the Second World War, when 4,000 acres are known to have been treated5, would help to reduce the chances of blowing in some areas, but this is a very expensive operation.

The processes of erosion in Britain are usually slow, continuous and unnoticed, but occasionally, as in 1967, conditions may combine to accelerate erosion which can be visually dramatic and economically serious. If the present trend in farming continues, and if more sandy areas are ploughed and more hedges are grubbed out, it is probable that very careful management will be necessary to minimize blowing and its consequences even under less extreme conditions.

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Origin of Low Energy Cosmic Rays from Outside the Solar System

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The abundances of heavy nuclei in the low energy primary cosmic ray flux can be accounted for by the co-existence of two components, one of which has undergone nuclear spallation reactions. The passage of primary cosmic rays through the ambient gas clouds thought to be associated with quasi-stellar objects is one way in which spallation on a sufficient scale may have occurred.

It is usually considered that cosmic rays have path lengths of 4-5 g.cm⁻². This is consistent with the production in the flux of primary heavy nuclei of LiBeB from CO in a ratio $\sim 1/3$. It is also consistent with the ratio $\sim 1/3$

observed at energies $> \sim 1$ GeV per nucleon for odd to even nuclear charges (z) with $z \ge 10$. Path lengths significantly greater than 5 g.cm⁻² are thought to be ruled out by the low value of the helium isotope ratio He³/He⁴

⇒ 0.25, for longer paths would lead to higher values of this ratio. The data at energies of more than ~1 GeV per nucleon have been reviewed by Webber¹, who remarks, "The value of 4 g.cm⁻² is consistent with our rather crude knowledge of the fragmentation parameters and ideas of the relative absence of odd nuclei in the source regions. Path lengths ≥ 10 g.cm⁻² for the regular diffusion model seem to be incompatible with the presently measured charge distribution".

An unexpectedly small value $\leq 1.4 \times 10^{-3}$ has recently been reported by Comstock, Fan and Simpson² for the ratio F/O at energies $< \sim 100 \, \mathrm{MeV/nucleon}$. These authors have pointed out that this determination casts doubt on the picture described in the previous paragraph, at least as far as low energy cosmic rays are concerned. Bernas et al.3 have measured a cross-section of 28 mbarn for the nuclear reaction $F^{19}(p, pn)F^{18}$ at a incident proton energy of ~ 150 MeV. We expect the same spallation reaction $Ne^{20}(p, pn)Ne^{19}$ to have a similar cross-section, for Ne^{20} has two neutrons outside the closed p shell, as does F^{19} . Thus the fraction of Ne²⁰ converted to Ne¹⁹, and then by $\beta\text{-decay}$ to F19, in a path of 4 g.cm-2 in hydrogen is $\sim 2\cdot 8 \times$ 10^{-26} . $(4/M_{\rm H})$ where $M_{\rm H}$ is the mass of the hydrogen atom. This fraction is ~ 0.07 . With Ne/O $\simeq 1/7$ we have an expected value ~ 0.01 for F/O arising from the spallation of Ne²⁰ alone. Allowance for some production from other nuclei, particularly Mg24, gives a discrepancy between the expected ratio and the observed upper limit equivalent to a factor of about 10.

Several workers (Y. Pal, private communication, and P. H. Fowler, private communication) have sought to escape from this difficulty by assuming a distribution of path lengths p of the form $\exp{(-p/p_0)}dp$ with $p_0 \simeq 5$ g.cm⁻². With this value of p_0 , however, there is still far too great a production of F. It seems to us that a very low value for F/O cannot be obtained unless there are virtually no path lengths of 4-5 g.cm⁻².

We wish to consider a model in which the low energy cosmic rays are a mixture of two components, one which has experienced little or no spallation and one which has experienced spallation corresponding to path lengths as great as 20 g.cm⁻². This model was first proposed by Comstock et al.², who discussed it briefly within the framework of the galactic theory of cosmic ray origin. The first component is necessary if we are to understand He³/He⁴<0.25, while the second component is necessary to account for the observed occurrence of LiBeB. We have the following situation

FIRST COMPONENT	SECOND COMPONENT
$He^3 = 0$	$\mathrm{He^3/He^4} \simeq 1$
LiBeB = 0	LiBeB ≃ (CO) before spallation
$\mathbf{F} = 0$	
He4, C, O without spallatio	n (CO) before spallation $\simeq 0$

If the first component is some two or three times more important in the mixture than the second component, we immediately obtain LiBeB/CO $\simeq 1/3$, He³/He⁴ $\lesssim 0.15$. Where F is concerned, there is none from the first component and comparatively little from the second component. This is because NeMgSi, which are the most important progenitors of F when the path length ~ 5 g.cm⁻², become too heavily attacked by spallation to yield much F at significantly greater path lengths. This point will become clearer from the following argument.

A nuclear species exposed to protons of several hundred MeV can be regarded as continuously affected by spallation according to the equation

$$\frac{\mathrm{d}A}{\mathrm{d}p} \simeq -\frac{A}{50} \tag{1}$$

where the atomic weight A is a function of the path length p (in g.cm⁻²). Equation (1) may be derived in two ways. An incident proton can be considered as having an average cross-section of ~35 mbarn for experiencing scattering by any one of the nucleons within the nucleus, provided A is not too large. The number of nucleons expelled per g.cm⁻² of path is therefore $\sim 3.5 \times 10^{-38}$ A more conventional way of looking at the same problem is to consider the target area mr. $r_0 \simeq 1.2 \times 10^{-13} A^{1/3}$ cm for collision of a proton with the nucleus, giving $\sim 4.5 \times 10^{-26}~A^{2/3}/M_{\rm H}$ collisions per g.cm⁻² of the path. Then, taking the number of nucleons expelled per collision as proportional to the radius of the nucleus in other words, to $A^{1/3}$ —we again obtain an evaporation rate proportional to A. The numerical coefficient is also the same as before, provided the average number of nucleons expelled per collision is close to $A^{1/3}$. Most of the nucleons will be expelled separately or as a particles. (It is important to recognize that this argument would require modification at higher proton energies, for π -meson production raises complications.)

Equation (1) shows that for a total path p the change ΔA of the atomic weight due to spallation is given by

$$\frac{\Delta A}{A_0} = 1 - \exp\left(-\frac{p}{50}\right) \tag{2}$$

where A_0 is the initial atomic weight. For p=20 g.cm⁻², $\Delta A/A_0=0.33$, giving $\Delta A\simeq 9$ for Si²⁸, showing that for p somewhat in excess of 20 g.cm⁻², the elements NeMgSi are converted by spallation to nuclei with atomic numbers below F. The progenitors of F must then largely belong to the SArCa group.

Now the usual abundance ratio SArCa/O is about 1/10, and this value refers for the second component to abundances before spallation. After spallation, O is lost, while the nuclei SArCa become spread over a range of atomic weights perhaps as wide as 20, of which only atomic weight 19 yields F. For the final abundances of F and O in the mixture of both components we therefore have

With the unspalled first component some three times more important than the spalled second component

(O) First component
$$\simeq 3$$
 (O) Second component before spallstion (4)

Combining (3) and (4),

$$\frac{\mathbf{F}}{\mathbf{O}} \approx \frac{1}{60} \cdot \left(\frac{\text{(SArCa)}}{\mathbf{O}}\right)_{\substack{\text{Second component} \\ \text{before spallation}}} \approx \frac{1}{60} \cdot \frac{1}{10}$$
 (5)

which is just consistent with the upper limit set by Comstock et al.². By increasing the importance of the first component still further, the F/O ratio could, of course, be reduced below this upper limit. The discussion given later suggests that equation (5) should be applicable when the energy per nucleon is about 150 MeV, but that F/O should fall below that given by equation (5) at lower energies.

Applying equation (2) for p=20 g.cm⁻², $A_0=10$, gives $\Delta A=5$, while for $A_0=12$, $\Delta A=4$. While these values of ΔA are to be interpreted as averages—there will clearly be an appreciable scatter in ΔA from one nucleus of given A_0 to another—it seems likely that most spallation LiBe is derived from C and that most B is derived from O. Because the C and O abundances are likely to be compar-

able before spallation we obtain

$$\frac{\mathrm{B}}{\mathrm{LiBe}} \simeq 1$$
 (6)

Indeed, if we take LiBe to be distributed according to the number of their stable isotopes* Li³, Li³; Be³ the approximate abundance ratios become Li:Be:B \simeq 2:1:3. Comstock et al.² give 3:2:4. It is interesting that the present model does not lead to as great an excess of B as would occur if the special reaction $C^{12}(p, pn)C^{11}$ were the dominant spallation process.

Next we consider the conditions under which this two-component model might be set up. We regard the spallation as having taken place within the source, not in the Galaxy, thereby avoiding the galactic diffusion model in the quotation mentioned here from Webber's article. In all known sources of cosmic rays, whether supernovae, quasi-stellar objects or radiogalaxies, there exist both the cosmic rays themselves and ambient gas which is detected spectroscopically. For quasistellar objects (QSOs) in particular, there are spectroscopic arguments4 for supposing that emerging light has a path of I g.cm⁻². Hence if QSOs are a major source of cosmic rays, those of high energy $> \sim 1$ GeV per nucleon should have paths of a few g.cm⁻² for the spiralling of the cosmic rays about magnetic field lines must increase the paths to a few times the path length for light. We take this to be the situation for the higher energy cosmic rays mentioned at the beginning of this article. Moreover, in QSOs there is evidence for the existence of discrete clouds of ambient gas. It is from the mixture of such clouds with higher energy cosmic rays that a two-component model for the low energy cosmic rays can be obtained.

In such a mixture, some higher energy cosmic rays will enter and be stopped in the discrete clouds of ambient gas. Consider a cloud exposed to an outwardly directed beam of cosmic rays with energies $> \sim 1$ GeV per nucleon. If the intensity of the beam is small, the cosmic rays will be quenched by the cloud without affecting its motion significantly, but if the intensity is great enough the cloud will experience a strong outward acceleration which may be sufficient to expel it from the system. The velocity of emergence in this case depends on the beam intensity. There could be low velocities of no interest in the cosmic ray problem; there could be velocities so near the velocity of light c that the cloud effectively joins the distribution of higher energy cosmic rays; and there could be the velocities ~ 0.5 c characteristic of low energy cosmic rays.

If we now turn the argument around by requiring the emergent cloud velocity to be ~ 0.5 c, we can estimate the ratio of the cloud mass M to the mass m of the beam. Initially, the outward momentum belongs to the beam and is $mc(1-\beta^2)^{-1/2}$, where $(1-\beta^2)^{-1/2}$ is the average time dilatation factor for the higher energy cosmic rays, say 4, corresponding to an average kinetic energy three times the rest mass. After impact, the outward momentum is required to be 0.5 (M+m)c. Assuming no appreciable deceleration of the cloud on its outward journey, we have

$$0.5(M+m)c = mc/(1-\beta^2)^{1/2}$$

$$M/m = 7 \text{ for } 1/(1-\beta^2)^{1/2} = 4$$
(7)

The heavy nuclei present in the beam experience spallation. Assuming that the chemical composition of the cosmic rays and of the cloud is the same, an equal spallation occurs by reciprocity for the material of the cloud. (Incoming nuclei undergo spallation in collisions with protons of the cloud, while some of the nuclei of the

cloud undergo spallation by incoming protons.) The resulting component therefore has mass 2m and the component unaffected by spallation has mass M-m. For the ratio obtained in equation (7), the unaffected component is three times more important than the component affected by spallation.

It follows from this argument that the relative importance of the two components is not an arbitrary parameter in the model. The relative importance is a function of the energy of emergence of the low energy cosmic rays. Write E as the kinetic energy per nucleon. The corresponding velocity is given by

$$1 + E = \left(1 - \frac{v^2}{c^2}\right)^{-1/2} \tag{8}$$

provided E is written in terms of the nucleon rest mass. In place of the first of equations (7), we now have

$$(M+m)v = mc/(\overline{1-\beta^2)^{1/2}}$$
 (9)

The relative importance is just (M-m)/2m. Equations (8) and (9) determine (M-m)/2m in terms of E, provided $(1-\beta^2)^{1/2}$ is specified. It is easy to see that the component unaffected by spallation becomes more important as E is reduced.

It is likely that the material of a cloud will become ionized. It may not be ionized to begin with, but will become so because of the impact of the high energy particles. The rate of energy loss per nucleon for a relativistic nucleus in ionized hydrogen is ~ 12 MeV per g.cm⁻². For the nuclei of importance in the fluorine problem, SArCa, this is ~ 100 MeV/g.cm⁻². Paths of ~ 20 g.cm⁻² therefore occur when the incident energy is ~ 2 GeV per nucleon. For the mean energy of cosmic rays, ~ 3 GeV per nucleon, the path length somewhat exceeds 20 g.cm⁻². It appears then that the path lengths for the component affected by spallation in this model are not arbitrary either.

To summarize:

(1) Low energy cosmic rays (~ 100 MeV per nucleon) arise from the interaction of higher energy cosmic rays ($> \sim 1$ GeV per nucleon) with ambient gas clouds.

(2) The gas clouds are only partially affected by spallation, so that low energy cosmic rays contain a corresponding component.

(3) The importance of the component affected by spallation is a function of the emergent energy per nucleon, the importance increasing as the energy is decreased. The abundance of LiBeB, which come from the spalled component, should therefore decrease as the energy is lowered. So also should the ratio He³/He⁴.

(4) The component affected by spallation of the low energy cosmic rays has experienced path lengths ~ 20 g.cm⁻², whereas higher energy cosmic rays (> ~ 1 GeV per nucleon) have experienced path lengths which probably do not exceed a few g.cm⁻².

(5) The abundance of F in low energy cosmic rays is small, in part because of the importance of the component unaffected by spallation, in part because of the large path lengths of the other component. The abundances of other odd nuclei, particularly Na and Al, should also be low. The situation here is different from the higher energy cosmic rays. The latter, with paths of $\sim 5 \text{ g.cm}^{-2}$, have odd—even abundance ratios of $\sim 1/3$.

(6) The abundances of the heavy metals of the Fe group should increase as the energy is decreased, because these nuclei come from the unspalled component which becomes more important at low energy.

We have formulated the description of the model in terms of QSOs because the spectroscopic data for QSOs are more extensive than they are for most other sources of cosmic rays. If the cosmic rays are indeed generated in QSOs they are of extragalactic origin and must fill space with an energy density of $\sim 10^{-12}$ erg cm⁻³. If the QSOs

^{*} In private discussion, Professor P. H. Fowler has indicated that Be' is likely to contribute to the Be abundance. Since Be', with its low neutron binding energy, has a very low productive cross-section in some experimentally measured spallation events, we consider that Be' should replace Be' in the text, which otherwise surgives.

are not cosmologically distant objects but lie within a distance of ~100 Mpc, the 105 QSOs estimated from observation have a density of one QSO per 10⁷⁵ cm³, about the same as galaxies^{5,6}. The energetic requirement is for $\sim 10^{63}$ erg of cosmic rays per object. For masses $\sim 10^{13}$ M $_{\odot}$, comparable to large galaxies, the requirement is for 10-3 of the rest mass energy to be converted into cosmic rays. This is a reasonable energetic requirement. If, on the other hand, the QSOs are taken to lie at cosmological distances, the observed density of QSOs is only 1 per 1079 cm3 and the energetic requirement would be raised to ~ 1067 erg per object if the only QSOs are the ones that are currently observed. It is, however, possible to argue that the lifetimes of QSOs are much less than the cosmological time scale of 1010 years, in which case the total number of QSOs over 1010 years would be much greater than those currently observed. Indeed, for an average lifetime of $\sim 10^6$ years the total space density of all QSOs is comparable to galaxies and the energetic

Either way, the requirement is the same as before. energetic requirement is very reasonable.

It may be possible to formulate a similar model for galactic supernovae as sources, provided it can be shown that the ambient gas in supernova remnants has the appropriate properties, and also provided the existence of nearby supernovae can be made plausible.

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Geomagnetic Polarity Zones for Icelandic Lavas

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Analysis of cores collected from a sequence of lavas in Eastern Iceland has made possible an accurate calculation of the average rate of reversal of the Earth's magnetic field.

DURING an extensive palaeomagnetic survey of Eastern Iceland1, cores were collected from twenty-one overlapping profiles (Fig. 1) so that the chronological sequence of lavas within each profile is known by superposition, and the relationship between the profiles is also known from stratigraphical correlations (Fig. 2). The cores are from a predominantly basaltic succession and range from oldest Tertiary in Eastern Iceland to young Quaternary in age.

The profiles, A to V, comprise a total thickness of 11.0 km of volcanic rocks; they are mostly lava flows and flow units, some 1,140 of which were sampled. (Some lava flows are made up of two or more flow units, all of which are the products of the same volcanic eruption; the units are separated by intervals of time ranging from several hours to several years.) Non-exposure accounts for some 4.5 per cent of the total thickness. Making allowance for repetition by overlap of one profile on another, this represents a succession 8.8 km thick, comprising some 900 separate lava flows and flow units.

Tertiary volcanic centres (Fig. 1) were avoided because of their impersistent stratigraphy, the paucity of basalts, the widespread and often drastic chemical alteration of the rocks and uncertainty as to whether the dip of the lavas is original or secondary. The lower lavas of profile G and the thin lavas of profile N are, however, believed to be parts of central volcanoes. A flexure zone in the area north-east of Nordurdalur (map, Fig. 1) was also avoided.

Interbasaltic clastic beds constitute some 10 per cent of the volcanic succession (Fig. 2). They include many thin red beds (regarded as wind-blown volcanic dust

deposits and usually less than 1 m thick), acid tuffs (including ignimbrites), palagonite tuffs and breccias, tuffs which have been relaid as sediments, conglomerates and tillite like beds. Although only one undoubted tillite has been identified in our succession, several of the clastic beds pass into tillite when traced along strike. Glacial conditions therefore prevailed at several times during the period represented by profiles P to V.

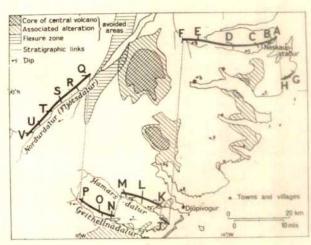


Fig. 1. Map of collecting areas in Eastern Iceland, showing location of

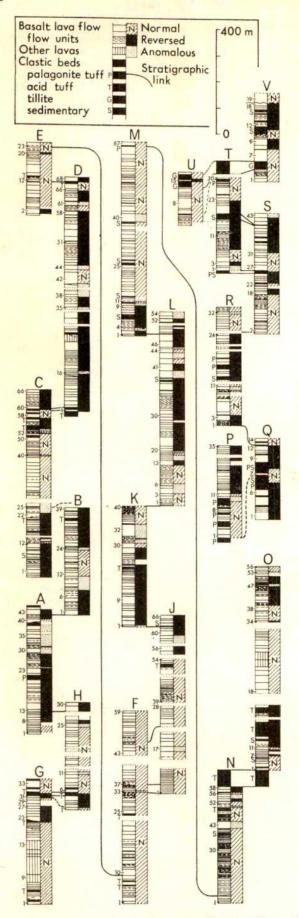


Fig. 2. The lava succession, showing stratigraphic links and magnetic polarity of the lavas.

It should be pointed out that correlation of one profile with another several kilometres away is not often possible because individual lava flows can seldom be traced far. The boundary between two groups of lavas of contrasted petrology or a prominent tuff bed may be used as the basis for a correlation. In neither case is it certain that any of the lava flows of one profile are found in the other, and in the former it is possible that the boundary is slightly diachronous. Of all the correlations, that between P and Q is the least certain, although there is little doubt that the groups of thick clastic beds in the lower parts of these two profiles are equivalent. So far we have collected and measured 2,200 oriented cores (usually two cores from each lava) from 1,070 lava flows or flow units. Except for one small break between profiles O and P, this gives a continuous sequence which starts with the oldest lava accessible in Eastern Iceland (profile G) and ends in the late glacial period.

The mean latitude of the collecting sites is $+64.9^{\circ}$ and, if the Earth's magnetic field is that of a dipole the axis of which is, on the average, coincident with the rotational axis, then the present or Normal field direction in this locality would be 0° E. of N., +76.8° (down), while the Reversed direction would be 180° E. of N., -76.8° (up). After a.c. demagnetization (of all the specimens by at least 150, 250 and 400 peak oersteds) the majority of the lavas give directions which cluster around these two modes, but a significant number have directions which do not lie near the two extremes. In determining the polarity we have defined a flow to be Normally (Reversely) magnetized if both cores give directions within 40° (absolute) of the Normal (Reverse) direction of the dipole field. We have defined as "anomalous" those flows for which the directions of both separately oriented cores agree and fall outside the other two classes. "Discordant" flows for

rejected. They will be studied further to try to resolve the difference. With this classification we find 551 Normal flows, 406 Reversed flows and seventy-three Anomalous flows, forty having had to be rejected.

which the directions of the two cores do not agree were

Nearly all the seventy-three flows with anomalous directions occur between sets of flows showing good Normal and good Reversed directions (Fig. 2). Fifty-five of them show such a high degree of stability that the directions found are considered to define the direction of the ancient field. This suggests that at least the fifty-five "good" anomalous directions are truly Intermediate and indicate how the direction of the Earth's field changed during the transition. If this is so, then the reversal does not simply involve a change in the sense of the dipole. The intermediate directions could be caused either by the continuous change of the dipole axis with possibly also a change in strength or by the reduction in strength of the dipole without change in orientation, allowing the nondipole part of the field to make a proportionally larger contribution and so give the anomalous directions. There are also indications that on some occasions a complete reversal does not take place but that the field finishes up with the same sense as at the start of the excursion. This phenomenon could either represent an only partially delineated event or an as yet undescribed characteristic field variation intermediate between secular variation and

The flows with anomalous directions generally have a much weaker magnetization than the Normal or Reversed flows (Fig. 3). Because it is unlikely that magnetically soft rocks always coincide with a period of transition, the weak average magnetization of the flows with anomalous directions could be caused by a decrease in the strength of the geomagnetic field as it undergoes a reversal. We are trying to measure ancient field intensities for the stable lavas with anomalous directions, so that by comparing the results with those normal and reversed intensities already collected³ the hypothesis can be tested. Sigurgeirsson⁴ has found forty-five lavas in Western Iceland

with anomalous directions which also show low intensity of magnetization and a positive correlation between reversals and low magnetic intensities has been reported in sea cores5-7.

Ten samples from six flows in the lower part of our sequence have been dated (Fig. 4). They indicate a maximum age of about 20×10^6 yr, but because Icelandic basalts are poor material for potassium-argon dating^{8,8}, we do not know precisely the total time span covered by our collection. Dates, however, have been obtained for other Icelandic materials, the oldest of which—10.6 × 106 yr for an obsidian by the potassium-argon method10-can be correlated with profile K of our succession. Edwards (personal communication), however, dates the same material at 6.2×10^6 yr by the fission track method. The ages of the Austurhorn granophyre intrusion, which cuts rocks in profile K and of a granite block in bedded agglomerate at about the same level, have been measured by Gale et al. $^{\circ}$ to be 8.8 and 8.9×10^{6} yr, respectively. It can be argued that these four dates all give minimum ages for the lavas, and lavas below our profile K must therefore be older than about 10×10° yr. Allowing for overlap and considering associated flow units to represent a single flow, our succession is made up of at least 726 independent flows, and so using the present low extrusion rate of 1 lava/40,000 yr (ref. 8) would give 28×10^6 yr as an upper limit. This evidence, taken together with the older basalt dating results, suggests that the oldest lavas in Eastern Iceland are about 20×10^6 yr old. Seven hundred and twenty-six lavas in 20×10^6 yr would mean that each lava represents on the average 28,000 yr.

The present analysis indicates at least sixty-one polarity zones, or sixty complete changes of polarity (Figs. 2 and 4), in our succession. (See results in Western

Iceland, refs. 4 and 30.)

The times at which reversals occurred cannot be defined, but the relative time span of each of the sixty-one zones in our sequence can be judged crudely by the number of the flows or total lava thickness in each zone after allowance has been made for the overlap of different profiles. An important structural characteristic of the basaltic pile of Eastern Iceland needs to be explained; there is a general up-dip attenuation of the lava pile, and at the same time there is a regular upward decrease in the dip of the lava flow averaging 1°/170 m increase in altitude. This means that the succession of lavas in the upper part of a profile is attenuated compared with that in the lower part. Measured thicknesses are therefore not proportional to the duration of time they represent. A correction based on the regular upward dip decreases was applied in the preparation of Fig. 4, to attempt to remedy this situation.

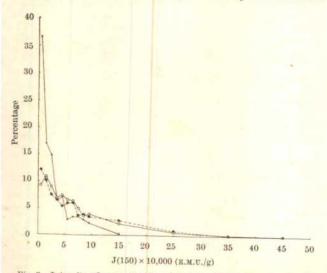


Fig. 3. Intensity of magnetization of samples after demagnetization in 150 oersted peak a.c. field. Each group normalized to 100 samples. ○—○, Normal; • · · · •, reversed; △—△, anomalous.

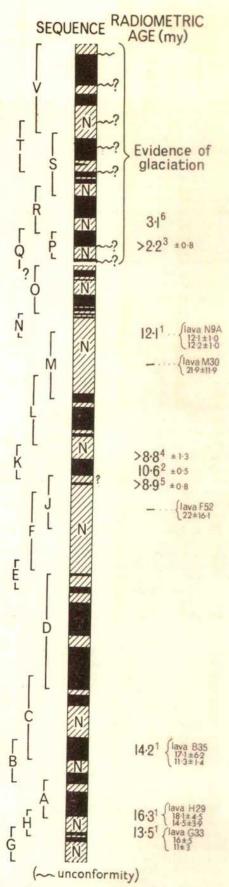


Fig. 4. Generalized succession of polarity zones with thickness modified as discussed in text. K-Ar age determinations: (1) basalt lavas (Grasty); (2) pitchstone (Grasty; Edwards (6:2±0·4)); (3) granophyre intrusion. Hvannadalur (Gale et al., 1966); (4) granophyre intrusion, Austurhorn: oldest of four samples (Gale et al., 1966); (5) granite block in agglomerate, Breidalur volcano (Gale et al., 1966); (6) McDougall and Wensink. 1966, correlation uncertain.

Our sixty inversions in 20×10^6 yr give an average rate of at least 3.0 inversions/10° yr in comparison with the well documented rate of 2.5 inversions/106 yr given by Doell et al.^{10,11}, which becomes 3·1 if the Gilsa⁸ event and a split Mammoth^{12,13} event are real. The sequence of polarity zones built up10,11 from discrete lavas has been amply confirmed by the recent sea core investigations^{5-7,13} which might be expected to provide a continuous record, although the resolution obtainable will depend on the rate of sedimentation. A succession like ours, made up of discrete lavas, cannot claim to be continuous in time, but it is possible, on the other hand, that very brief events will be recorded either if they coincide by chance with a period of extrusion, or if the average rate of extrusion is high enough. Given 40,000 yr/lavas as a low estimate of the average rate, one has an average of twenty-five spot evaluations of the field each 10° yr; 28,000 yr/lava would give thirty-six points. Then many events and epochs should be recorded if there are only an average of, say, 3.0-3.5 inversions/10° yr as shown here. One might even observe a higher rate of inversion than is intrinsically resolvable in some sea cores.

For six polarity zones there is only the evidence from a single lava (N53, O10, O11, O34, S22 and T3). In these cases there are unexposed areas or clastic beds above and/or below the single odd polarity lava flow which indicates that some considerable time may have elapsed between the statement of

between the extrusion of successive lavas.

Two of the polarity zones revealed by our work are particularly noteworthy because of their length (Figs. 2, 4 and 5); E21-23, F1-59, J1-54 contains about seventysix non-overlapping lavas (~ 980 m), while L54, M10-67, N1-52 contains 101 non-overlapping lavas (~ 850 m). Both these are Normal periods. The sixty-one zones shown in Fig. 4 are represented by 726 flows so that there is a mean sequence of twelve flows per polarity zone. The more recent zone in profiles M and N is made of many thin lavas on the flank of an ancient volcano. This suggests a faster than usual rate of extrusion so that the time involved might not be as long as suggested by the number of flows. An incomplete magnetic profile across the same part of Iceland as covered by our survey has been obtained14 using the field mapping technique. At least thirty polarity zones were found, including a long normal zone 1,500 m thick, in the mountains just north of Reydarfjordur. This zone is almost certainly to be identified with the long normal zone in our profiles E, F and J. Several recent studies of magnetic anomalies over ocean ridges have shown them to have remarkable linearity, and symmetry about their axis. The original hypothesis of Vine and Matthews15 that the anomalies are produced by successive strips of normal and reversed material spreading outwards from the axis has now been shown16-18 to be consistent with the polarity epochs reported by Doell et al.10,11 if a suitable rate of spreading is assumed. Pitman and Heirtzler^{17,18} have analysed their results for the Reykjanes ridge, which is a continuation of the central active zone running N.E-S.W. through Iceland. Using a spreading rate of 1 cm/yr^{17,18} (or rather less¹⁹) which compares reasonably well with the rate of 0.6 cm/yr independently suggested by Bodvarsson and Walker²⁰ for Iceland, Pitman and Heirtzler have suggested a polarity sequence back to 107 yr and reaching to 100 km east of the ridge axis. This involves thirty-four reversals or 3.4/10° yr. Their profiles extend beyond 10° yr, and show two relatively long normal zones at BB, CC (Fig. 4, ref. 18), the youngest one beginning just after the thirty-four reversals at 9.0 × 106 yr, or about 90 km from the axis of the ridge. A similar long normal zone is reported between 9×10^6 and 10^7 yr and thirty-eight reversals from the ridge axes in the East Pacific Rise and on the Juan de Fuca Ridge¹⁹. If the top of our succession lies in the Jaramillo normal event (see later discussion), then thirty reversals from that event or thirty-two from the present epoch would take us back into the younger of our two long

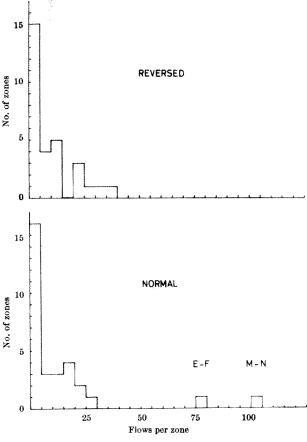


Fig. 5. Histograms showing the distribution of polarity zones according to the number of flows they contain.

normal zones already discussed, which is also about 100 km from the centre of Iceland's active zone. Lava N9A in this youngest normal zone has been dated at 12.1×10^6 yr (Fig. 4), but this lava is known to be quite altered so the date may not be reliable and stratigraphically older lavas in profile K are thought to be only about 10⁷ yr old. The older long normal zone suggested by the Revkjanes ridge profiles CC, but not discussed by Pitman and Heirtzler, starts at about 12.5×106 yr. It is not possible at present to correlate each Normal and Reversed zone in our land survey and in the ocean ridge survey, but it is tempting to correlate our two long normal zones with those found at sea. If this is a world-wide phenomenon one might eventually hope to establish a pair of marker horizons for dating purposes near to 107 yr. It is possible that these zones are associated with the discontinuity in spreading rate of the ocean floor suggested by Ewing and Ewing29

An extrusion rate of 2.8 × 104 yr/lava would suggest 2.0×10^6 and 2.5×10^6 yr for the duration of these two Normal zones M-N and F-J, respectively. McDougall and Wensink's estimate of 4×10^4 yr/lava would give 2.8×10^6 and 3.6×10^6 yr. This is not unreasonable, for evidence21 suggests a much longer reversed epoch during Permo-Carboniferous times, and nearly all the Tertiary lavas collected in Britain have reversed magnetization. Material from the Tertiary collections^{22,23} in Great Britain, and in Kenya²⁴, has been dated. Of twenty-five samples between 20.7×10^6 and 73.7×10^6 yr old, all except two Scottish dykes of $45.0 \pm 1.3 \times 10^6$ yr and $51.0 \pm 6.2 \times 10^6$ yr are reversed. While it is possible that more inversions exist in the lower Tertiary, these results statistically suggest one or more long reversed epochs during the early Tertiary. Vine19 suggests that there may be a decrease in the frequency of reversals at about 25×10^6 yr. The rapid rate of reversal found for the past few million years

may not be a general phenomenon throughout even most of geological time. The rate may vary widely and often; an example is seen between N52 and O11 in our sequence, where there are six inversions in seventeen lavas but none for the next 100 lavas. The inversion rate itself rather than simple polarity might eventually become the characteristic quantity associated with various intervals

of geological time28.

Wensink^{26,27} has reported a palaeomagnetic survey in Jokuldalur some 30 km north-west of our collecting area. Five of his lavas have been dateds and the palaeomagnetic stratigraphy appears to correlate his lava sequence with the Matuyama (R_1) , Gauss (N_2) and Gilbert (R_2) epochs between 1.5×10^6 and 3.5×10^6 yr. The sedimentary bed below the base of profiles P and Q in our sequence is also found near Hofteigur which is below the lowest flow of Wensink's column. Our youngest lava, V19, was collected from the top of the plateau Fljotsdalsheidi, and is normally magnetized but, at the base of Snaefell, about 10 km to the west, a reversally magnetized breccia was found which is apparently younger (personal communication from Einarsson). Although the record of the polarity zones in this area of peneplanation may not be complete, the time gap between the plateau and the breccia is not thought to be very long. This suggests that V19 represents either the Jaramillo or Olduvai normal events. In either case we expect our sequence to include all of

According to Wensink's palaeomagnetic map (Fig. 1, ref. 14) our sequence R to V in Fljotsdalur should lie in the Gauss and older epochs and certainly include the area near Klief, said by Wensink to represent the complete Gauss (N_2) epoch. In fact, Wensink's Kleif profile (Fig. 1, ref. 14) should be our profile T. It shows a single reversed flow in the middle, where we find a reversed zone with twentyfour flows or flow units almost 200 m thick. Wensink describes this as "an inversely magnetized basalt flow intercalated in the N_2 series" and does not include it in his map. The map is based partly on magnetic mapping in the field using undemagnetized samples and it is possible that this method has not revealed all the existing We find it essential to demagnetize all samples before being certain of their polarity, even if the natural remanent magnetism directions of all samples from the same flow are coherent. After thorough a.c. demagnetization, we find sixteen different polarity zones in about ninety-six flows (allowing for overlap) from profiles R-V, which would represent 2.6 × 106 yr at 27,600 yr/flow or 3.8×10^6 yr at 40,000 yr/flow⁸. In the absence of dated materials for this part of our sequence, it would be wrong to try to correlate our series with Wensink's.

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Porphyrin Abiogenesis from Pyrrole and Formaldehyde under Simulated Geochemical Conditions

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Experiments with gas mixtures intended to simulate primeval atmospheres have yielded a great many types of chemicals of biological interest. It has now been shown that metal porphyrin complexes are commonly produced in these experiments.

THE emergence of photosynthetic tetrapyrrole pigments was fundamental to the establishment of advanced plant life on Earth. In addition, the generation of oxygen in the photosynthetic processes undoubtedly contributed to the accumulation of free oxygen in the atmosphere and thus played a part in the evolution of the oxidizing atmosphere of the Earth. Studies of the primitive atmosphere have shown pathways by which other vitally

important biochemicals may have arisenof systems containing methane, ammonia and water assumed to represent the primordial atmosphere of the Earth¹ involving ultra-violet light or electric discharges has been successful either directly or in stepwise experiments in producing urea², amino-acids³, dipeptides⁴, polypeptides⁵, proteinoids⁶, macromolecules⁷, adenine⁵, guanine⁹, pentose sugars¹⁰, 11, deoxyadenosine¹², ATP¹³ nucleotides and constituents of nucleic acids¹⁴. Allen and Ponnamperuma have also produced fatty acids by this method¹⁵. Thus there is reason to believe that these important constituents of living matter can arise under primitive-atmosphere conditions.

In reporting evidence for porphyrin pigments in extraterrestrial carbonaceous meteorite samples we suggested a biogenic origin for at least part of the carbonaceous matter. The porphyrins in such bodies, however, are not necessarily indicative of fossil life but may alternate

tively represent a pathway by which life arose.

Tetrapyrrole pigments such as porphyrins and dihydroporphyrins (chlorins) are formed biosynthetically from δ-amino levulinic acid and an asymmetrical succinyl derivative¹⁷. Two molecules of the acid are then condensed by a dehydrase enzyme to form porphobilinogen; condensation of porphobilinogen produces the tetrapyrrole

structure uroporphyrinogen III.

For abiogenic production of the porphyrin structure two general approaches are possible. One is the levulinic acid approach using ultra-violet light instead of the enzyme, as demonstrated by Szutka¹⁸. The other approach is to use pyrrole and formaldehyde to produce the porphin ring. In this connexion, pyrrole has been made from acetylene (and ethane) with ammonia at high temperatures (300° C)¹⁹, and preliminary evidence from Ponnamperuma indicates the formation of pyrrole from methane and ammonia in electrical discharge reactions. Rothemund²⁰ in early work on porphyrin synthesis used a mixture of 5 molar pyrrole in methanol, an equal volume of pyridine and a ten-fold volume of 2 per cent formaldehyde in methanol. After heating for 30 h at 90°-95° C in sealed tubes, the reaction yielded free porphin in a 0·1 per cent yield.

The purpose of the present study was to demonstrate the ready formation of metal porphyrin pigments from products of primitive-atmosphere experiments. Reaction conditions simulated geochemical conditions starting with experiments closely related to the Rothemund synthesis²⁰ and leading into an aqueous mineral suspension system in which metal porphyrin complexes were produced under reasonably mild geochemical conditions. The metals selected for the study were those of primary concern in the geochemistry of terrestrial porphyrins^{21,22}

Four experiments were carried out using redistilled pyrrole and paraformaldehyde in evacuated sealed tubes. In these initial experiments with about 2 ml. of pyrrole and 0.5 g of paraformaldehyde, an attempt was made to examine the effect of added cations by including nickel, cupric and vanadyl salts in three of the tubes in amounts of 0.5 g. After heating at 84° C for 3 h the tubes were opened and the contents extracted with benzene to remove any porphyrin compounds that may have formed. For elution chromatography on silica gel, the benzene extract was reduced to dryness under nitrogen and extracted with n-hexane. The hexane solution was put on a silica gel column and eluted with n-hexane to give an n-hexane fraction. In a similar fashion, the benzene residue was next extracted with a mixture of n-hexane and benzene (in equal volumes) and chromatographed giving a corresponding column eluant. In turn, further eluants were obtained: benzene; a mixture of benzene and methanol (in equal volumes); and methanol.

Each of the eluants was evaporated to a few ml. under nitrogen for spectral examination. None of the chromatographic fractions from the reaction tube free of cations showed absorption features recognizable as porphyrin, soret or non-soret peaks. Significant yields of porphyrin pigments, however, were observed in each of the other reaction tubes containing added cations. The pigments were present in all eluant fractions, but predominantly in the benzene eluants. This is in keeping with the general chromatographic behaviour of naturally occurring porphyrins²³. The pigments were evidently free porphyrins rather than metal complexes, as indicated by the typical

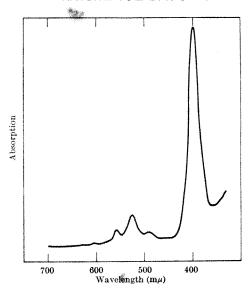


Fig. 1. Spectrum of free porphyrin from reaction of pyrrole with formaldehyde for 3 h at 84° C.

soret and four non-soret peaks of Fig. 1, and by the fact that the pigments fluoresced (red) under 3650 Å ultraviolet light, and complexed readily with copper in acetic acid according to the method of Fischer and Stern²⁴.

The reaction system with added nickel showed the greatest yield of porphyrin pigments, 7.5 μg ; copper gave 1.7 μg , and vanadyl only 0.16 μg . While these yields are exceedingly low (0.002–0.001 per cent) the significant observation is that the formation of the pigments took place and was promoted by the presence of cations, even though the corresponding metal complexes did not form in the course of the reaction.

A second series under reaction conditions of 20 h at 135°C produced principally metal complexes of porphyrins. Although the vanadyl and cation-free tubes produced only enough pigment to make the soret band detectable, the pigment obtained in the copper system had absorption peaks of 3960 Å, 5180 Å and 5520 Å, and that obtained from the nickel system had peaks at 3940 Å, 5100 Å and 5410 Å. Recoveries in this series were: for the copper reaction, 11·8 µg (0·003 per cent); Ni, 4·1 µg (0·0008 per cent); VO, 0·08 µg (<0·00002 per cent); no cation, 0·05 µg (0·00001 per cent).

Water was introduced in later reaction systems to simulate more meaningful geochemical conditions. In reaction mixtures similar to the foregoing, with water added in varying amounts (0·01, 0·1 and 1·0 ml.) to the 2 g mixtures of pyrrole and paraformaldehyde, the presence of water appeared to diminish the yield of metal porphyrin complexes. In this series, the nickel system was the more productive, yielding 1·3, 1·1 and 0·9 µg of metal porphyrin in the systems containing 0·01, 0·1 and 1·0 ml. of water, respectively; the copper yielded little or no porphyrins after 20 h at 135° C.

To explore the effect of other cations in the same reaction conditions, additional experiments with water were carried out. The nickel system produced most pigment although suppressed by the presence of water (yield: 7 μg against 4 μg for the reaction containing 1·0 ml. of water). The copper reaction resulted in the formation of slightly less pigment and vanadyl produced only a trace of porphyrin. Neither ferrous nor ferric cation reactions yielded porphyrin pigments. A mercuric chloride system differed in that it produced a reduced porphyrin, a chlorin, as indicated by non-soret spectral features at 6320 Å and 6650 Å; the soret band was at 3930 Å. Aqueous sealed-tube reactions carried out with copper and nickel at low temperatures, that is at 97° C (for 42 h), produced little pigment materials, again showing the suppressive effect of the water.

To simulate more realistic geochemical conditions pertinent to the abiogenesis of porphyrins, the reaction system was modified so that the pyrrole and paraformaldehyde reactants were present only in small amounts in aqueous reaction systems. Thus a series of aqueous reflux experiments was set up involving nickel, cupric, mercuric, ferrous, ferric and cation-free reactions. The pyrrole, paraformaldehyde and cation contents were about 0.005, 0.003 and 0.005 molar respectively; reaction times were 64 h. Nearly 8 µg of porphyrin complexes was recovered from the reaction with nickel (3890 Å, 5070 Å, 5390 Å). Lack of fluorescence and acid-insolubility clearly showed it to be a metal complex. Small amounts of porphyrin were formed in the reactions with the iron salts, with the yield being slightly greater in the case of the ferric salt.

More effective geochemical simulation calls for the presence of mineral constituents of rocks, primarily to provide solid surfaces which may hinder or promote the formation of porphyrins. As a first approximation, without any direct regard for the rock composition of primitive systems, a supply of crushed rock was prepared from a number of rock samples which had been used in a related study of the occurrence of porphyrins in terrestrial rocks. The rocks were shales, sandstones and carbonates. To prevent the introduction of organic matter (particularly terrestrial porphyrins), the extracted rock samples were ignited at 600° C for a few hours to burn off organic matter.

Relatively low concentrations of the minerals were used—about 0.3 per cent by weight. All six foregoing cations were included as well. About 100 µg of a metal complex (after refluxing for 125 h at 97° C) was obtained (3930 Å, 5180 Å, 5500 Å) (Fig. 2). Spectrally it was similar to a copper complex made from free porphyrin in a related experiment. Mass spectra showed parent peaks at 373 and 371 mass units corresponding to the copper isotope distribution to be expected in copper complexes of porphin (308 mass units). Optical spectroscopy confirmed the copper in the porphyrin complex. Indications were also obtained for parent peaks for the porphyrin at values greater than those of porphin, namely 385 and 387 mass units. The spectral character of the metal complexes was somewhat unusual in that the intensities of the non-soret peaks were reversed, that is $\beta > \alpha$, in comparison with the porphyrins of terrestrial rocks. Such a reversal was observed by Falk²⁵ and related to inherent porphyrin instabilities. Furthermore, it is significant that the cupric complex was produced rather than the nickel favoured in the earlier non-aqueous experiments.

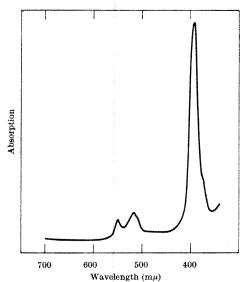


Fig. 2. Spectrum of metal complexed porphyrin produced during aqueous reflux of pyrrole, formaldehyde and added minerals.

A subsequent experiment in which the mineral content was much higher, that is about 10 per cent by weight. and to which about 2.5 per cent of 12 normal ammonium hydroxide was added rather arbitrarily to simulate an ammonia atmosphere, showed similar results. Again an apparent metal complex evolved, even though no cations were added to the system. The spectral features were at 3990 Å, 5260 Å and 5580 Å—somewhat higher than those for the copper complex of the foregoing experiment. In addition there was an appreciable yield of free porphyrin. Thus free porphyrins evidently also form under these conditions, and proceed to metal complexes by taking up cations from the suspended minerals, as noted for free porphyrins in earlier work26 in which standard clay minerals provided complexing cations for biogenic free porphyrins in aqueous systems at room temperature. In all cases, the identity of the complexing cations was unknown, but there was evidence to suggest that they were divalent cations such as copper, zinc or cobalt. The role of magnesium is not clear in this context, although it is known to be very unstable in chlorin complexes as in the chlorophylls and their immediate degradation

In an attempt to determine the identity of the complexing cations in the abiogenic experiment, samples of the free porphyrin formed in the experiment were complexed with vanadyl, iron, silver, copper and magnesium. The absorption spectra of these complexes, as summarized in Table 1, did not, however, correspond with the unknown complexes.

Table 1. ABSORPTION MAXIMA OF METAL COMPLEXED PORPHYRINS Vanadyl Iron Silver Copper Magnesium 3890 Å 3890 Å 5170 Å 3880 Å 5160 Å 4050 Å 3870 Å Soret Non-soret II (β) Non-soret I (a) 5140 Å 5370 Å 5160 Å 5490 Å $5500~{\rm \AA}$ 5640 Å

Some terrestrial porphyrins show evidence in their chromatographic and gel permeation behaviour of biogenic residues, presumably lipids and proteins, associated with the porphyrins. The abiogenic porphyrins in the present study showed no such indications and chromatographic column cluants were strikingly free of background absorption. In the same manner, gel permeation studies on a nickel porphyrin prepared from the free porphyrin in this experiment gave no indication of molecularly associated material with the pigment.

The experiments in this study show that porphyrins can arise under mild geochemical conditions from low concentrations of pyrrole and formaldehyde in aqueous systems. The presence of divalent cations, particularly nickel and cupric, promote the formation of the porphyrins. Cupric and other cations are readily scavenged from minerals in simulated geochemical systems.

The molecular weight of the synthesized perphyrins range from 308 for the simplest perphyrin. Molecular size measurements based on gel permeation show a regular sequence of free and metal perphyrins. Typical spectra for the perphyrins formed under the simulated geochemical condition are somewhat displaced from those for biogenic pigments, the peaks being about 100 Å lower in wavelength for the abiogenic perphyrins.

If porphyrins arise under such mild simulated thermal geochemical conditions, they may, of course, be expected under related conditions involving the input of irradiation energy. This has been shown to be the case for pyrrole and benzaldehyde²⁷, and also for levulinic acid in the generation of pyrrole¹⁸. There is reason therefore to believe that porphyrins might arise directly in primitive atmosphere experiments. In some of his classic electric discharge experiments with methane, ammonia and water vapour, Miller²⁸ found red substances containing components with absorption peaks at 3900 Å and 5400 Å. While the latter were established to be something other than (free) porphyrins which should have shown fluorescence, they were probably metal porphyrin complexes. Their

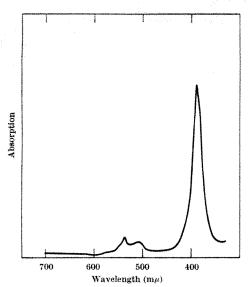


Fig. 3. Spectrum of non-fluorescing nickel complex of an abiogenically synthesized porphyrin.

spectrum was in good agreement with those of simulated geochemical abiogenic synthesis in the present study, such as that shown in Fig. 3.

Thus there is evidence to permit the addition of porphyrins to the already long list of biologically vital compounds which arise under prebiotic primitive-atmosphere conditions. It is important to point out, however, that such an "explosive" porphyrin formation is far removed from the step-wise process of biogenic porphyrin synthesis common to all forms of life at the present time. It is also clear that porphin-like porphyrins generated under abiogenic conditions, such as those in the present study, are considerably short of specific biogenic porphyrins, for example, protoporphyrin IX. The significant point, however, relates to the prebiotic systems, particularly to

the indications that the basic porphyrin structure can emerge directly from simple precursors without dependence on the fairly complex biological steps presently in use, which may have evolved subsequently in developing living organisms.

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Numerical Specification of Biological Form

by

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R. BROWN Botany Department, University of Edinburgh In the study of topics such as morphogenesis, numerical specification of form is particularly important. The authors outline a method, using Walsh functions, which can be used to define a two-dimensional form.

The numerical specification of form is important particularly in the study of morphogenesis. The development of an organ or organism is characterized by a succession of stages each of which may involve distinctive metabolic states and morphological patterns. The change in metabolic state is now being exhaustively examined with considerable success. It is generally recognized that the metabolic state expresses a particular protein pattern. The nature of the pattern and the mechanism involved in determining it can now be examined with some precision.

Change in the morphological pattern, however, still eludes precise definition. Simple inspection shows that change in form is as pronounced as change in metabolic state, but while the one can be examined analytically the other cannot. The difference undoubtedly arises from the fact that, while in one connexion certain attributes of the end product can be expressed quantitatively, in the other they cannot. The total quantity of protein and the relative amounts of different types of proteins can be assessed in numerical terms. The particular characteris-

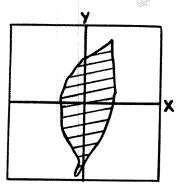


Fig. 1. Object in frame as function.

tics of the morphological pattern are not, on the other hand, amenable to extensive numerical treatment. The change in morphological pattern can only be appreciated in terms of a series of diagrams, which do not provide the basis for an analytical characterization of the change or for an examination of the factors by which it is determined.

Moreover, the present limitation imposes another restraint on the analysis of the morphogenetic process, as at present it is difficult to determine the interaction between the two changing aspects. It cannot be doubted that the metabolic state is linked in some sense to the form by which it is accompanied. Certain metabolic states are likely to promote certain forms of expansion and not others, and localization of metabolic states is likely to lead to differential growth rates which will promote the development of a particular form. The work on the genetical determination of readily distinguishable forms (in, for example, tomato leaves¹) indicates as much. At the same time it is not inconceivable that the change in metabolic state may itself be affected by the change in spatial configuration.

Clearly an elaboration of the techniques available for defining biological form is desirable, and the present paper is intended as a contribution to this problem. The situation is simplified when the pattern is twodimensional and attention has therefore been restricted to foliage leaves; an analysis of change in form with time, however, has not been attempted. The primary purpose has been to elaborate a technique which defines certain quantitative characteristics of the mature form. Several investigations have been concerned with the factors involved in determining leaf shape in particular species, or at different levels along the shoot of a single plant. Melville² described a technique for analysing shape in leaves of species of Ulmus and several workers have examined the change in shape along a shoot by measuring the lengths of particular axes (Njoku³). These techniques are not, however, generally applicable, and the purpose here has been to elaborate a technique which is applicable to all leaves and all stages of development.

In the method described here the two-dimensional pattern is represented by a function taking the values 1 and 0. This function is analysed into a sum of weighted basic pattern functions. The process is very similar to spectral analysis, and in fact the sinusoidal functions of the latter and our basic functions are particular examples of the very useful class of orthonormal functions. The method can be straightforwardly generalized to 3-dimensional forms. An initial application of the method to six leaves of Althea species and two of Coleus species is described.

Consider a two-dimensional form like a leaf, enclosed in a square frame with position specified, say, by Cartesian rectangular co-ordinates, x and y. If we assign the value 1 to points where the object is and 0 elsewhere, we shall have defined a function of position F(x,y), taking only 1 or 0 as possible values, which completely fixes the object in the frame. If as in Fig. 1 we shade those portions of the

frame where F(x,y) has the value 1, we have a vivid representation not only of the object but of this function also. This function can be expressed as a weighted sum of certain "basic" functions which we denote by means of two indices, as follows

$$f_{0,0}(x,y), f_{0,1}(x,y), f_{1,0}(x,y), f_{0,2}(x,y), f_{2,1}(x,y), \dots, f_{i,j}(x,y), \dots \text{ etc.},$$

which take only the values l and -1. Fig. 2 gives a representation of a selection of these basic functions with the regions of value l shaded in. If

$$c_{0,0}, c_{0,1}, c_{1,0}, \ldots c_{i,j} \ldots$$
, etc.,

are the respective weights of the basic functions required for representing the object, then the object function is given by

$$F(x,y) = c_{0,0} f_{0,0}(x,y) + c_{0,1} f_{0,1}(x,y) + \ldots c_{i,j} f_{i,j}(x,y) + \ldots$$

The weights $c_{i,j}$ can be calculated in a rather simple manner and have a simple intuitive interpretation: $c_{0,0}$ equals the area A of the form, while every other weight is a measure of the correlation between the object pattern and the corresponding basic pattern; it is in fact equal to one-half the correlation coefficient, positive values indicating positive and negative values negative correlation.

The weights tend to get smaller as the indices i and j of the basic function increase. This will always be the case and is in fact a necessary condition for the success of the method, for the set of basic functions is actually infinite in number and one requires that the sum converges to a suitable degree of approximation to its correct value in a finite number of terms. The closeness of the approximation can be easily judged, for if one includes only a finite number of the weights, it can be shown that the pattern reconstituted from the corresponding basic patterns bears a correlation to the true pattern given by the ratio of the sums of the squares of the weights to the area A. Thus if all the weights are used, the sum of their squares should be precisely equal to A. So the defined ratio can be used to give the percentage accuracy of the representation.

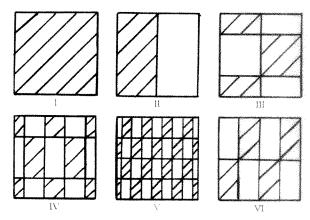


Fig. 2. Selection of basic Walsh patterns; I, $f_{9,0}$; II, $f_{1,0}$; III. $f_{1,0}$; IV. $f_{4,2}$; V, $f_{8,3}$; VI, $f_{1,3}$.

Qualitative features of the forms are reflected in the values of the weights. For example, patterns which are symmetrical about one of the axes must have certain weights zero, and small-scale features in the form reveal themselves in correspondingly large values of some of the higher-order weights. It is clear that it would not be difficult to devise numerical measures of qualitative features of the pattern such as degree of symmetry or

degree of fine-structure, in terms of the squares of the

weights.

The weights calculated do not as they stand specify the form. This can be seen immediately, for if one merely shifted, or rotated, or uniformly contracted or expanded the leaf pattern of Fig. 1, the resulting correlation with the basic function patterns would obviously alter, and so the calculated weights would alter. There are two ways in which one may deal with this problem.

The first method, while involving a considerable computational load, provides an exact specification of form with no arbitrary features. Suppose we fixed on one of the basic functions, say, $f_{i,j}$, and calculated the values of $2c_{i,j}$ for all possible positions, orientations and changes of size of the leaf form within the frame, one would obtain a set of values lying in the range -1 to +1. The algebraically largest of these, that is, the most positive one, would be a measure of the resemblance of the leaf to this basic $f_{i,j}$ pattern, for it gives the maximum correlation possible when one has abstracted the irrelevant factors of location, orientation and size. For a given form, and a given set of basic functions, these maximal weights are uniquely determined, thus constituting an appropriate set of numbers for specifying the form.

In the second method, for a given series of forms—for example, different stages in the development of a leaf—one fixes on some standardization of size, location and orientation in the frame. For a series of the kind mentioned, if one chooses the standardization skilfully, the results can be meaningful and useful. If, however, one wishes to compare one series of forms with another considerably different, it becomes much more difficult to choose suitable standardization for both sets, which would make comparisons meaningful. For the weights calculated are not determined only by the shape of the objects concerned, but also by the particular choice of standard size, position and orientation. However skilful this choice, there is always an element of arbitrariness about it.

There is little doubt that the maximal-weight method is an unequivocal and better method than one using standardization. Because of the latter's comparative simplicity in respect of computation, however, it was used in the initial work reported.

Some experience has now been obtained in analysing leaf patterns. Typically, a coarse analysis giving only the first 16 weights might yield 35 per cent accuracy, while finer ones might give 70 per cent for 64 and 90 per cent for 256 weights. One may therefore have to deal with as many as 256 weights or more.

For purposes of initial and overall survey, it is useful to condense the information contained in the weights. This can be done by grouping weights corresponding to a given structural resolution as follows: with each basic function, $f_{i,j}$, one associates a "resolution", defined as the average distance between successive discontinuities, in both the x and y directions, in the corresponding pattern. If the side of the frame is 1, then

Resolution =
$$\frac{2}{i+j}$$

One adds the sums of the squares of the weights corresponding to a given resolution, and plots these against resolution grouped over equal intervals as in Fig. 3.

These histograms give an intuitively satisfying presentation of the analysis. The large-resolution end of the histogram gives the coarse-structure, "area" type of information about the form; the small-resolution end gives information about fine-structure. The analogy of these histograms to graphs of optical spectra is clear. The abscissa chosen is analogous to wavelength in optics. If the reciprocal of resolution had been used for the abscissa, the parameter would have been analogous to

frequency; this, however, appeared to be rather unsuitable, for it represents the fine-structure components as being orders of magnitude smaller than the coarse-structure ones. This is inappropriate, for it is the fine-structure components which really characterize the shape.

Grouping also has an incidental advantage in that it tends to reduce the effects of the arbitrariness introduced by standardization.

Analysis of Leaves

The preliminary results presented refer to six successive leaves taken from a shoot of Althea species and two leaves taken from the same shoot of a plant of Coleus species. The outlines of the leaves were traced and to simplify computation in the initial stages were symmetrized. With the Coleus leaves the apex was displaced to bring it into the same line as the axis of the leaf. With the Althea leaves the right side was made a mirror image of the left. In the Coleus leaf the serrations at the edge were disregarded and the margin was taken to be a line drawn through the serrations. In all cases the form was taken to be that defined by the lamina and the outline that was analysed was drawn through the point of attachment of the petiole to the lamina.

Table 1. The results from which fig. 3 was plotted Nos. I to VI are leaves from the species *Althea*, and Nos. VII and VIII are from the *Coleus* species.

Resolution				(Weight	$(s)^2 \times 10^5$			
		Coleus						
	1	H	Ш	IV	V	VI	VII	VIII
0-0.067	0	0	0	0	0	0	- 0	0
0.067 - 0.133	876	933	1,216	1,057	1,288	963	702	865
0.133 - 0.2	1,231	1,597	2,102	1,777	2,998	3,497	1,933	1,695
$0 \cdot 2 - 0 \cdot 267$	1,581	1,248	976	1,377	791	510	599	801
0.267 - 0.333	3,200	2,471	3,515	3,116	5,083	5,798	1,515	1,494
0.333-0.4	2,238	2,375	1,917	2,539	2,335	2,004	779	978
0.4-0.467	0	0	0	0	0	0	0	0
0.467 - 0.533	1,600	2,036	1,489	795	669	480	1,052	754
0.533 - 0.6	0	0	0	0	0	0	0	0
0.6-0.667	39	63	232	222	79	227	697	1,328
0.933-1	8,496	7,940	5,885	3,727	3,710	4,436	11,711	10,104
1.933-2	1,893	2,347	3,192	5,464	2,733	1,036	1,487	2,638

The results are given in Table 1 and plotted in Fig. 3 in histogram form. Each histogram is accompanied by a tracing of the leaf from which it is derived. The histograms give only the "low resolution" weights in the range 0-0.667. The large resolution weights are less significant because they are heavily affected by the particular standardization chosen. The six Althea leaves (I-VI, Fig. 3), although they are taken from successive nodes, show a progressive change from a form which is almost entire to one that is sharply palmate. This is reflected in the tendency in the histograms of certain fine structure components to become more prominent. two Coleus leaves (VII and VIII, Fig. 3) although they are also entire are nevertheless lanceolate. Their histograms are similar to each other but in type are remarkably different from those of the first two leaves of the Althea series. It may be noted that the change in the form of the Althea leaves is a reflexion of a corresponding change in the morphogenetic activity of the terminal meristem from which they are derived. The progressive change in the histogram pattern is therefore a numerical characterization of the change in the morphogenetic activity of the meristem.

Mathematical Basis

The basic pattern functions $f_{i,j}$, which are functions of two variables x and y, are synthesized from certain functions of a single variable which were introduced into mathematical analysis by Walsh⁴. Walsh's set of functions constitute what is known as a complete ortho-

normal set; it is this property which they share with the sine and cosine functions of Fourier analysis, which makes them suitable for our purposes.

Each of the basic pattern functions $f_{i,j}(x,y)$ is defined as

$$f_{i,j}(x,y) = f_i(x) f_j(y)$$

where f_i and f_j are the Walsh functions of index i and j

respectively. $f_i(x)$ is defined as follows: Let $i=d_1d_2\ldots d_r$ be the binary representation of the index i, where the d's are 0 or 1, but d_1 is 1. Let s be the value of 1 plus the binary number $d_2d_3 \dots d_r$. Then

$$f_i(x) = \varphi_r^i(x)$$

where $\varphi_{*}(x)$ is defined recursively as follows. Two integral indices n and k are used in the definitions

$$\varphi_{z}^{z}(x)$$
: For $-\frac{1}{2} \leq x < 0$ $\varphi_{1}(2x + \frac{1}{2})$

$$0 \leq x < \frac{1}{2}$$
 $\varphi_{1}(2x - \frac{1}{2})$

For all n > 1 and values of $k, 1, 2, 3 ... 2^{n-1}$:

$$(2x\varphi_{n+1}^{2k-1}(x)): \quad \text{For } -\frac{1}{2} \leqslant x < 0 \qquad \varphi_n^k(2x + \frac{1}{2})$$

$$0 \leqslant x < \frac{1}{2} \qquad (-1)^{k+1}\varphi_n^k(2x - \frac{1}{2})$$

$$\varphi_{n+1}^{2k}(x): \quad \text{For } -\frac{1}{2} \leqslant x < 0 \qquad \varphi_n^k(2x + \frac{1}{2})$$

$$0 \leqslant x < \frac{1}{2} \qquad (-1)^k\varphi_n^k(2x - \frac{1}{2})$$

This definition appears complicated, but the construction of the functions is actually quite simple. The functions are defined for values of x between $-\frac{1}{2}$ and $+\frac{1}{2}$. The The first function φ₀ has the value 1 over this domain. next function φ_1 has the value 1 over the first half of the domain and the value -1 over the second half. The next functions squeeze φ_1 into the first half of the domain and, respectively, φ_1 and $-\varphi_1$ into the second half of the domain. The next four functions repeat the same process on each of the previous two. The following sixteen functions repeat the same process on each of the previous four, and so on, and so on.

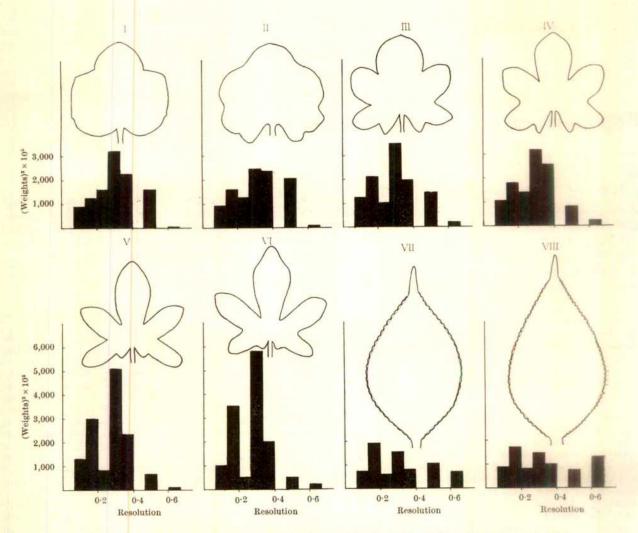


Fig. 3. Outlines and histograms of squared weights for six leaves of species of Althea (I-VI) and for two leaves of species of Coleus (VII and VIII).

The orthonormal properties of the functions are expressed by the following conditions

Orthogonality: $\int f_i(x)f_j(x)dx = 0$ for $i \neq j$ Normality: $\int f_i^2(x)dx = 1$ for all i,

where the integral is over the domain $-\frac{1}{2}$ to $+\frac{1}{2}$ of x.

Our indexing of these Walsh functions is a very convenient one, for it turns out that fi has precisely i discontinuities, that is, jumps between +1 and -1, and that the parity of the function is equal to the parity of the index i.

It follows from the orthonormality of the one-dimensional Walsh functions that the basic pattern functions

$$f_{i,j}(x,y) = f_i(x)f_j(y)$$

are also orthonormal, with the integral concerned now being a double integral over a square of side equal to 1. From the orthonormality it follows that the weights $c_{i,j}$ are given by

$$c_{i,j} = \int \int F(x,y) f_{i,j}(x,y) dx dy$$

To find how this weight is related to the correlation between the object pattern and the basic function pattern, we set

$$g(x,y) = 2F(x,y) - 1$$

thereby ensuring that g(x,y) represents the object pattern by 1's and -1's, unlike f(x,y) which represents it by 1's and 0's. Hence

$$c_{i,j} = \iiint \left[\frac{1}{2} + \frac{1}{2}g(x,y) \right] f_{i,j}(x,y) \, dx \, dy$$

= $\frac{1}{2} \iint g(x,y) f_{i,j}(x,y) \, dx \, dy$

provided i and j are not both 0. It is also easy to see that $c_{0,0}$ equals the area of the form represented by F(x,y). Note that it also follows from the orthonormality that

$$A = \iint [F(x,y)]^2 dx dy = c_{0,0}^2 + c_{0,1}^2 + c_{1,0}^2 + \ldots + c_{i,j}^2 + \ldots$$

where A is the area; hence $c_{i,j}$ tends to 0 as the indices i and j tend to infinity.

It is easily seen, from the parity properties of the Walsh functions, that for a form symmetrical about the y-axis the weights $c_{i,j}$ are zero for all odd i, and for one symmetrical about the x-axis they are zero for all odd j. From the oscillation properties one also sees that small-scale features of a form will be indicated by the $c_{i,j}$ with high indices—the latter obviously will not be much affected by large scale features.

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Repopulation of Peyer's Patches in Mice

by

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When irradiated mice were injected with bone marrow and lymphoid cells carrying distinct marker chromosome, the pattern of cellular repopulation of the Peyer's patches resembled that of lymph nodes, not that of thymus. This supports the view that the patches are not the analogue of the avian bursa of Fabricius.

WE have already described the patterns of cellular repopulation that are seen in various parts of the lymphomyeloid complex, that is bone marrow, spleen, thymus, lymph nodes and peripheral blood, when myeloid and lymphoid cells (distinguishable by the presence of one or two marker chromosomes) are injected simultaneously into lethally irradiated mice¹⁻³. The thymus was found to differ strikingly from the lymph nodes in showing negligible proliferation of injected lymph node or thymus cells: it was almost entirely repopulated by cells derived from the injected bone marrow. The thymus of mammals differs from the lymph nodes in a number of other ways: it is a lympho-epithelial organ; it plays an important part in both early and adult life in the genesis of immune reactivity. Nevertheless, it does not normally participate directly in immune reactions by, for example, producing immunoblasts, germinal centres, plasmocytes and immunoglobulins. In birds there is, in addition to the thymus,

another important lympho-epithelial organ, the bursa of Fabricius. In birds the thymus seems to control the development of cellular reactivity, while the bursa is responsible for the development of humoral antibody formation⁴. In mammals, too, the thymus is chiefly (although not exclusively) involved in the cellular kind of immunity (allograft rejection and delayed-type hypersensitivity), and Good and his co-workers have therefore been interested in the possibility that there may be an analogue of the avian bursa in mammals⁵⁻⁷. Their recent suggestion⁷ that the Peyer's patches of the intestinal wall might represent such an analogue prompted us to find out whether the Peyer's patches differed from lymph nodes in their pattern of repopulation.

Male CBA mice were exposed to lethal whole-body X-irradiation (1,000 rad) and injected intravenously with $10^5 (CBA \times CBA - T6T6) F_1$ bone marrow cells (containing a single marker chromosome) and $10^7 CBA - T6T6$ lymph

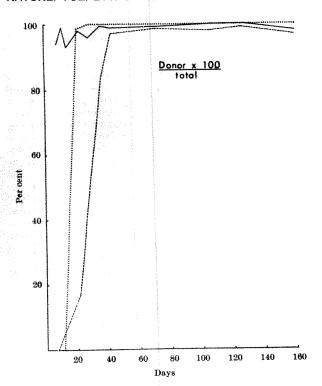


Fig. 1. Percentage of donor-type mitoses in lymphoid tissues of *CBA* mice after lethal irradiation and injection of 10⁵ hone marrow and 10⁷ lymph node or thymus cells. _______, Lymph nodes; ______, Peyer's patches, ______, thymus.

node or thymus cells (containing two marker chromosomes), as described before2. Pairs of recipients (one thymus injected and one lymph node injected) were killed on days 8, 11, 14 (two pairs), 21 (two pairs), 28, 35 42 and 70. Additional individual mice were killed on days 70, 106, 127 and 162. 'Colcemid' (Ciba) was injected intraperitoneally 1.5 h before killing. The mitotic cells in the thymus, pooled subcutaneous and mesenteric lymph nodes and pooled Peyer's patches of each individual were examined cytologically. Methods of preparing lymph node and thymus for examination have been described in Peyer's patches were prepared in detail elsewhere⁸. essentially the same way. It was usually possible to cut them away cleanly from the surrounding intestinal mucosa. Some contamination with intestinal epithelium was nevertheless unavoidable, but control experiments showed that mucosal epithelial cells were not dissociated and spread out by the technique used, so that their mitoses did not contribute to the total scored in the Peyer's patches. Two preparations of thymus and one of Peyer's patches provided less than twenty mitoses suitable for scoring and were omitted from the results. Apart from these, 2,888 mitoses were scored from thymus (mean 131/mouse), 3,520 from lymph nodes (mean 147/ mouse) and 2,793 from Peyer's patches (mean 121/ mouse).

Fig. 1 shows the pattern of repopulation of the three tissues by the donor myeloid and lymphoid cells taken together. In the lymph nodes, donor cells greatly out-numbered host cells throughout the experimental period. In the thymus, host cells were in the majority on days 8 and 11, and in three of four animals on day 14. But by day 21, 98-100 per cent of the cells were of donor origin in all four animals examined, and no further host mitoses were recorded. In the Peyer's patches, host cells remained in the majority for a longer time, forming 63-94 per cent of the total in the four mice examined on day 21, and they were still in a substantial minority in one of two animals on day 35.

Considerable numbers of mitoses derived from the donor lymph node inoculum were found in the lymph nodes and Peyer's patches (Fig. 2). Mitoses derived from the thymus were relatively rare in Peyer's patches; while they were dominant in the lymph nodes the patches were still largely populated by host cells. Only two cells originating from the lymph node or thymus inocula were detected in the thymus, which was repopulated by cells derived from the relatively small bone marrow inoculum.

Once repopulation of the Peyer's patches had begun, the proportion of donor mitoses in them derived from the lymphoid inoculum was at first similar to the proportion present in the lymph nodes of the same individual, except in one mouse, killed at 21 days, which may not have been representative. Tests of homogeneity showed that five of the individual differences were significant. from the mouse just mentioned, all the significant differences were observed in mice killed 42 days or more after treatment.

The proportion of donor cells derived from the lymphoid inoculum decreased rapidly in the first few weeks after their first appearance in both lymph nodes and Peyer's patches. Thereafter the proportion seems to have become stabilized, but at a significantly lower level in the patches than in the lymph nodes. Comparison of the mice given LN cells and killed at and after 70 days revealed no significant heterogeneity either in the lymph nodes $(\chi_*^2 = 2\cdot 13)$; P = 0.35) or in the Peyer's patches ($\chi_{\frac{3}{2}}^2 = 2.68$; P = 0.28). but the difference between the two tissues based on the combined counts for three mice was very highly significant ($\chi_1^2 = 46.49$; P infinitesimal). Similar comparisons for the parallel series of mice given thymus cells and killed at or after 35 days also revealed no significant heterogeneity in the lymph nodes ($\chi_4^2 = 7.45$; P = 0.11) or in the Peyer's patches ($\chi_4^2 = 2.15$; P = 0.71). The difference between the two tissues based on the data from five mice was again highly significant ($\chi_1^2 = 18.85$; P = 0.00002).

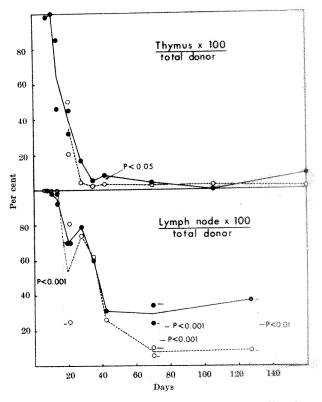


Fig. 2. Proportion of donor cells derived from the lymphoid inoculum in the lymph nodes (♠—♠) and Peyer's patches (○···○) of CEA mice after lethal irradiation and injection of 10⁵ bone marrow and 10⁷ lymph node or thymus cells.

The decrease of the lymphoid component was more rapid in the mice given thymus cells and the final level was significantly lower, although the numbers of lymph node and thymus cells in the original inocula were equal, and their proliferation was measured against the proliferation of an identical bone marrow inoculum. As far as the thymus and lymph nodes are concerned, the results agree with our previous findings2. These tissues were included in the present investigation simply as a yardstick against which to measure the Peyer's patches.

The Peyer's patches differed from the other tissues (and from bone marrow and spleen which were studied previously2) in maintaining a high proportion of host-type mitoses for a longer time after radiation. In the absence of data about the cellularity of the Peyer's patches we can only speculate about an interpretation. Thus we do not know whether the repopulation by donor cells begins later in Peyer's patches than elsewhere, or whether a larger proportion of host cells escape fatal radiation damage in the Peyer's patches than in other lymphoid tissues. A further formal possibility is that relatively undamaged lymphoid cells from various parts of the body tend to circulate after irradiation and become localized preferentially in the Peyer's patches. But whatever the reason for the exceptional persistence of host cells, donor cells finally achieve the same degree of dominance as they enjoy in other tissues.

If the descendants of the lymphoid and bone marrow inocula finally reach a stable equilibrium it is not surprising if the level differs significantly between Peyer's patches and lymph nodes, because the factors influencing repopulation may well differ, if only in degree, between the one type of tissue and the other. At any rate, there is little, if any, discrimination (such as exists in the thymus) against the entry and proliferation of injected lymphoid cells. As far as entry is concerned, this result was predictable from the findings of Gowans and Knight⁹ in rats; they observed that transfused radioactively labelled lymphocytes migrated into the Peyer's patches in the same way as into the lymph nodes.

If we follow the distinction between "central" and "peripheral" lymphoid tissues, the pattern of repopulation of the Peyer's patches aligns them with the peripheral type (lymph nodes) rather than with the one central site (thymus) that has so far been definitely established in mammals. Other features which (at least in guinea-pigs) distinguish the Peyer's patches from the thymus are poor

development at birth, the presence of germinal centres in adults, and failure to develop in germ free conditions¹⁰. In adult guinea-pigs¹¹ and rats (personal communication from G. N. Cooper) the Peyer's patches indeed contain particularly prominent germinal centres. In birds, the bursa of Fabricius resembles the thymus in being well developed at hatching (in contrast to the spleen)12, in not possessing germinal centres¹³, and in developing normally in germ free conditions14. It is not clear exactly how strong an argument against the function of Peyer's patches as a mammalian analogue of the avian bursa these facts constitute. Good \check{et} al. have put a persuasive case for the existence of some kind of analogue⁶ and produced some preliminary evidence supporting this function for the Peyer's patches?. On the other hand, it seems difficult to regard the Peyer's patches as first-order lymphoid tissue on a par with the thymus, and their possession of recirculating lymphocytes, and germinal centres endows them with two important characteristics of "peripheral" or "effector" lymphoid tissue. It remains possible that each patch is in effect two organs—one forming antibody, and the other exercising some undefined influence on antibody synthesis in the lymphoid tissue as a whole.

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Controlled Fracture of Brittle Solids and Interruption of Electrical Current

by

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Controlled high speed fracture of conducting solids can provide a feasible method of breaking alternating currents at zero current.

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A FULL understanding of brittle fracture growth is a problem of both theoretical and experimental complexity. Experimentally, the difficulties arise because fracture frequently starts at a defect of submicroscopic size, and then propagates at velocities reaching a considerable fraction of the stress wave velocities for the material.

With the continued development of high-speed photography and other sophisticated techniques, however, it has been possible over the past few years to study the propagation of fracture in some detail.

Much of the earlier work on fracture was by Schardin', who with Struth² first observed that a maximum crack

velocity of about 1,500 m/sec exists for glass like solids. More recently, in this laboratory, a study has been made of the mechanism of crack propagation in a wide range of materials3-6. One point of significance that emerges is that there are many brittle solids in which cracks can propagate at several thousand metres per second. In order to reach these very high velocities and obtain a clean single crack which runs straight through the solid it is necessary to use carefully controlled conditions. Controlled brittle fracture clearly offers a means for dividing a solid extremely quickly.

We have used the fracture of a brittle conducting solid as a method for interrupting electric current in a short interval of time, typically of the order of a microsecond. With alternating current it has proved possible to use a synchronized fracture process to interrupt the current at (or very close to) zero. These experiments are interesting because they provide a new method for circuit breaking. The normal method for opening a 50 cycle alternating current circuit is by the mechanical separation of metallic This is a comparatively slow process as the breaking time may be milliseconds, and the break may occur at any point on the current cycle. Another method is by the operation of a fuse, and again the interruption is a comparatively slow process.

Table 1. FRACTURE VELOCITY MEASUREMENTS

Material	V _F (m/sec)	$V_F \\ C_1$	VF CR	Comments
Glass (commercial soda-lime)	1,580	0.27	0.51	Isotropic solid; branching of crack at high velocities
Magnesium oxide	5,100	0-56	0.88	Cleavage fracture; branching suppressed
Diamond	7,200	0-4	0.6	Limited number of specimens. VF could be higher
Sapphire	4,500	0.4	0.8	VF for cleavage on {1010}. Fractures frequently con- choidal
Tungsten (rolled, polycrystalline)	2,200	0.43	0.85	Rolling causes some orienta- tion, branching partly sup- pressed

 $V_F = Maximum$ fracture velocity. C_1 = Dilatational stress wave velocity. CR = Rayleigh stress wave velocity.

Table 1 summarizes the results of our fracture velocity measurements. The velocities given are the maximum velocities for the growth of a single fracture (the word "single" is emphasized because situations can arise where a fracture apparently travels at the velocity of one of the stress waves, invariably because the stress wave is initiating many small cracks which subsequently join up to give one fracture). In the case of diamond, relatively few specimens were fractured; the figure for diamond therefore proves that cracks can travel at this speed, but does not rule out the possibility of an even higher

propagation velocity. In the case of the other results are based on the fracture of many Fracture growth was followed by using either and Whitley (Model 189) high-speed camera ultrasonic technique based on a method first Kerkhof and his co-workers (for a review see high-speed camera records were made by the crack edge-on or, alternatively, by growth of the fracture face. This latter me photographing down through the solid, but the detailed development of all the features on face, and shows clearly where measurements growth of a single fracture.

The ratios of crack velocity VF to the stress wave velocity C1 and the Rayleigh s velocity C_R are also given in Table 1, because theoretical estimates of maximum crack usually given in terms of a ratio with one or stress waves. A fuller discussion of these re given in a separate paper yet to be publi general point which emerges is that cracks rea higher velocities in crystalline solids (for magnesium oxide V_F is very close to the Ra velocity) than in isotropic solids like glasses falls midway in this classification; cleave when it occurred, was found most comm {10I0} planes, but many fractures were of nature.) It is interesting to note that at his fractures tend to bifurcate. This bifurcation much more difficult in solids with well-defiplanes. It seems possible that bifurcation limits the velocity of crack propagation in gla but has a smaller effect in solids with well-d of fracture. In these solids velocities app Rayleigh stress wave velocity are attainable is discussed in more detail later in the artic

The surface features which are found on surfaces of crystalline materials have been length in the literature. Gilman7, for exampl screw dislocations can give rise to cleavage then either cancel or add to give the well l pattern". Forty⁸ discusses the formation of fracture surfaces. The present evidence processes occur at relatively low crack veloci Knudsen and Walsh quote a limit of 60 m/s

Two other distinct mechanisms may also roughening or gross bifurcation of a fracture either when the fracture reaches a high velo stress waves interact with the stress field Fig. 1 illustrates the branching whi high crack velocities in glass fractured at

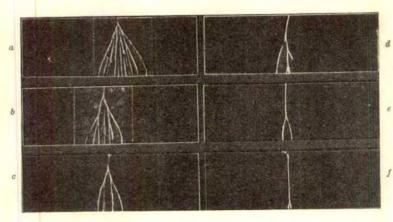


Fig. 1. Glass slides (3 in. \times 1 in. \times 0.05 in.) fractured in a Hounsfield tensometer at different stress levels. The specimens a to f broke at successively lower stress levels showing that the lower the stress level at fracture the longer the path length before crack branching.

of glass slides (3 in. $\times 1$ in. $\times 0.05$ in.) was at the mid-point of one edge of each speciotch sizes were varied as much as possible. s were then fractured in a Hounsfield tensoe and the stress at fracture recorded. As he notch was in the top edge and the speciulled along their length, so that the tennormal to the notch. In la the specimen had notch, which gave only a small stress connd consequently the specimen fractured ess level. As can be seen, there is only a ath before the first bifurcation, and this in ed by further branching. In the remaining specimens were fractured at lower stress they had successively more serious notches) length before branching increased. In stured at lower stress levels than If a single crossed the specimen.

ely, the results show that the length before s inversely proportional to the stress in the re fracture, σ_f , squared, that is

$$\lambda \sigma_f^2 = \text{const.}$$
 (1)

appears to be of broad applicability, for as have been found for other materials and of experimental conditions 11-13. It is not t an equation of the form of equation (1) e, for stress and length are similarly y formulae associated with fracture growth. ergies, equation (1) tells us that the energy before fracture determines the length of

rface roughening and bifurcation.

Bly convincing explanation for branching ent, though two mechanisms have strong first is based on the theoretical work of howed that when a crack reaches a high ress field at the crack tip changes. The ation is usually based on an energy argu-

ment, a point eventually being reached when there is sufficient energy to produce two cracks (see, for example, ref. 13). In our measurements of crack velocities, we find that the crack is still accelerating, albeit slowly, at the branching point. This observation favours the view that branching imposes a maximum velocity in isotropic materials, rather than that the maximum velocity is reached before the bifurcation point. Furthermore, the measured values for the branching velocity have a slight spread. If, however, the two cracks which form the bifurcation are activated by the stress field at the crack tip, then this spread can be explained in terms of a distribution of defects of varying sizes throughout the bulk of the solid. In crystalline solids with well-defined cleavage planes, it is clearly more difficult for cracks to form at an angle to the primary cleavage direction. This could explain the relatively higher fracture velocities in these materials.

The interaction between a stress wave and a fracture may also cause branching. A common situation where this is observed is when fracture is the result of impulsive loading. The dilatational stress wave, set up by the loading, runs ahead of the fracture and reflects at the boundaries of the solid forming a wave of tension which, on its way back through the solid, interacts with the advancing fracture front, causing branching6. In crystalline solids, the available cleavage planes influence the branching process. In diamond the interaction with the stress wave can cause the primary (111) cleavage crack to branch onto two (111) planes at angles to the primary direction. In magnesium oxide, the change in crack direction may be from one (100) plane onto one at right angles to it; this is shown in Fig. 2. The loading in this example was applied by a small explosive detonator at the mid-point of the top edge.

The reflexion of stress waves at the boundaries of the crystal can be prevented by backing the solid with material of the correct acoustic impedance. This is illustrated in Fig. 3, which shows a magnesium oxide

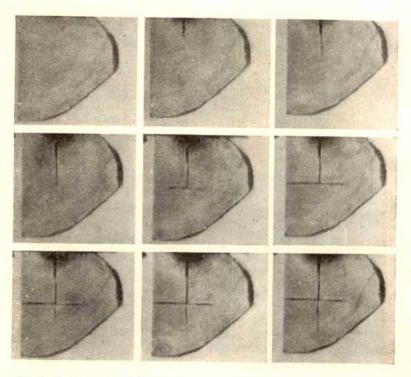


Fig. 2. The fracture of a single crystal of magnesium oxide. The cracks which cut across the specimen from frame 4 onwards are caused when the reflected dilatational wave interacts with the stresses at the tip of the primary crack. The interval between frames is 2-0 microseconds. The length and thickness of the crystal are 3-3 cm and 0-25 cm, respectively.

crystal loaded in a manner similar to that in Fig. 2, but with the crystal mounted on a steel anvil. This time the dilatational wave does not reflect at the lower surface as a wave of tension, and the primary cracks develop smoothly down the specimen.

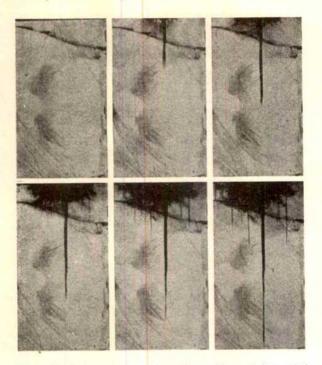


Fig. 3. The fracture of a magnesium oxide crystal mounted on a steel anvil. This time no reflected wave passes back up the specimen and secondary cracking is absent. Frame interval 1-0 µsec.

For a measurement of the speed of fracture growth in a conducting solid, it is possible to devise methods based on the change of one or other of the solid's electrical parameters15,16. In our initial experiments with metals, the change of resistance of a specimen as a crack cut through was measured. This method has the advantage that it gives a continuous measure of crack velocity, but has the disadvantage that the change of resistance is not a linear function of crack length: this restricts the accuracy. Considerably more accuracy can be achieved with the method depicted schematically in Fig. 4a, in which a high frequency a.c. signal is fed onto terminals, one on either side of a notch in the specimen. Under these conditions the "skin effect" operates and the current path is from one terminal, around the notch and to the second terminal (Fig. 4b). When the specimen fractures and a crack extends from the notch, the current path The changing electrical impedance of the specimen is eventually displayed on an oscilloscope as a voltage/time trace from which the crack velocity data can be determined. The frequency of 5 Mc/s was chosen because then the current path followed the shape of the crack without going to the extreme of "cutting corners" (too low a frequency) or of following any surface roughness of the fracture face (too high a frequency). A further advantage of this method is that direct currents or low frequency alternating currents can be passed through the specimen without affecting the crack velocity measurement. The electrical methods are not necessarily restricted only to conducting solids. We have, for example, measured crack velocities in glasses by evaporating conducting films of uniform thickness on to the glass.

The conducting solids so far investigated have been polycrystalline tungsten, molybdenum and silicon iron. The specimens were in strip form, and had notches of chosen size spark eroded into them. Specimen thicknesses

and widths ranged from 0.15 mm to 0.6 mm and 1 cm to 3 cm, respectively. As a general rule, the specimen length was always three times greater than the specimen width; this ensured that the fracture process was over before stress waves reflected from the grips returned to interact with the crack. All the specimens were fractured in a Hounsfield tensometer machine. The work with the brittle metals has shown that cracks can divide the specimen in a few microseconds. As with the glass slides (Fig. 1), bifurcation occurred at high velocities. spread in the crack velocity measurements at which this process took place was, however, greater than with glass. This effect appears to be related to the fact that some metals, when produced in strip form by a rolling process, do have a preferred orientation. X-ray diffraction showed that with tungsten the crystals had effectively a (001) face in the plane of the specimen and a [110] direction parallel to the direction of rolling. When tungsten specimens were cut at various orientations from a large strip, the crack branching occurred more or less readily, depending on this orientation. Measurements of the angles of branching indicated planes of weakness at roughly ±45° to the rolling direction. In specimens cut so that the fracture followed a plane of weakness, the branching was suppressed (compare with crystalline solids discussed earlier) and high velocity single cracks could be produced (see Table 1).

Fig. 5a shows a typical tungsten specimen used in the experiments. It is 1·3 cm wide and 0·15 mm thick. The notch was made by spark erosion and the metal terminals were spot welded into position. Fig. 5b is an example of a tungsten specimen cut so that a plane of weakness lay along the fracture direction. The resulting high velocity fracture was comparatively straight and clean.

Experiments have been performed in which specimens were fractured with steady currents of known size passing through them. As the fracture cuts through the specimen a difference in voltage builds up between the two fracture faces, while the current has to pass through a junction of rapidly decreasing size. Arcing was detected when the voltage exceeded about 18 volts: because the specimens were broken in air under normal atmospheric conditions, this was the value expected. No attempt has been made so far to open up the gap between the fracture faces quickly, or to fracture the specimen in either a high vacuum or in the presence of other gases such as sulphur hexafluoride, or in oil (all procedures which could inhibit arcing).

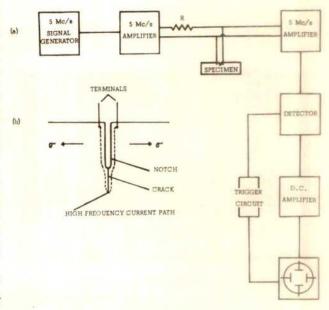


Fig. 4. High frequency fracture velocity method.

The restriction of the current path produces heating in the unfractured part of the solid. Because any heating will make plastic flow at the crack tip more likely with a consequent slowing of the crack, it is important to know the extent of this effect. In a series of experiments with tungsten specimens of width 1.3 cm and thickness of 0.15 mm, we have broken currents of varying size. For currents of less than about 20 amp, no slowing of the crack was detected. For currents of 20 amp and greater, the crack velocity decreased towards the end of its path. Examination of these specimens showed evidence of plastic deformation in the grains near the fracture faces. It is interesting to estimate the current density in the unfractured material when "crack slowing" was detected. For a current of 20 amp, the crack only began to slow down when it was typically 80 per cent across the specimen. This gives a value for the current density of about 5×10^3 amp/cm² in the unfractured solid. It should be emphasized that, even though the crack was slowing down in these experiments, it was still, by normal standards, travelling very fast. It was only when currents greater than about 100 amp were used that crack stopping was occasionally found.

Fig. 6 shows schematically the record on the oscilloscope in an experiment in which a current of 20 amp is interrupted. The top trace is from the apparatus shown in Fig. 4 and gives effectively the length of crack against time from which the velocity is calculated. The lower trace measures the current through the specimen and this is broken in the very short time (less than a microsecond) at the end of the fracture's path through the specimen.

In breaking alternating current it is clearly desirable to do so at as near current zero as possible. An apparatus has been built which fires a pulse at a predetermined point on the alternating current cycle. The pulse can be used to start the fracture process by, for example, firing a small explosive detonator (the reproducibility of our detonators was within 1.5 usec). To prevent the detonator affecting the terminals of the velocity measuring apparatus (see Fig. 4b), it was found convenient to send the pulse from the detonator down a chisel which sat in the notch. It was possible, using this method, to fracture alternating currents sufficiently near the current zero point to produce fast brittle cracks.

From the discussion of fracture it is clear that many intriguing points still need to be settled: enough is now known about fracture, however, to make it a controllable event. The application of a sudden pulse to a notch by, for example, an explosive detonator (a variety of other means is possible) can initiate a fracture in a brittle solid at a required time. The path of the fracture can then be controlled, either by having the specimen already under tension, or by having a plane of weakness in the solid; in

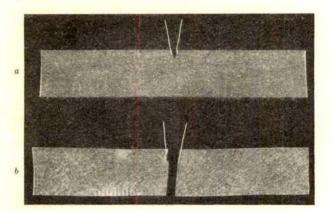


Fig. 5. a, A typical tungsten specimen of width 1.3 cm, 0.15 mm thickness. Terminals are spot welded on either side of the notch for the application of the high frequency current. b, A specimen fractured by a fast fracture. The specimen was prepared so that a plane of weakness lay along the fracture direction.

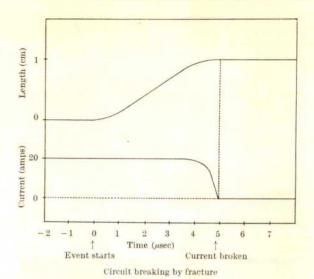


Fig. 6. A schematic record of the fracture of a tungsten specimen carrying a current of 20 amps. The top trace gives length of crack versus time, while the lower trace gives the current through the specimen. The current is interrupted very rapidly.

a crystalline solid the cleavage plane itself would suffice. The velocity of fracture (Table 1) has been measured for a variety of solids, and it is possible to predict fracture velocities with reasonable accuracy in other brittle solids by knowing their elastic properties, and whether fracture is conchoidal or cleavage. The effects of crack bifurcation are reasonably well understood. Stress wave interference can be eliminated, either by careful choice of specimen shape, or by acoustic matching at the boundaries of the solid. The branching which occurs at high velocity obeys equation (1), and therefore if branching is undesirable (in some applications it may not be) and occurs too readily, then the driving stresses can be reduced to give a large path before bifurcation. Experimental evidence shows that a crack quickly accelerates if sufficient energy is available and, for a considerable path length before a branching point, is very near the maximum velocity.

The experiments on metals have shown that high crack velocities can be reached in conducting materials. It is clearly desirable to break alternating currents at zero current and the experiments show that this is feasible. The experiments with direct current indicate the levels for voltage breakdown and the current density when crack slowing begins. These figures give an idea of the tolerance that is required for fracturing near current zero. These observations may be of practical interest for they provide a new method for the very rapid interruption of electrical current.

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LETTERS TO THE EDITOR

ASTRONOMY

Microwave Background in a Steady-state Universe

Ir has been widely claimed that the background microwave radiation observed by Penzias and Wilson¹ and Roll and Wilkinson² must originate from a radiation dominant, early phase of the Universe. Hoyle and Wickramasinghe³ have, however, discussed the possibility of a more local origin for this radiation. They point out that the energy density arising from the conversion of hydrogen to helium in all galaxies is about 10-13 erg/cm3, close to the energy density of the 3° K blackbody field. If an appreciable fraction of this energy could be thermalized by dust grains the observed "black body" microwave spectrum might be explained. The requirement is that quantum oscillators covering much of the frequency range 0.01 cm⁻¹ to 10 cm-1 are present in the grains. While arguments may be given for the presence of localized oscillation modes within this frequency range, the strongest evidence at the present time would seem to be for the existence of oscillators close to about 10 cm⁻¹ (Sievers⁴, Sievers and Takeno⁵). Such frequencies may be appropriate for the oscillation of weakly bound substitutional impurities in crystals. In a typical case the oscillator strength could be about 0.1 and the line width about 1/10 of the central frequency. We shall consider here the effect of a few strong emission bands at about 1 to 10 cm-1 which may be present in grains. From a total of about 1055 grains in the Galaxy each containing approximately 105 impurity oscillators we could obtain an infra-red emission at about 10 cm-1 equal to approximately 1/3 of the entire luminosity of the Galaxy. This flux of Galactic radiation at v~ 10 cm⁻¹ (1 mm wavelength) could produce the observed excitation of the J=1 rotational levels of cyanogen (CN) (Field and Hitchcock⁶), but it would not account for the microwave observations1,2.

We shall consider the consequences of a similar process operating in other galaxies. It does not seem likely that dust is a phenomenon in any way peculiar to our own galaxy. There is evidence of interstellar polarization in M31 and of interstellar reddening in SMC and LMC as well as of conspicuous striations in many extragalactic objects—all of which point to the presence of dust. If dust is present, it is also reasonable to find effects arising from impurity atoms in the grains.

For a steady-state model of the Universe we shall assume that in every element of proper volume an appreciable fraction of the galaxies contains interstellar dust which can efficiently absorb starlight and re-emit about 30 per cent of the total luminosity of the galaxy through impurity induced resonances at about $10~\rm cm^{-1}$. Quanta emitted from a galaxy at $v\approx 10~\rm cm^{-1}$ will be red-shifted to $\lambda=7.35~\rm cm$ and $3.2~\rm cm$ at red-shifts $z\approx 73~\rm and$ 31, respectively. While galaxies can also absorb radiation at $v\approx 10~\rm cm^{-1}$, the mean free path of an emitted quantum between galaxies is large, about $10^4~\rm mparsec$. This corresponds to a red-shift $z\simeq 3$; thus a quantum with original frequency $v=10~\rm cm^{-1}$ would be red-shifted well outside the width of the absorption band when it encounters another galaxy.

Let N denote the number of galaxies per unit proper volume in the universe. Then the number of galaxies

observed at present with red-shifts between z and z+dz is given by

$$dn = 4\pi \left(\frac{c}{H}\right)^3 N \frac{z^2}{(1+z)^3} dz$$
 (1)

where H is the Hubble constant and c the velocity of light. We shall assume that a fraction α of these galaxies contains interstellar dust and takes part in the thermalization process described. Suppose the amount of radiation emitted per unit time by a typical galaxy in this manner is L, and that this radiation has a normalized spectral function $I(\gamma)$, that is,

$$\int_{0}^{\infty} I(\mathbf{v}) d\mathbf{v} = 1 \tag{2}$$

Because of the expansion of the universe, the observed normalized spectral function from a galaxy of red-shift z is

$$I(z,\mathbf{v}) = (1+z)I(\mathbf{v}.\overline{1+z}) \tag{3}$$

The observed radiation flux per unit time per unit frequency range from a galaxy of red-shift z is therefore given by

$$S(z, \mathbf{v}) = \frac{LH^2}{4\pi c^2 z^2 (1+z)^2} I(z, \mathbf{v})$$

$$= \frac{LH^2}{4\pi c^2 z^2 (1+z)} I(v.1+z) \tag{4}$$

Adding contributions from all galaxies in the universe we get

$$\alpha \int S(z, \mathbf{v}) dn = \alpha \left(\frac{c}{H}\right) LN \int_{0}^{\infty} \frac{I(\mathbf{v} \cdot \overline{1+z}) dz}{(1+z)^{4}} = \mathcal{J}(\mathbf{v})$$
 (5)

 $\mathcal{J}(\mathbf{v})$ dv denotes the total radiation crossing unit area per unit time in the frequency range dv.

We shall first consider the simple case in which the thermalization mechanism in the interstellar dust of each galaxy results in the radiation of a sharp line of frequency v_0 . This corresponds to

$$I(\mathbf{v}) = \delta(\mathbf{v} - \mathbf{v}_0) \tag{6}$$

From equation (5) we get

$$(v) = \begin{cases} \alpha \left(\frac{c}{H}\right) LN \frac{v^3}{v_0^4}, v \leq v_0 \\ 0, v > v_0 \end{cases}$$

$$(7)$$

Thus although each galaxy emits a sharp line, the net contribution from all galaxies results in a continuum spectrum given by equation (7). It is of interest to note that the spectrum continues only on the long wavelength side of the line emitted by a typical galaxy.

Because a general function can be represented as a superposition of suitably weighted delta functions, we can consider the more complicated forms of I(v) by superposing functions of the form in equation (7). The crystallographic properties of interstellar grains are yet to be fully investigated for us to give a precise form for I(v) against ln v. The curve will have a slope of three at the very long wavelengths $(v\rightarrow 0)$. As the range of v overlaps that of I(v), however, the slope will be reduced.

The case where $I(\nu)$ consists of two sharp lines at the original frequencies ν_1 , ν_2 is shown schematically in Fig. 1. We envisage a situation where $\nu_2 \approx 1-10$ cm⁻¹ and $\nu_1 \approx 0.2$ cm⁻¹, the latter frequency lying between the observations of Penzias and Wilson¹ and of Roll and Wilkinson². There is crystallographic evidence for the presence of impurity oscillators around both such frequencies—the former arising from bodily oscillations of a weakly bound

impurity atom, and the latter due to hindered rotations of a diatomic molecule in a crystal^{8,9}. The width of the lines is not relevant because we do not require a coverage by individual lines. It is clear from Fig. 1 that two suitably placed emission lines may give rise to a microwave continuum with a slope of about 2 between $\lambda = 7.35$ cm and $\lambda = 3.2$ cm, in accord with the observations.

It remains to determine the magnitude of the flux arising from our model. From equation (7) the flux arising from a line at $\nu_0 \approx 10$ cm⁻¹ is $\sim 10^{-31}$ erg/cm³ (c/s)⁻¹ at $\lambda \approx 7.35$ cm, assuming $\alpha \approx 1$, $L \approx 10^{43}$ erg/sterad, $N \approx 1$ mparsec⁻³, $H^{-1} \approx 10^{10}$ yr. With $\nu_0 \approx 1$ cm⁻¹ (refs. 8 and 9), and the same values for the other parameters, we get a flux of about 10^{-27} erg cm⁻³ (c/s)⁻¹ at $\lambda \approx 7.35$ cm which is in accord with the observations to within a power of 10

In the preceding calculation the value of total luminosity of the galaxy of about 1043 erg/sterad was assumed. This estimate is based on the observed stellar radiation at visible wavelengths. The energy output of a galaxy in the ultra-violet at $\lambda \le 2000$ Å is uncertain. In young spiral galaxies it could not be ruled out that the total stellar emission in the ultra-violet exceeds the visible output by a factor 10-100. Dust grains present in such galaxies would absorb this radiation with an efficiency factor of about five times greater than for visible light. Thus a galaxy having an optical depth of order unity for visible light will have $\tau \approx 5$ in the ultra-violet. The result is that no ultra-violet radiation can escape from the galaxy. The grains which absorb this energy will reradiate it in the microwave spectral region in the manner described here. The microwave power of galaxies could thus exceed the visible power by a factor of about 100.

Hoyle and Tayler's ¹⁰ estimate of the He/H ratio from the energy output of stars is of interest in this connexion. Taking $L\approx 4\cdot 10^{43}$ erg/sterad as the optical energy output of all the stars in the galaxy they obtain a He/H ratio of about 0·01, resulting from the conversion H \rightarrow He throughout the age of the galaxy. The discrepancy between this estimate and the observed value He/H ratio of about 0·1 would be resolved if the total energy output of the galaxy is about 10 times in excess of that deduced from the optical emission alone.

Our final remarks concern the close equality between the energy density of the microwave background and that of several galactic fields (cosmic rays, galactic magnetic field, etc.) which was pointed out by Hoyle and Wickramasinghe³. It would be a remarkable coincidence if the primordial radiation from a big bang universe possessed the same energy density as these galactic quantities. The present explanation of the microwave background has the

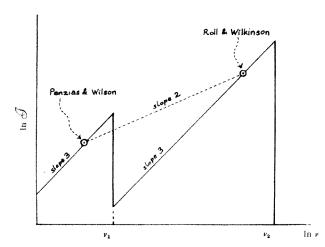


Fig. 1. Schematic representation of a 2-line spectrum with lines at v_1 and v_2 . The dotted line shows how a slope of 2 may be measured between two observational points.

advantage of linking the observed flux with processes currently operating in galaxies.

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Diameters of Some Quasars at a Wavelength of 66.9 cm

The measurement of the diameter of radio sources in the range of 10^{-2} sec of arc or less has recently been made possible by a new technique in radio interferometry. Independent stable local oscillators are used to convert the signals at the two stations to frequencies which are recorded on magnetic tapes. In principle, these long baseline interferometers (LBI) can operate at any separation.

Measurements at 49 cm wavelength with a baseline of 220 km $(4.6 \times 10^5 \lambda)$ have been reported by the NRAO—Arecibo group¹, and at the 18 cm hydroxyl line wavelength with a baseline of 845 km $(4.7 \times 10^6 \lambda)$ by the MITNRAO group². Measurement at 66.9 cm wavelength with baselines of 183 km $(2.7 \times 10^5 \lambda)$ and 3,074 km $(4.6 \times 10^6 \lambda)$ have also been reported by the Canadian LBI group³. A further series of observations has been made at 66.9 cm with the 3,074 km baseline during the period July 26–29, 1967, in which fringes of amplitude greater than 4 flux units have been detected from eight sources.

Table 1 shows the fringe visibilities determined from both the $2.7 \times 10^5 \lambda$ baseline (position angle 103°) and the $4.6 \times 10^6 \lambda$ baseline (position angle 95°). The details of the interferometer have been described previously4. Most of the observations were taken near the instrument meridian. The fringe amplitudes were determined from artificial fringes produced by the introduction of coherent c.w. signals at each receiver. The level of these signals was commensurate with the total power output from sources of known flux value, taking into account the bandpass characteristics of the system. The fringe visibilities were then derived from comparison with adopted zero spacing flux values as shown in Table 1. The three highest visibilities at the $4.6 \times 10^6 \lambda$ spacing and the nine highest visibilities at the $2.7 \times 10^5 \lambda$ baseline were nearly equal, and close to unity. A slight adjustment was therefore applied in deriving the values in Table 1 so that the average of each of the groups was normalized to unity. The estimated probable errors of the results are included with each entry. The last column lists source diameters computed on the assumption of a Gaussian model. For sources observed at both separations the data from the longer baseline were used. About forty other sources were observed in the July programme. More elaborate data processing will be necessary, however, to determine their visibilities.

At small elongations interplanetary scintillation could cause a significant reduction in fringe amplitudes. At the time of observation, however, the sources closest to the Sun $(CTA\ 21,\ 3C\ 273B,\ 3C\ 279$ and 1127-14) had

elongations from 60° to 75°. From published data⁵ it is estimated that the effect of scintillation on fringe amplitude is small. The high visibility of 1127-14 supports this conclusion. The sources, 3C 273B, 3C 279, 3C 286, 3C 309·1 and CTA 21 are apparently partially resolved. The detection of fringes at the shorter spacing for the extra-galactic source $\breve{3}C$ 274 supports the suggestion of a small diameter components.

			Table 1		
		S_{448}	Fringe v $2.7 \times 10^{6} \lambda$	isibility 4·6×10°2	Diameter (sec. of arc)
•	CTA 21 1127-14	10·5 5·0	*	$0.5 \pm 0.1 \\ 0.9 \pm 0.2$	0.02 ≤0.01
	$\frac{3C}{3C}\frac{273B}{274}\dagger$	19·0 520	$\begin{array}{c} 1 \cdot 1 & \pm 0 \cdot 2 \\ 0 \cdot 02 \pm 0 \cdot 01 \end{array}$	0.5 ± 0.1	0.02
	3C 279 3C 287	12·3 11·8	$\begin{array}{ccc} 0.9 & \pm 0.2 \\ 1.2 & \pm 0.2 \end{array}$	0·3 ± 0·1	0·03 ≤0·1
	3 <i>C</i> 286 3 <i>C</i> 298	22·7 23·0	$\begin{array}{ccc} 0.9 & \pm 0.2 \\ 1.0 & \pm 0.2 \end{array}$	0.2 ± 0.1	0-03 ≤0-1
	3C 309·1 3C 345	$\substack{14.1\\9.0}$	$0.7 \pm 0.2 \\ 0.9 \pm 0.2$	0·3 ± 0·1 1·0 ± 0·2	0.03 ≤0.01
	NRAO 530 3C 446	6·5 11·4	$\begin{array}{cc} 0.9 & \pm 0.2 \\ 1.0 & \pm 0.2 \\ \end{array}$		≤0·1 ≤0·3
	CTA 102 3C 454·3	$7.3 \\ 14.0$	$\begin{array}{ccc} 1.1 & \pm 0.2 & \pm \\ 0.4 & + 0.1 & \pm \end{array}$	1.0 ± 0.2	≤0.01 0.6

* Source not included in observing programme. † It is assumed that 3C 273A is completely resolved at both spacings. † These visibilities were derived for observation laid at the following effective base lines and position angles: 3C 446, 10×10^5 λ and 120° ; CTA 102, 1.5×10^5 λ and 139° ; 3C 454-3, 1.7×10^5 λ and 143° .

The variations of relative local oscillator phase necessary to keep a source on the same interference fringe for sources 3C $27\overline{3}B$ and 3C 345, observed at the longer baseline, were analysed in detail. Over a period of 15 min the variations of apparent local oscillator phase difference with time were fitted with a third order polynomial which approximated the expected variations. The r.m.s. errors were 25° and 70°, respectively. This indicates that even with the relatively low stability of rubidium clocks, valuable information can be derived from phase data for such problems as the synchronization of time and frequency standards at different locations, the determination of relative positions on Earth, the precise measurement of source position and the analysis of source structure. With the more stable hydrogen clocks, the long baseline interferometer will make many new investigations? possible.

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PLANETARY SCIENCE

Attitude Determination of the Ariel III Satellite

ARIEL III, the third UK/US satellite, was successfully placed into orbit on May 5, 1967, with apogee and perigee approximately 600 km and 500 km, respectively, and inclination 80°. It had been suggested some time before launch that the spin axis direction required for the interpretation of data from some of the experiments could be monitored by observing fluctuations in the telemetry AGC recordings as the satellite rotated; this method had been adopted for the Ariel II satellite1, but the analysis proved difficult and because the telemetry polar diagrams for Ariel III were found to be nearly circular this technique was regarded as unpromising. An alternative method was therefore proposed similar to that used on Telstar²—namely, observing the glints of solar reflexions from the solar cell panels and from special mirrors placed on the spacecraft. Solar aspect sensors already included in the payload would determine the angle between the spin axis of the satellite and the direction of the Sun, thus defining a conical surface containing the spin axis. To position the spin axis on this cone another frame of reference would be required and the mirror glints would define this. The method was adopted successfully for Ariel III, and spin axis direction to better than $\pm 2^{\circ}$ has been calculated together with coning angle and spin

Six echelon mirrors each with an effective reflecting surface at 30° to the spin axis direction were placed around the body of the spacecraft which was launched with a spin rate of 30 r.p.m. When the spin axis is correctly related to the directions of the Sun and the observer. there are three reflexions every second from the mirrors: similarly, the body solar cells reflect glints at a rate of six a second with the appropriate directions to the spin axis, and the double-sided boom solar cells at a rate of two a second. As an example, with the Sun's direction normal to that of the spin axis there are glints at angles of 25. 60, 90 and 155 degrees to the spin axis direction. The mirrors are rhodium plated and have a 76 per cent reflexion for normally incident light in the visible spectrum while the solar cells have a reflectivity of 20 per cent for normal light. If the mirrors had been optically flat only two or three glints would have been detected during a pass over an observer on the Earth because the Sun's reflexion would illuminate, at any instant, an area of only 5 km in diameter, so it was decided to manufacture them flat to only 1°, thereby giving a 2.5° dispersion of the Sun's image. According to calculations, about ten 7 msec glints of first magnitude would be observed at a ground station in a period of about 4 sec from mirror reflexions while glints from the solar cell panels, which had greater surface irregularities, would each have about 25 msec duration with their glint train lasting for at least 15 sec and the most intense magnitude glint also being first magnitude. As the eye has an integration period longer than the individual glint duration, these glints seem to be about two magnitudes lower to the eye, although photographic and photoelectric devices record the magnitude correctly. An experiment carried out in the laboratory showed that the mirror glints would be easily visible to the human eye through binoculars of magnification 10, although reasonably accurate predictions of the azimuth and elevation of observation would be required. Owing to the number of reflecting surface angles, some glints would be visible on each pass over any station provided the Sun was at least about 10° below the horizon and the satellite was illuminated by the Sun.

A number of organizations agreed to co-operate in the observations and they are listed, together with the type of equipment used, in Table 1.

Two teams of amateur observers controlled by the SAO Moonwatch group and Radio and Space Research Station were also alerted. It is difficult to predict the position along the orbit where glints are expected at a particular station, and so the photoelectric cameras which are capable of tracking the spacecraft from horizon to horizon are the most satisfactory recording system.

Fig. 1 shows the echelon mirrors mounted on the satellite body. They could be adjusted in angular position, and alignment tests were carried out on a flight satellite. The spacecraft was vibrated to the level expected during lift off and the resulting change in alignment was only 1/6°. The final flight model alignment was carried out just before launch when the spacecraft doors had been screwed down for the last time and the full dispersion characteristics of each mirror were determined. The mirrors were found to exhibit different dispersion characteristics, and it is possible on analysis of ground

photoelectric photometry records to indicate which mirror has led to a particular flash.

Table 1

National Aeronautics and Space Administration (NASA)

Smithsonian Astrophysical Observatory (SAO)
United States Air Force (USAF)
Royal Observatory, Edinburgh

Aerospace Research Laboratories USAF (ARL) NASA Optical Facility Worldwide network of Minitrack Optical Tracking System (MOTS) cameras

Worldwide network of Baker-Nunn cameras

Baker-Nunn cameras Kinetheodolites at Edinburgh and Malta

Photoelectric camera at Ohio

Photoelectric camera at Washington

Since launch there have been many reports of glint observations from satellite tracking cameras and amateur observers all over the world. A sample record from a Baker-Nunn camera in stationary mode is shown in Fig. 2 in which the satellite travels from right to left. The camera shutter was opened just after the satellite entered the field of view. Some remarkable photoelectric records from the Aerospace Research Laboratories, Ohio, have

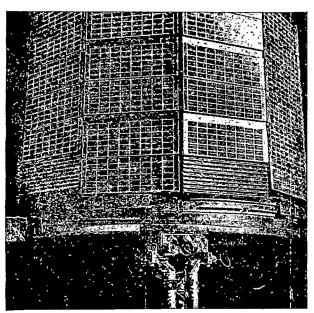


Fig. 1. The Ariel III satellite showing the echelon mirrors fixed to the body of the spacecraft.

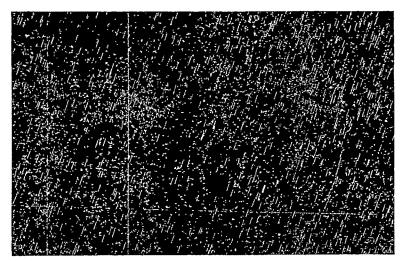


Fig. 2. Glints from body solar cells on Ariel III. Photographed by an SAO Baker-Nunn camera at Island Lagoon, Australia, May 14, 1967.

led to the calculation of the satellite's coning angle, which is the angle between the geometric and dynamic axes, and have shown it to be less than 1°. There is an r.m.s. error in the analysed spin axis direction of about 2° which is no doubt caused by an inability to identify the peak magnitude in the glint train either on photographs or during visual observations by amateurs. The photoelectric data from Ohio, however, have allowed this peak magnitude position to be determined to an accuracy of about 1/6 sec of time. This type of recording is therefore to be recommended in any future use of the optical glint technique. Such a recorder, with azimuth and elevation angle indicated correct to 0·1°, enables the spin axis direction to be measured to better than $\pm 1^{\circ}$ and the coning angle to $\pm 0.1^{\circ}$. Furthermore, a series of measurements of the glints is sufficient to measure these angles without the use of solar aspect sensors, particularly when more than one set of glints can be observed on a single pass. By this means the use of telemetry channels for transmitting solar aspect data is avoided.

The results of spin axis direction during the first 45 days are shown in Fig. 3, the day of the year being marked periodically on the curve with launch occurring on day

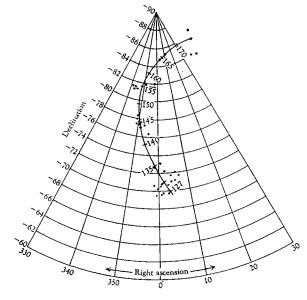


Fig. 3. Ariel III spin axis direction from launch on day 125 to day 170.

Day 125 is May 5, 1967.

Further papers will describe more results and illustrate technical and mathematical features of the technique in more detail.

I thank NASA, SAO, USAF and the Royal Observatory, Edinburgh, for their continuing help in monitoring glints from the satellite and also to the many amateur observers for their valued assistance, the British Aircraft Corporation for their co-operation in carrying out the installation and mechanical tests on the mirrors, and Dr D. E. Smith and M. Colbourne for their help. The Ariel III international co-operative project was managed and supported by the Science Research Council.

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Ionospheric Electron Content and the [OI] 6300 A Nightglow

SEVERAL emission features of the night airglow in the tropics are measured regularly at the Haleakala Observatory of the Institute for Astronomy, University of Simultaneously, determinations of the ionospheric electron content are being made by the Department of Electrical Engineering, University of Hawaii, in Honolulu.

The electron content is determined by the Faraday rotation of VHF signals from the synchronous satellite, Syncom III, as received in Honolulu. Determinations are made every 5 min1. A photoelectric photometer with an interference filter2 is pointed at the ionospheric point of the satellite—the point at which the ray path of the satellite signals intersects the ionosphere at a certain altitude on the way to the receiver. We have chosen an altitude of 250 km, at the base of the night time F region and near the height of peak emission rate of the [OI] 6300/6364 Å airglow. The intensity of the 6300 Å emission is measured

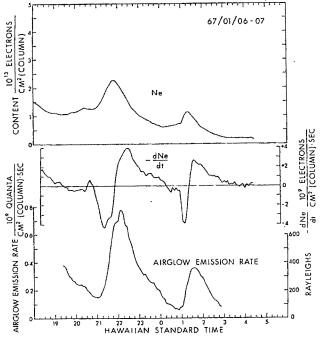


Fig. 1. Observations made in the Hawaiian Islands of the electron content, its time rate of change, and the [OI] 6300/6364 Å nightglow emission rate. The values shown are local zenith values.

every 5 min during the night. Contaminating continuum radiation is eliminated by the so-called two colour method.

Fig. 1 shows the results of these simultaneous observations on the night of January 6/7, 1967. The large night time increases in the intensity of the airglow are peculiar to the tropics3 and these results show that they are preceded by nocturnal increases in electron content. Also shown in Fig. 1 is the negative time rate of change of content, which is expected to be closely related to the airglow emission rate if this large content change occurs in the F region of the ionosphere4. Ionosonde records from Maui, Hawaii, show this to be the case. True height electron density profiles show that the ionization density increases throughout the bottomside F layer, and that the layer falls, during these content increases. The airglow reaches its highest intensity when the ionization reaches its lowest altitude.

From Fig. 1 it may be inferred that about 0.15 quantum is produced for each electron lost in the night time ionosphere. This number includes both the 6300 and 6364 Å emission features. In computing this efficiency, care must be taken to ensure that the ionization source has decayed sufficiently so that the time rate of change of content is primarily a measure of electron loss and not just the difference between production and loss.

The tropical nightglow has several morphological aspects in common with those of the so-called equatorial anomaly of the F region of the ionosphere⁵. This implies that the explanation of the nocturnal increases in electron content is to be found in the peculiar ionization distributions and motions found in the tropics, and not by invoking a night time ionizing agent.

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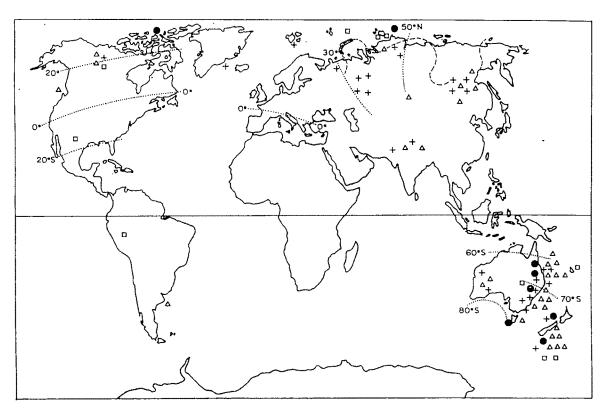
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Cool-water Faunas from the Permian of the Canadian Arctic

A LONG established tenet supporting continental displacement has been the presence of Upper Palaeozoic glacial sediments associated with peculiar, presumably cold-loving, faunas and floras in the continents of the southern hemisphere, and the absence of glacial or coldloving biota from the northern hemisphere, even from the Arctic. This suggests that continents now occupying the northern part of the world once lay far to the south, beyond the reach of any northern ice-cap which counterbalanced the southern ice. Thus faunas recently described from the Permian of Greenland¹ and the Canadian Arctic² have virtually nothing in common with the Permian faunas of eastern Australia, where glaciation was particularly intense, to judge from the abundance of glacial varves, and tillites associated with the fossils. Arctic faunas described previously resemble much more closely the faunas of the Zechstein of Germany and Magnesian Limestone of England, where associated salt deposits suggest that waters were warm. Low latitudes for northern Canada are apparently confirmed from palaeomagnetism, Irving³ placing this region between about 10° and 30° north of the Permian equator (Fig. 1). with the North Magnetic Pole situated far away in a position now corresponding with China.



New discoveries of extensive Permian brachiopod faunas by the Geological Survey of Canada cast doubt on this picture, for they include a number of genera allied to long ranging cold-water forms of the Australian Permian. These faunas come partly from

Permian. These faunas come partly from the Arctic Islands, such as Devon Island, and also from the northern Yukon, where Irving³ indicated a palaeolatitude of 20° to 25°. There are three principal cold-water elements, and a fourth more characteristic of slightly warmer waters. Most arresting are distinctive Strophalosiid brachiopods with fine spines and a wedge-shaped dorsal valve as in Wyndhamia Booker (Fig. 2A and D). Wyndhamia is reliably known only from eastern Australia and New Zealand; I have never seen it in collections from Indonesia, south-east Asia, India, Pakistan, Europe or the remainder of North America.

Also abundantly represented are members of the sub-family Licharewiinae. These are transverse spiriferids, usually with pustulose ornament and internal details comparable with the closely allied genus Spiriferella. Licharewia or Permospirifer is common in the Yukon (Fig. 2C), and Pterospirifer and "Spirifer" osborni Harker and Thorsteinsson in the Arctic. This sub-family is prolific in the Permian faunas of eastern Australia, including many so-called Spirifer species, such as forms referred to as vespertilio, phalaena and stutchburi.

The third group are brachythyrid plicate Yukon shells (Fig. 2B) allied to Martiniopsis, and probably congeneric with Tomiopsis Benediktova⁴, or Ambikella Sahni

and Srivastava⁵. The last genus is probably identical with *Ingelarella* Campbell⁶, represented by about fifteen species in both Queensland⁶⁻⁸ and New Zealand⁶⁻¹³. The relationship between *Tomiopsis* and *Ambikella* is not

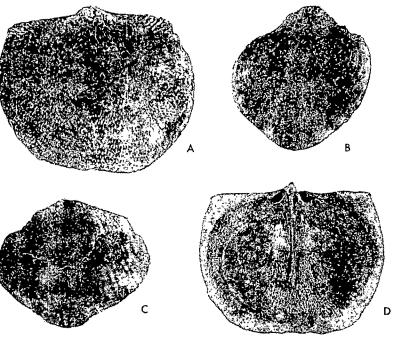


Fig. 2. Permian brachiopods from the Canadian Arctic. A and D are ventral exterior and dorsal interior aspects of? Wyndhamia, from the Assistance Formation (sensu lato). A is from locality 35317, Melville Island; D is from locality 76029, Cape Fortune, Cameron Island, North-west Territories; B is Tomiopsis, dorsal aspect of exterior, from below the Fantasque Formation, locality 36539, Tetsa River area, north of Alask Highway, mile 382, Yukon; C is Licharewia species dorsal aspect of exterior, from same locality. All specimens reproduced natural size, kept at Geological Survey of Canada, Ottaws.

fully established. In Tomiopsis the dorsal median septum seems to arise at the base of the laminated myophore face of the cardinal process¹³ as in the Yukon species. In New Zealand, Indian and Australian Ambikella, the septum commences a little in front.

Another genus represented in the Yukon is Attenuatella Stehli. Although first named from Texas, it is extremely rare in this region (personal communication from G. A. Cooper, Smithsonian Institution), and especially typical of Novaya Zemlya and Taimyr¹⁴ and New Zealand⁹. It also occurs in Peru¹⁵, New Caledonia¹⁶ and to a limited extent in eastern Australia¹⁶.

Whatever the exact generic identities may be, we must conclude that elements of the Canadian brachiopod faunas compare closely with Australian cold-water forms. Canada is not unique among northern countries in this respect. The home of the Licharewiinae and Tomiopsis is the eastern Russian Platform and north-east Siberia, and forms possibly akin to the Canadian Wyndhamia-like shells have been described from Taimyr¹⁴. Furthermore, some of the peculiar Australian bivalve genera seem to be represented in north-east Siberia¹⁷⁻¹⁸, as pointed out by Dickins²⁰. There thus appears to be evidence of a bipolar fauna, with cool or cold-water elements in both Australasia and the Canadian-Siberian Arctic. A latitude as low as 20° N. for the Canadian faunas seems unlikely, palaeomagnetic evidence notwithstanding. In overall appearances the faunas show some similarities to those of northern Queensland, and especially New Zealand. Judging from the distribution of faunas and glacial sediments, Queensland possibly occupied palaeolatitudes of about 70° to 55° S. in the Permian. This accords well with palaeomagnetic data²¹, except that faunas and sediments suggest that only eastern Australia suffered a mid-Permian (Baigendzinian) glaciation, so that the South Pole may have been to the east of the Great Australian Bight, not in the middle as shown by Irving. No palaeomagnetic data are available for New Zealand. The faunas and floras and sediments from this country indicate palaeolatitudes only 10° or so from its present position, perhaps between 55° and 45° S. A slightly lower position would seem acceptable for the Yukon, or even much the same palaeolatitude, if allowance is made for the greater extent of ice in the southern hemisphere. This does not accord well with the palaeolatitude assigned by Irving³, but agrees more closely with palaeolatitudes for north-east Siberia (ref. 3, Fig. 9.9, page 197). While such disagreement may indicate the crudities and risks involved in trying to calibrate palaeolatitudes through faunal studies, it seems feasible to propose that neither Siberia nor northern Canada has moved in phase with the regions where palaeomagnetic work used by Irving was carried out. The tectonic and stratigraphic maps of the Soviet Union suggest buckling of geosynclines on a huge scale, so that if that part of the continent has moved, it has not moved as an undistorted plate, but has crumpled severely. It is certainly difficult to accept palaeomagnetic reconstructions showing the Texas Permian at 15° S. in a palaeolatitude almost comparable with that of the Yukon and Canadian Arctic at 20° N. Dunbar²² also emphasized the considerable difference between his Greenland faunas, supposedly with a palaeolatitude close to 20° N., and those of Texas.

In conclusion, it seems from the nature of faunas that the seas covering northern Canada and north-east Siberia in the Permian occupied temperate rather than subtropical palaeolatitudes, high enough to support a number of cool or cold-water loving genera, at least from time to time, presumably when glacial spasms gripped the southern hemisphere.

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Palaeomagnetic Evidence for the Ordovician Geomagnetic Pole Position

I have obtained evidence which supports a pole position in the region of 165° E., 13° N. (antipole 15° W., 13° S.) on the present latitude-longitude grid and relative to the British Isles. This agrees with the palaeoclimatological evidence reported by Spjeldnaes¹, whose studies, based chiefly on the global distribution of shallow water shell deposits, led him to suggest that the Ordovician climatic zones could be deduced from the faunal provinces and that polar ice caps existed during this period. Spjeldnaes1 infers a rotational pole lying in or west of Africa which would correspond to the palaeomagnetic pole at 15° W., These data are not in conflict with Wegener's hypothesis of continental drift, for the positions of the climatic zones on either side of the Atlantic are improved by moving the continental blocks together. There is little corresponding information for the 165° E., 13° N. pole because the data available for China are too few to say whether the palaeolatitude and palaeomagnetic latitude are in agreement. Spjeldnaes concluded from a consideration of Runcorn's2 palaeomagnetic polar wandering curves that a magnetic pole at about 165° E., not far from the Equator, in the region of 0°-25° north of it, would correlate well with the available data.

Oriented hand samples were collected from seven lava flows in the Eyeott group in the Borrowdale volcanic series of Llanvirn-Llandeilo age. Cylinders were cored out of these in the laboratory and an astatic magnetometer was used to measure the remanence of the cores.

			Table 1					
Eycott	Long.	Lat.	α .	Circular standard deviation	Circular standard error	No. of samples	R	Polarity
(1) (2° 56′ W., 54° 40′ N.)	165° E.	14° N.	11·4°	15·1°	5·3°	7	6.794	N.
(2) Llanelwedd (3° 24' W., 52° 9' N.)	162° E.	15° N.	13.9°	21.0°	7-0	9	8-453	s.
(3) Stockenray Bay (4° 54′ W., 55° 11.5′ N.)	168° E.	11° N.	9.50	17.40	5•0°	12	11.495	s.

(Note: Fisherian's statistics used R, σ .)

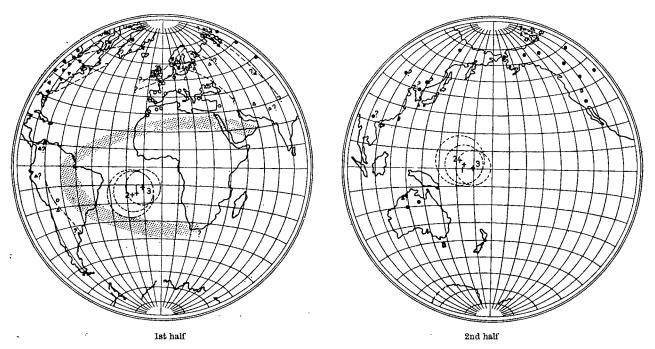


Fig. 1. Comparison of the proposed pole position with Spieldnaes' climatic data. • Tropical faunas; A, warm temperate faunas; A, cold temperate faunas; O, Arctic and Antarctic type faunas. Numbers 1, 2 and 3 refer to the sites in Table 1.

These samples were then subjected to a.c. and thermal demagnetization tests. On the basis of these tests three hand samples were rejected in calculating a mean direction of magnetization. Two of these included the Earth's present field direction within the circular standard deviation of their field directions at the site. The third was strongly magnetized in a direction remote from the remaining samples, but its mean direction began to migrate towards the main group on a.c. cleaning in fields above 560 peak cersteds, moving into the same quadrant after 900 peak cersteds. The specimens initially had a geometric mean intensity 58.7×10^{-4} (S.D. logs 0.37) gauss, susceptibility of 16.0×10^{-3} (S.D. logs 0.42) gauss/cersted, and intensity to susceptibility ratio of 0.37 (S.D. logs 0.37) cersted. A pole position corresponding to the mean direction of magnetization (after cleaning in 85 peak cersteds) was calculated for each flow and a mean pole (Table 1) for the site calculated from these (Fig. 1 (1)).

Evidence supporting this direction was obtained from hand samples of Upper Llanvirn³ age from the Builth volcanic series collected at Llanelwedd in the Builth Wells-Llandrindod inlier. Samples were subjected to a.c. and thermal demagnetization tests on the basis of which three hand samples out of twelve had to be rejected. The mean directions from the other hand samples were combined to yield the mean pole position (Fig. 1 (2) and Table 1 (2)). Most of these samples were very weak with geometric mean intensities in the range 1.2-20.7 × 10-6 gauss and susceptibilities in the range (12.7-52.0) × 10-6 gauss/oersted.

Twelve samples were also collected from four sites in the Arenig lavas in Stockenray Bay south of Girvan. These had a geometric mean intensity of $75 \cdot 0 \times 10^{-6}$ gauss (S.D. logs 0·11), susceptibility of 119×10^{-6} gauss/oersted (S.D. logs 0·18), and intensity/susceptibility ratio of 0·75

Table 2. THE MAGNETIZATION OF SAMPLES FROM STOCKENRAY BAY

	Dec.	Inc.	No. of samples	\boldsymbol{R}	Circular standard deviation	standard error
Horizontal plane Bedding plane	184° 202°	54° 32°	12 12	10·674 8·621	28° 46°	8° 13°
After 85 oersteds Horizontal plane Bedding plane	189° 202°	40° 21°	12 12	11·511 9·216	17° 42°	5° 12°

(S.D. logs 0·12) oersted. It was found that a.c. demagnetization reduced the scatter between samples from the same site and the scatter of the directions between sites, particularly when these directions were referred to the horizontal plane rather than the local bedding direction. This demonstrated that the magnetic age probably corresponds to the age of the folding of these rocks which occurred before the deposition of the Bala sediments which overstep the Arenig rocks of the Girvan⁴ region. This could therefore be an Ordovician direction.

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PHYSICS

Growth and Destruction of Ice Filaments in an Electric Field

Modification by electric fields of the growth of ice crystals has been observed under various conditions. Schaefer¹, who was the first to report rapid growth of ice in the form of whisker-like aggregates at high electrical gradients, observed that super-cooled water droplets were projected outwards from the whisker tips. Marshall and Gunn² found that the growth of ice crystals in relatively weak electric fields was initiated as opaque sprouts, which later grew many irregular branches of small cross-section and of no recognizable crystal habit. More recent observations³¹⁴, including studies of the growth of ice crystals in a diffusion cloud chamber, have yielded essentially similar results.

There are three chief factors which indicate the importance of studying the effect of electric fields on ice crystal growth from the vapour phase. First, electric

fields in thunderstorms are most intense, reaching 104 V/cm in the vicinity of the liquid and solid hydrometeors; these charge separations are usually considered to be produced by ice crystal collision and growth mechanisms within the active updraft regions of the cloud. Second, it might be expected not only that the growth of ice crystals would initiate charge separation, but also that this electric field might in turn modify the further development of the ice, thereby altering the charge separation at later stages in the lifetime of the storm. This sort of feed-back process has recently been suggested to account for the rapid, avalanche-like charge generation frequently observed in growing thunderclouds^{6,7}. Finally, as suggested by Bartlett et al.4, the habit variation of ice crystals under the influence of electric fields might lead to an understanding of the basic mechanism of ice crystal growth.

The initial tests were carried out in the absence of an electric field in order to evaluate the crystal growth rates to be expected. The apparatus consisted of a small cold cell, about 5 cm3, in which a water drop could be suspended and viewed from outside. Temperature control was provided by circulating a mixture of petrol-dry ice over two sides and the bottom of the cold cell. water vapour pressure within the cell was maintained at an ice saturation value corresponding to the cell floor temperature; this could be varied between 0° and -40° C and was in general within about 1° C of the working level some 2 cm higher up.

In each trial, a drop of distilled water, 1-2 mm in diameter, was suspended from a fine wire thermocouple and lowered slowly into the cell. This drop served as a source of water vapour, even after its temperature reached that of the surrounding air. It would begin to evaporate slowly, with the water vapour condensing out on the floor of the cell. Immediately after inserting the drop a small ice crystal, attached to a glass fibre, was brought to within a millimetre of the drop, and it commenced to grow rapidly in the vapour field surrounding the drop. This growth was viewed from outside the cell using a low power microscope, and was recorded by time-lapse photography.

The drop usually became nucleated and began to freeze if it was allowed to cool below -15° C; above that temperature it could be artificially nucleated by suspending silver iodide particles in the distilled water. Freezing produced an ice surface at 0° C for a short period of time and resulted in very rapid growth of the ice crystal, almost entirely along the drop-crystal axis. This growth slowed as the drop freezing proceeded and stopped entirely once the completely frozen drop cooled to the ambient Usually the thin filament-like ice air temperature. crystals had bridged the gap before this stage was reached. For a cell temperature of -15° C, the growth rates averaged about 0.1 µ/sec initially when the drop was unfrozen, rose rapidly to 0.8 u/sec as the drop became nucleated and warmed to 0° C, and then decreased, as shown in Fig. 1. As expected, the peak was less pronounced at warmer temperatures, but the difference was not great.

Rough calculations can be made to determine what growth rates to expect in these circumstances. The basic equation is

$$rac{\mathrm{d}m}{\mathrm{d}t} = 4\pi C D (\,
ho_e -\,
ho_e)$$

where ρ_{ϵ} is the environmental vapour density, ρ_{ϵ} is the vapour density at the ice crystal surface, D the diffusivity, and C a shape factor which assumes different values depending on the geometry of the crystal. This may be brought, by the use of the gas law, Clausius-Clapeyron and equivalent heat flow equations, to the form

$$\frac{\mathrm{d}m}{\mathrm{d}t} \approx 4\pi C \frac{\sigma}{f(T)}$$
...

where σ is the supersaturation of the environment relative of ice and f(T) is a function of temperature only. For the initial stage, where the unfrozen drop at -15° C provides the supersaturation, $\sigma/f(T) = 3 \times 10^{-8}$ g/cm sec. value will increase by a factor of ten once the source becomes a frozen drop at 0° C. For a long thin needle, where the length, a, is much greater than the diameter, C may be approximated to by a/3. Thus if the growth is assumed to take place entirely along the needle's length, the growth equation becomes

$$\frac{\mathrm{d}a}{\mathrm{d}t} \approx 4.4 \times 10^{-8} \frac{a}{r^2} \text{ cm/sec}$$
 $\approx 0.44 \text{ }\mu/\text{sec}$

with the diameter of the needles, r, taken as 0.1 mm and the length as about 1 mm.

This is considerably greater than the rate of growth observed with the drop unfrozen at -15° C. conceivably result from thickening of the ice filament as it grows. On the other hand, the observed ratio of 1:8 between growth with the drop unfrozen and that after nucleation is reasonably close to that expected from the equation.

Two parallel brass plates 2 cm apart were used to establish an electric field within the cold cell; the negative electrode was grounded. The drop and the ice crystal suspended from the fibre were introduced as before, midway between the electrodes.

With fields below 200 V/cm no change in the appearance or rate of growth of the ice crystal could be observed. Above this value the growth rate increased rapidly, to 25 μ/sec at 250 V/cm, and reached 150 μ/sec with fields of 1,500 V/cm and the crystals were more numerous, thinner and more fragile; typical examples are shown in Fig. 2.

Another significant feature was the alteration in appearance of these needles according to whether the ambient temperature within the chamber was above or below -12° C. In the former case, opaque sprouts were observed to grow rapidly as thin needles towards the supercooled drop. Under the colder conditions the long thin needles were replaced by even finer, many branched dendritic filaments. These fractured very readily and apparently spontaneously, to be succeeded quickly by a second dendrite, usually growing from the residual stub of its predecessor. In this manner as many as ten dendritic filaments could be observed to grow across the gap between drop and fibre and to shatter one after the other

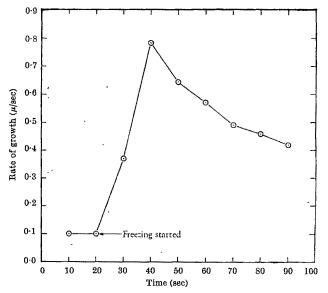


Fig. 1. Typical growth rate curve for the ice crystals in the absence of an electric field. Distance between the drop and crystal tip= 256μ at Distance between the drop and crystal tip=256 μ at T=0. Air temperature = -15° C.

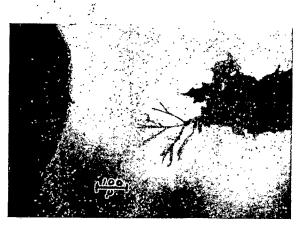




Fig. 2. Branched ice crystal filaments growing in an electric field of 250 V/cm at an ambient temperature of -15° C. The time interval between the two photographs is 10 sec.

during the course of a single drop freezing. This shattering did not occur so readily with the needles grown above -12° C. On the other hand, when such a needle was cooled below -12° C, and allowed to resume growth, it produced the fragile dendritic filaments. Changing the polarity of the electrodes did not in any way alter these observations, the growth being simply towards the vapour source. This does not agree with recent Russian experiments⁸ in which a polarity difference was observed.

A consideration of the electric field distortion around the tip of a thin ice needle or filament can give some indication of the force likely to be responsible for the rapid growth and ultimate shatter. For a long prolate spheroid, the electric field, E, at the tip can be given approximately as

$$Epprox rac{a^2}{b^{ar{2}}}rac{E'}{\lograc{2a}{b}-1}$$

where E' is the externally applied field and a and b are the major and minor semi-axes as before. This equation suggests that the electric field at the growing tip is proportional to $(a/b)^2$, as the term in the denominator varies comparatively slowly. Now, vapour in the vicinity of the tip will be rapidly accreted and will produce further local distortion of the field. In this way a cumulative effect is produced as the crystal grows, until the fragile ice filament shatters.

From the observation of the trajectories and masses of the ejected splinters some idea of the charge carried by them may be obtained. With a field strength of 1,500 V/cm, the largest splinters—about 40µ long—apparently carried average charges of 2×10-4 E.S.U. This value may be compared with that found by Mason and Maybank³ of 10⁻³ E.S.U. after the freezing and shattering of a supercooled water drop, with this total charge being

divided among the twenty to fifty ice crystals ejected by the violent disruption. Hence, the charge carried by each crystal appears to be four to ten times higher in our experiments than in those involving the shatter of a freezing drop.

The possible meteorological significance of the ice needle shattering process may be outlined as follows. As cloud droplets are carried aloft they will be in the presence of an electric field. If any of the theories concerning warm cloud electrification are valid—and a few observations of lightning from clouds entirely warmer than 0° C suggest that they are—these fields could be fairly intense. passing the freezing level, a few ice crystals will begin to appear, either through nucleation onto ice nuclei that were previously dry or, more likely, through the freezing and shatter of a few of the larger droplets. This will produce the combination of factors discussed heresupercooled droplets, ice crystals and an electric field. The ice should grow rapidly as we have observed and, through the action of the electric field, shattering should be common. This will produce increased numbers of ice crystals and, depending on the distribution of the positive and negative charges on the splinters, might further enhance the electric field. This process would tend to increase in frequency as a cascade effect and could produce the rapid glaciation of cloud tops observed by Koenig⁶.

One further point should be noted. The alteration in appearance of the ice needles near -12° C is expected from the changes in crystal habit with temperature described by Mason⁵. The very rapid increase of shattering which was observed when growth took place below this temperature suggests that cascade glaciation might be pronounced only after the cloud top reaches -12° C.

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THE SOLID STATE

Morphology of Poly-L-alanine **Spherulites**

Geil¹ and Keith and Padden² prepared small spherulites and hedrites of poly-L-alanine. We have now studied the morphology and structure of such spherulites of larger size and much greater perfection which were formed in essentially 100 per cent yield by casting poly-L-alanine (40 mg) from solution (4 ml.) of trifluoroacetic acid and trifluoroethanol (1:1 v/v). The solvent was allowed to evaporate very slowly (1 ml./12 h) at room temperature from a large watch glass.

The spherulites were subjected to X-ray diffraction, light-scattering, surface replica electron microscopy, infrared spectroscopy and microscopy under polarized light. Fig. 1 is a photomicrograph of two typical spherulites in white light between crossed polarizer and analyser. The

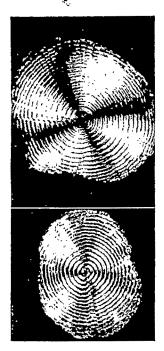


Fig. 1. Typical poly-L-alanine spherulites between crossed polarizer and analyser.

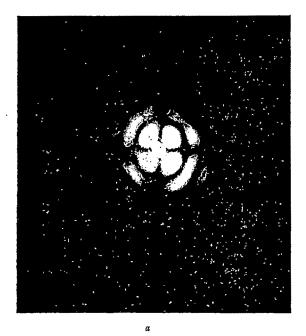
dark cross characteristic of spherulites is clearly visible. The ring and spiral structures are the result of periodic thickness variations, and can be made to disappear by microtoming the spherulite. The average spherulite thickness is about 8μ . The ring-to-ring distance ranges from 6.5 to 9.5 μ and is quite constant for each casting. Spherulite diameters vary from a few tenths of a millimetre to two millimetres. Mechanically, the spherulites are strong but brittle, and cannot be drawn or rolled. The rings apparent in Fig. 1 can, however, be separated quite easily, somewhat like the rings of an onion slice. This suggests that few, if any, polymer chains connect adjacent rings—this is supported by the observation of

cut and tilted spherulites in the polarizing microscope, using a $\lambda/4$ plate—regions of material with very little birefringence were observed to separate the rings. The spherulites are very stable, and have not degraded under ambient conditions during 6 months in this laboratory. A small negative birefringence is observed by Becke line inversion, with $n_r \cong 1.485$, $n_t \cong 1.490$.

The X-ray powder diffractometer pattern shows a very intense line at 7.00 Å, and several poorly defined peaks at 3.73, 4.26, 4.37 and 4.79 Å. These cannot be assigned unequivocally to the α - or β -form of the polypeptide3. A stack of whole spherulites, oriented similarly by pressing into a pellet, gives a normal powder pattern for all orientations relative to the X-ray beam. suggests that there is no preferred chain orientation in the whole spherulite. Only diffuse small-angle scattering is observed for this sample. Diffraction patterns of a group of similarly oriented spherulite segments, however. show a pseudo-fibre pattern. When the radius of the segment is along the equatorial direction of the film, the most intense pair of arcs (7 Å spacing) is also equatorial. If the main chain direction were radial, the arcs would correspond to the fibre repeat of the ordinary β -structure.

Infra-red spectra show the N-H deformation band at 1,525 cm⁻¹ (ref. 4) which is usually associated with the β -form of poly-L-alanine. The carbonyl absorption peak is too wide for assignment to either form.

Small-angle light-scattering from a single spherulite results in the patterns of Fig. 2, obtained with a Spectra-Physics 130 laser on Polaroid 57 film. The observed well defined four-lobe pattern is predicted theoretically by Stein et al. for two-dimensional spherulites with unique radial and tangential polarizability; the pattern therefore indicates that these structures have a high degree of perfection. The photographs of Fig. 2 were taken in the H_{ν} mode—that is, vertically polarized incident light and a horizontal analyser between sample and recording film. The additional arcs just beyond the four principal lobes are not predicted for small-angle scattering from a spherulitic structure. The concentric rings just visible at high angles in Fig. 2b have irregular intensity along the azimuth. Theory predicts maxima at azimuthal angles of 45° corresponding to the ring texture, if the latter is



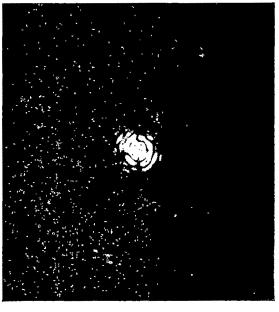
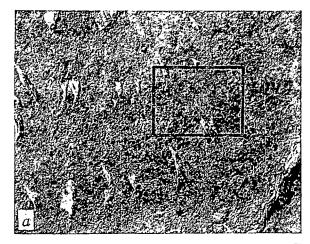


Fig. 2. a, H_v-mode light scattering from a poly-L-alanine spherulite immersed in liquid of refractive index n = 1.488. Distance from sample to plate is 47 cm; exposure time is 1/100 second (×4). b, Same conditions as a. Exposure time is 1/50 second. The weaker high-angle reflexions are now clearly visible (×1).



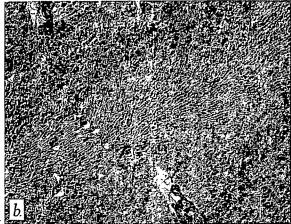


Fig. 3. a, Electron micrograph of surface replica of part of a poly-Lalanine spherulite. (×3,000.) b, Enlargement of the section indicated in a.

due to the periodic anisotropy fluctuation in the radial direction. Because the wide-angle rings in Fig. 2b are equivalent to spacings of $6.5-9.0\mu$, in agreement with the ring-to-ring distance in the micrographs (Fig. 1), it is logical to associate them with the same periodic anisotropy in the radial direction. The same wide angle rings were present in the V_v patterns. The reasons for the large number of lines in the scattering pattern and for some barely visible fine structure in the central lobes of the scattering pattern are not yet clear. Spherulite sizes measured from the radial extent of the lobes agree with those obtained microscopically.

Electron micrographs of carbon replicas show well defined tangential ridges corresponding to the ring structures of Fig. 1. Fig. 3a is a typical photograph; the width of one ring is defined by a series of fissures which arise from strains caused by loss of solvent. An enlarged section of one ring is shown in Fig. 3b.

On the basis of this evidence—primarily the small negative birefringence, separability of rings, X-ray diffraction from spherulite segments and electron micrographs—we suggest that the spherulite in Fig. 1 consists of tangential, twisted lamellar ribbons of irregularly folded poly-L-alanine chains. The polymer chains fold within the plane of the ribbon with their long axes normal to the ribbon axis. The data do not allow an unequivocal assignment of the poly-tr-alanine to the α - or β -configuration. The morphology of the interstitial material between rings also is not yet clear. Both these questions may be answered by electron diffraction experiments now in progress.

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Oxidation of Iron-Chromium Binary Alloys

RECENT results1 show that for the oxidation of ironchromium alloys containing 16-38 per cent chromium, in oxygen at atmospheric pressure, the process is controlled by diffusion during the first 2 h of exposure at temperatures between 650° and 950° C. The parabolic rate constants, derived from the linear relationship between the square of the gain in weight and time, showed the expected increase with increase in temperature, but there seemed to be an unexpected minimum in the rate, as a function of alloy composition, at about 20 per cent chromium, as shown in Fig. I. The same trend is shown by weight gain values after longer periods of oxidation and also by the results of another, less detailed study of iron-chromium oxidation2. There is also some evidence that the minimum shifts slightly from 20 per cent chromium to higher chromium contents at lower oxidation temperatures.

The tentative explanation put forward to explain the small increases in oxidation rates at the higher chromium contents was an extension of a suggestion by Mortimer and Menzies3. The scales on the alloys rich in chromium are thought to consist of nearly pure chromic oxide, while the films formed on the iron-chromium alloys, showing the slowest oxidation rates, are chromic oxide doped with small quantities of ferric oxide in solid solution. The pure chromic oxide has p-type conduction4,5 and predominantly cationic vacancies and diffusion6,7. Increasing substitution in the chromic oxide of iron, capable of existing to some extent as Fe2+, would progressively reduce the cation vacancy concentration and sup-

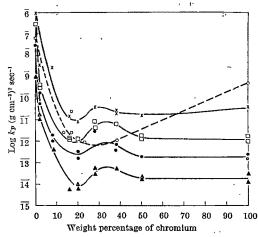


Fig. 1. 'Parabolic rate constants for oxidation of iron-chromium alloys. ×,950° C; ☐,850° C; ♠,750° C; ♠,650° C; ○,900° C (ref.2),

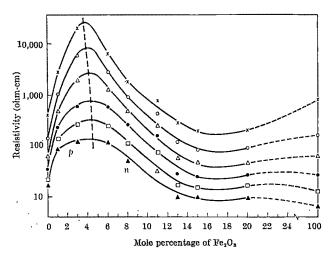


Fig. 2. Electrical resistivity of sintered Fe₂O₃-Cr₂O₃ compacts (1,500° C, 16 h). ×, 600° C; O, 700° C; △, 800° C; ●, 900° C; □, 1,000° C; ▲, 1,100° C.

press the p-type conductivity. At higher iron contents there might be an approach to the ferric oxide structure with anion vacancies, increasing anion diffusion, and n-type conduction, No direct evidence was available for this change in defect structure, however, nor for the composition at which it might occur. Such a process might have important effects on the stresses, formed in the oxide films and consequently on the long-term oxidation resistance of these alloys. We present here further evidence for this mechanism and indicate the chromic–ferric oxide composition at which the p to n transition occurs.

Samples of ferric-chromic oxide were prepared by cold pressing and sintering intimate mixtures of the 'Analar' Homogeneity was confirmed by X-ray grade oxides. diffraction and electron probe analysis and the density was found to be about 90 per cent of the theoretical value in every case. The d.c. electrical conductivities were measured by a two-point contact method over a range of temperatures similar to those used in the oxidation studies. For each composition, identical resistances were recorded at both increasing and decreasing temperature intervals. The results of a.c. measurements were within 5 per cent of the d.c. values. The absence of surface leakage and contact resistance effects was confirmed by three- and four-point d.c. experiments. Polarization effects were negligible, as would be expected from the very low ionic transport numbers¹³.

The experimental results, giving resistivity as a function of ferric oxide content, are plotted in Fig. 2 and show that maxima are obtained at all temperatures at about 3 mole per cent of ferric oxide. Fair agreement is obtained with the results of other workers on samples of pure chromic oxide^{4,14} and ferric oxide^{11,15}. The positions of the maxima shift slightly to higher ferric oxide contents at the higher temperatures. This indication that higher doping levels of ferric oxide are required to obtain the maximum resistance at higher temperatures is in qualitative agreement with the observation that higher iron levels conferred maximum oxidation resistance on the alloys at the higher temperatures. After the resistivity-composition curves of Fig. 2 had been obtained the signs of the thermoelectric powers of the samples were determined by establishing a temperature gradient along them. It was found that the semi-conductivity changed from p-type on the low ferric oxide side of the maximum to n-type on the high ferric oxide side exactly as suggested in the original paper¹.

The only remaining evidence required to confirm the mechanism put forward to account for the minimum

oxidation rate is a measurement of the iron concentrations in the oxide scales formed on the alloys. This is, of course, very difficult for the thin scales formed on the slowly oxidizing alloys (16 per cent chromium and above), and in any case there is some variation of composition with time of oxidation. Fig. 3 shows a plot of the iron contents of the scale forming in the first few hours of oxidation at 950° C as a function of the chromium content of the alloy. There is a remarkable correlation, the alloy with the maximum oxidation resistance forming the oxide with maximum high temperature electrical resistivity.

It is interesting to note that the electrical conductivities14 in the analogous chromic-aluminium oxide solid solution system¹⁶ show a trend similar to the ferricchromic oxide results. Despite some uncertainty^{17,18}, the anion vacancy is thought to be the predominant defect in aluminium oxide at temperatures below 1,200° C¹⁹⁻²¹, but because the defect concentration is less than that in ferric oxide it is understandable that 10 mole per cent of aluminium oxide is required to achieve the p-n transition compared with only 3 mole per cent in the case of ferric oxide (Fig. 4). The difficulty with measurements of electrical resistance, however, is that the ionic and electronic components are not easily separated and direct measurements of cation and anion diffusion rates as functions of oxide composition are desirable. In spite of this, providing that electron/hole mobilities do not vary greatly with composition, the density of the charge carriers, and hence the conductivity, is directly proportional to the ionic defect concentration. Consequently the change in the sign of the charge carriers and the minima in conductivity and oxidation rates do suggest that at this critical level of 3-4 mole per cent of ferric oxide the vacancy concentration is at a minimum and the oxide

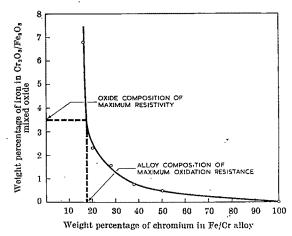
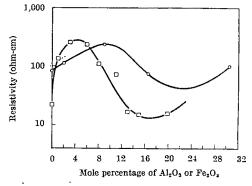


Fig. 3. Composition of oxide forming in 0-18 h oxidation at 950°C.



. Fig. 4.. Effect of Al₂O₂ (O, ref. 14) and Fe₂O₃ ([], this work) additions on resistivity at 1,000° C of Cr₂O₃.

composition approaches perfect stoichiometry. The relatively small associated decrease in oxidation rate of alloys forming oxide of this composition may indicate that vacancies of both types are present throughout the whole composition range and that a small excess of one kind is replaced by a small excess of the other.

A surprising feature of these results is that the transition from p to n occurs at such low ferric oxide contents. Attempts to calculate this critical composition, from experimentally measured defect concentrations 5,11 or stoichiometries, gave values from 0.6 to 20 mole per cent depending on the values selected and their interpretation.

The measurements of high temperature conductivity and thermoelectric e.m.f.s reported here provide a satisfactory explanation of the minima observed in the oxidation rate versus alloy chromium content curves. This is the first direct experimental evidence that oxide scales with anion defects give way to scales with cation defects as the chromium content of the alloy is increased above 20 per cent. While at the critical composition of 3 mole per cent ferric oxide the two types of defect may balance, the greater mobility of the cation vacancies may result in the flux of this species predominating to higher iron con-This proposed change in ionic transport mechanism will have important consequences on the integrity and adhesion of the oxide scales formed. Our preliminary measurements have indicated lower compressive stresses in oxides grown on pure chromium and on a 50 per cent chromium/50 per cent iron alloy than in the oxide forming on the 28 per cent chromium/iron alloy, in broad agreement with recent qualitative observations by Caplan and Cohen¹². These measurements have already been used by Noden et al.22 to explain their observations of the growth of fuel element cans during oxidation in carbon dioxide and oxygen. The predominating ionic transport mechanism will probably vary as the oxidation proceeds, for it is known that the oxide composition changes during growth.

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MOLECULAR STRUCTURE

Distribution of an Arginine-Lysine Interchange in the Invariable Half of Human L-Type Bence-Jones Proteins

SEROLOGICAL reagents have been used to identify allelic antigenic determinants of the peptide chains of immunoglobulins1. Several allotypic determinants of the heavy chains of human immunoglobulins have been identified2 and their distribution in human populations3 has been investigated. Only a few allotypic determinants of the K-type light chain of human immunoglobulins have been identified2 and one of these has been correlated with an amino-acid interchange in the invariable half of these peptide chains4-6. No homologous serological reagent has been available for the study of the L-type light chain.

Ein and Fahey' described a heterologous antiserum, obtained from the rabbit, which is valuable for identifying two sub-types of L-type human light chains. These subtype determinants, designated Oz(+) and Oz(-) respectively, were found to be present in Bence-Jones proteins and in all types of myeloma proteins tested, as well as in pooled immunoglobulins7. Appella and Ein8 have correlated the Oz antigenic type of 12 L-type Bence-Jones proteins with an amino-acid interchange in the invariable COOH-terminal sequence of these proteins. proteins were shown to have lysine, whereas Oz(-) proteins had arginine at position 190 (ref. 8).

We have studied the distribution of the arginine or lysine residue at position 190 in several L-type Bence-Jones proteins. The relative frequency of this aminoacid interchange presumably reflects the relative frequency of the corresponding allotypic determinants. present investigation has thus provided some information on the relative gene frequency of these determinants, which, although direct genetic analysis is still lacking, may be thought to be allelic.

Forty Bence-Jones proteins of type L have been examined for the presence of arginine or lysine at position 190. The proteins were obtained from the sources described before¹⁰. The proteins were isolated, purified, aminoethylated and digested with trypsin, also as previously described10. The analytical methods used before to obtain peptide maps of Bence-Jones proteins10 failed to separate clearly the tripeptides Ser-His-Lys and Ser-His-Arg which are obtained by tryptic digestion from the two sub-types of L-type Bence-Jones proteins, respectively, and which correspond to residues 188-190 (ref. 11). Furthermore, free arginine, which is sometimes present in the tryptic digest of Bence-Jones proteins, overlaps in part with these tripeptides.

The tryptic digest of Bence-Jones proteins was analysed by high voltage ionophoresis at pH 4.7 (ref. 12) and the ionograms were stained with ninhydrin and with reagents specific for arginine and histidine¹⁰. There was clear separation of a peptide positive for histidine and arginine from a peptide positive for histidine only (Fig. 1). All the Bence-Jones proteins analysed contained either peptide. The peptides were eluted from the ionograms, hydrolysed and their amino-acid composition was analysed¹¹. Ser-His-Arg and Ser-His-Lys, respectively,

Of the forty proteins analysed thirty-five had arginine in position 190 and five had lysine. The relative frequency of the allotypic determinants corresponding to these amino-acid residues is thus presumably close to 87.5 per cent and 12.5 per cent, respectively.

Appella and Ein⁸ have reported that of twelve proteins examined seven had lysine and five had arginine at position 190. The relative frequency of these amino-acid residues corresponded well with the relative frequency of Oz(+) and Oz(-) proteins in a sample of 22 L-type proteins7; twelve of these (55 per cent) were found to be

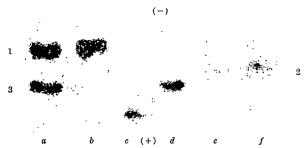


Fig. 1. Ionophoresis at pH+7 (ref. 12) of the tryptic digests of Bence-Jones proteins. Only that area of the ionophoresis where the most positively charged peptides and amino-acids run is shown. a and b, Digests of Bence-Jones proteins 1 and 69; e and f, digests of Bence-Jones proteins 87 and 134; e, 0-02 μ moles of arginine; and d, 0-02 μ moles of histidine. The lonophoresis was run at 5 kV for 1 h and 40 min on a 1 m strip of paper. The paper was stained with ninhydrin, and the peptides were eluted and analysed. 1 contains Ser-His-Lys; 2 contains Ser-His-Arg; 3 is a peptide which contains neither histidine nor arginine.

Oz(+), while 10 (45 per cent) were Oz(-). The relative frequency of the two sub-types of L-type chains, however, was different in a sample of twenty-two myeloma proteins. Of these eight (36 per cent) were Oz(+) and fourteen (64 per cent) were Oz(-).

The relative frequency of arginine and lysine at position 190 in the large sample of Bence-Jones proteins which we examined is quite different from that found by Appella and Eins. A possible explanation for this discrepancy is that the small sample of Bence-Jones proteins examined by Appella and Ein is not representative, although it seems unlikely that such a difference in the relative frequency of the two sub-types of Bence-Jones proteins can be explained by a sampling bias. Another explanation is that the Bence-Jones proteins were obtained from human populations differing in the relative frequency of the two sub-types. The Bence-Jones proteins analysed by us came from many countries 10 and it is impossible to establish any correlation with a given human population.

The relative frequency of valine/leucine at position 191 in Bence-Jones proteins of type K parallels the gene frequency Inv(a-)/Inv(a+) (ref. 5). The frequency of arginine/lysine at position 190 of Bence-Jones proteins of type L is similar to that of valine/leucine of K-type proteins, and so we might expect that the relative frequency of the corresponding genes is also similar to that of the Inv(a) genes.

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CHEMISTRY

Effect of an Applied Electrical Field on the Gamma-radiolysis of Gaseous Hydrogen Chloride

Previous publications¹⁻³ on the gamma-radiolysis of hydrogen chloride gas provide evidence for the presence of two hydrogen forming species. One of these is termed

a "hot" hydrogen atom and may be due to the excitation process

$$HCl \longrightarrow HCl^* \longrightarrow H^* + Cl$$
 (1)

where * denotes an excited species. The other, referred to as a "thermal" hydrogen atom, may or may not have a The initial ionizing thermalized electron as its precursor. action should produce both excited HCl molecules (reaction (1)) and HCl+ ions along with electrons

$$HCl \longrightarrow HCl^+ + e$$
 (2)

In the hope of interpreting the subsequent reactions of the ions produced in reaction (2), a study of the effect of an applied electrical field during radiolysis was carried out. A 'Pyrex' cylindrical glass cell which had its parallel end faces coated with colloidal graphite was used. Before the cell was filled with hydrogen chloride, it was baked out overnight at 150° C and evacuated for several hours to a pressure of 10-6 cm mercury. The faces were connected through the glass by platinum leads and served as the electrodes. The applied potential across the electrodes was supplied by a Hamner model N-413 high voltage power supply. A Keithley 410C micro-microammeter was used to measure the current. Both these instruments were connected to the irradiation cell by coaxial cables. Pressures of hydrogen chloride gas ranged from 150 to 700 torr and a field gradient from 0 to 6 V/cm/torr was applied. The samples were irradiated with a 1,200 c. cobalt-60 source.

Fig. I shows plots of ion pair yield of hydrogen production against applied field, and ionization current against applied field. It can be readily seen from these plots that the applied electrical field has no effect on the production of hydrogen either in the ion recombination region or in the saturation region for the range of the applied field investigated. This can be interpreted to mean that ion recombination reactions play an unimportant part in the formation of hydrogen4.

At this point the possible reactions of the electrons produced in reaction (2) will be considered. electrons should rapidly become thermalized and disappear by any of the following processes: ambipolar diffusion to the walls of the vessel; ion-electron recombination; and attachment to neutral molecules to form negative ions.

The first order decay constant for electron-ion loss by ambipolar diffusion is given by D/Δ^2 where D is the ambipolar diffusion coefficient and Δ is a length characteristic of the vessel geometry. A typical value of D $(0.09 \text{ cm}^2/\text{sec} \text{ at 1 atm.})$ (ref. 5) and $\Delta(\sim 1 \text{ cm})$ for the system used leads to $D/\Delta^2 = 70/P$ torr per sec This gives a diffusional loss time of at least I sec for pressures greater than 70 torr (1018 molecules/cc).

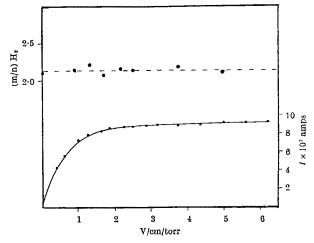


Fig. 1. Plots of ion pair yield of hydrogen production against applied field (dashed line) and ionization current against applied field (continuous line).

In these experiments, the rate of ion formation was about 10^{11} ions/cc/sec. If an ion electron recombination coefficient of 10-6 cc/sec is assumed6, then the calculated lifetime of the electron in this process is of the order of 10-3 sec.

The electron, on its way to becoming thermalized, can form Cl- ions by the ion pair formation process

$$e + HCl \longrightarrow H^+ + Cl^- + e$$
 (3)

or else may undergo the dissociative electron capture reaction

$$e + HCl \longrightarrow H + Cl^{-}$$
 (4)

The energy thresholds for these two processes are 14.5 and 0.6 eV (ref. 7), respectively. It has been shown that only about 5 per cent of the electrons will react by process (4) and that the capture cross-section for reaction (4) is at least one order of magnitude greater than that for the reaction (3) (ref. 3). Davidows has obtained evidence that the electrons disappear in a trimolecular reaction, the rate constant of which is in the range 2×10^{-32} cm⁶/ molecule²/sec. This gives a lifetime for the thermalized electron of the order of 10⁻⁸ sec at 600 torr pressure of hydrogen chloride. The three body processes, which could be depicted thus

should therefore be the principal process for electron dis-

Now the HCl+ ion can undergo charge neutralization by reacting either with an electron or the Cl- ion or else take part in the ion molecule reaction

$$HCl + HCl^+ \longrightarrow H_2Cl^+ + Cl$$
 (6)

This reaction has a specific rate constant of about 4×10^{-10} ce/molecule/sec (ref. 9). A lifetime of about 10^{-9} sec for the ion when the pressure of hydrogen chloride is about 700 torr (1019 molecules/cc) can be calculated. This is much faster than the ion recombination reaction and therefore the fate of the HCl+ ion would be chiefly to form the H₂Cl⁺ ion. This latter ion would be the cation collected on application of an electrical field.

The recent work of Kebarle et al.10 on ammonia and water has shown that the $\mathrm{NH_{4}^{+}}$ and $\mathrm{H_{3}O^{+}}$ ions are heavily clustered at atmospheric pressures. In view of this, clustering of the H2Cl+ and Cl- ions would be expected to occur, followed by a non-dissociative recombination of these clustered ions thus

$$H_2Cl^+_{(nHCl)} + Cl^-_{(mHCl)} \longrightarrow (n + m + 2)HCl$$
 (7)

This reaction is in agreement with previous evidence³ and is supported by the fact that there is no change in the ionic yield in the ion recombination region (Fig. 1) during the application of an electric field. Another explanation of the applied electric field results could be that reaction (7) produces hydrogen and that in the presence of the electric field the H₂Cl+ ions form H or H₂ on neutralization at the carbon electrodes. At present very little is known about reactions occurring at the electrodes in the gas phase and until more is known the first explanation of the results seems more acceptable.

At a field strength of 10 V/cm/torr, the effective electron energy in gaseous HCl is estimated at 0.14 eV (ref. 11). This energy is much too low to cause dissociation of the HCl molecule by either of the two processes below

$$e + HCl \longrightarrow H^+ + Cl^- + e$$
 (8)

$$e + HCl \longrightarrow HCl^* + e \longrightarrow H^* + Cl + e$$
 (9)

The first requires an energy of at least 14.5 eV and the second at least 4.5 eV. This means that a reduction of hydrogen yield caused by neutralization of ions at the electrodes cannot be compensated for by the processes described here.

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BIOLOGY

Technique for rearing Marine Calanoid Copepods in Laboratory Conditions

Allen and Nelson¹, who succeeded in keeping several Calanus alive for a few weeks in laboratory conditions, stated that it should be possible to rear Calanus in the laboratory without difficulty. Since then it has become apparent that the marine calanoid copepods are hard to rear and breed in laboratory conditions, and Crawshay² has discussed some of the early difficulties. A. G. Nicholls (unpublished results) reared three adult female Calanus from eggs3, and Conover4 reared two adult female Calanus with considerable mortality. It is only recently, however, that marine calanoid copepods—Acartia tonsa Dana⁵ and Acartia clausi Giesbrecht⁶—have been bred through the first filial generation and beyond.

A simple laboratory technique for rearing calanoid copepods under laboratory conditions has been used in present investigations on the rate of development of copepods. Adults of Temora longicornis (O. F. Müller) and Pseudocalanus minutus (Krøyer) have been successfully reared from egg to adult for the first time, and Pseudocalanus stage 1 nauplii have been reared to copepodid 1 at about 0°, 3°, 7° and 12° C.

Adult female copepods were selected from plankton tows and placed in 30 ml. Petri dishes kept at 7° Recently hatched nauplii were transferred to 20 ml. vials which were filled with one-third fresh sea water and onethird Erdschreiber medium containing a unialgal culture of the marine crysomonad Isochrysis galbana Parke, which gave a final cell concentration of 300,000–600,000 cells/ml. The vials were placed in a water bath at $12^{\circ} \pm 0.01^{\circ}$ C and kept under continuous weak illumination. The vials were checked regularly and fresh algal medium was added periodically to maintain a dense concentration. The water in the vials was not changed throughout the duration of the experiment. Eleven adults of the two species have been reared with a mortality of between 50 and 70 per cent, most of the deaths occurring in the naupliar stages. In one experiment, six of the fourteen nauplii which died did so on the surface film of water, which suggests that mortality could be further decreased by increasing the volume to surface ratio in experimental cultures. A male and female of Temora were reared in one experiment; these adults were fertile, for they produced first filial generation nauplii. It has not been possible to determine whether the *Pseudocalanus* were capable of producing a first filial generation because only females have so far been reared. It was observed, however, that these females laid infertile eggs.

Previous work showed that although the adults of Pseudocalanus elongatus Boeck and Temora longicornis

could be kept successfully in the laboratory fed on a culture of Isochrysis galbana, the larval stages could not be so kept because successful moults could not be obtained. I have since found that the flagellate Isochrysis galbana is a suitable food for the first generation larval stages in these species. The temperature and illumination are not critical factors, for early larval stages of Pseudocalanus have been reared at temperatures from 0° to 12° C, and a few adults have been reared in the dark.

The success of this laboratory technique appears to result from one or more of the following conditions: the use of fresh sea water taken from the same area of the sea as the adults; the use and maintenance of a high concentration of Isochrysis galbana as food; and the use of a simple technique with minimal disturbance of the copepods by not changing the sea water in experiments. C. J. CORKETT

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Oxygen Consumption of Ectoprocts

Mangum and Schopf¹ have reported oxygen consumption rates for a representative species of ectoproct, Bugula turrita. They found considerable discrepancy between their measured value and that predicted from a model they proposed based on ectoproct morphology. discrepancy led them to hypothesize that the rhythmic expansion and contraction of the tentacles of the animals was required as a circulatory mechanism to supply oxygen to the tissues.

Because of the small size of Bugula turrita, however, a hemi-cylinder 0.16 mm in diameter and 0.6 mm long, it would be expected that sufficient oxygen could be obtained from the environment by a diffusion process alone. Here we treat the model proposed by Mangum and Schopf by

standard mathematical techniques as a diffusion system containing a chemical reaction, and show that the measured oxygen consumption rate can be completely accounted for by diffusion alone; no assumption of internal circulatory mechanisms is required. The model is shown in Fig. 1, a, b, c and d. The ectoprocts are divided into three parts for analysis. The body or zoecium has a flat base, composed of chitin reinforced with calcium carbonate, which is impermeable to oxygen. On this base three annular layers of epidermis, coelomic fluid and tissue, surround the core of coelomic fluid. Three concentric annuli surrounding a coelomic core are also proposed for the tentacle sheath. The fourteen tentacles are composed of a layer of epidermis cells surrounding a coelomic core.

In the present analysis it is assumed that the oxygen consumption takes place entirely in the tissue layers (see Fig. 1, b, c and d). No oxygen consumption takes place in the coelomic regions (clear regions in Fig. 1, b, c and d), which are primarily sea water. The whole animal oxygen consumption rate 4.5 × 10-4 µl./animal h, reported in ref. 1, was recalculated to be 4.87×10^{-7} g/cm³ soft tissue sec by assuming that all oxygen consumption was by soft tissue only. This oxygen consumption rate was then apportioned to the various soft tissue parts of the animal on the basis of the fractional volume of each part.

In cylindrical co-ordinates, the general steady-state diffusion equation can be written

$$D\left[\frac{\mathrm{d}^2 c}{\mathrm{d} r^2} + \frac{1}{r} \frac{\mathrm{d}c}{\mathrm{d}r}\right] - Q = 0 \tag{1}$$

where D is the diffusivity of oxygen in cm²/sec; c is the concentration of oxygen in g/cm^3 ; r is the distance from the axis of the cylinder in cm; and Q is the oxygen consumption rate in g/cm³ sec.

The oxygen concentration is more conveniently expressed as an oxygen tension through the Henry's law equation

$$c = kp \tag{2}$$

c = kp (2) where k is the Henry's law constant in g/cm³ mm mercury, and p is the oxygen tension in mm mercury. Substitution of equation (2) into equation (1) gives

$$\frac{\mathrm{d}^2 p}{\mathrm{d} r^2} + \frac{1}{r} \frac{\mathrm{d} p}{\mathrm{d} r} - \frac{Q}{D k} = 0 \tag{3}$$

Diffusion in the axial direction is neglected. If axial diffusion were to be included it would increase the delivery of oxygen by diffusion, thereby strengthening the argument for diffusion as the sole transport process

To obtain the general solution of equation (3) we let R=r/a, where a= maximum radius of a particular part of the ectoproct, and let $P=p-p_0$, where p_0 is the oxygon tension in sea water. Using standard methods of solving equidimensional linear differential equations2 the general form of the solution is

$$P = A \log R + B + \frac{Qa^2}{4Dk} R^2 \tag{4}$$

Equation (4) is applicable to each of the layers in the three parts. To evaluate the unknown constants A and B, for each particular region, the following boundary conditions are imposed. (i) At the outer surface of the ectoproct the oxygen tension is assumed equal to the equilibrium concentration of oxygen in sea water; that is,

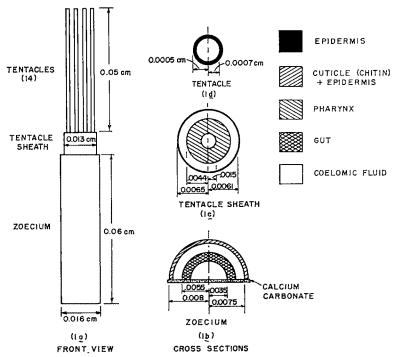


Fig. 1. Diagrammatic representation of morphology of Bugula turrita.

	Table 1	
Parameter	Value	Ref.
Diffusivity of oxygen through water	$1.8 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$	3
Diffusivity of oxygen through tissue	0.5×10^{-5} cm ² sec ⁻¹	4
Diffusivity of oxygen through chitin and epidermis	$3.0 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$	4
Henry's law constant	4.7×10^{-8} g/cc mm mercury	5
Equilibrium concentration of oxygen in sea water	7.4×10^{-6} g/cc (155 mm mercury oxygen pressure)	5
Oxygen consumption rate in soft tissue	4.87×10^{-7} g/cm ³ tissue sec	1

R=1, P=0. This boundary condition implies that the resistance to oxygen transfer in the sea water is small enough to be neglected. In view of the relatively high resistance in the outer layer of the animal, and since ectoprocts are usually found in well-aerated regions near the surface of the water, this appears to be a reasonable assumption. (ii) At steady state, the flux of material into the interior core of sea water is zero (for all three parts); that is, at the boundary, (dP/dR) = 0. This condition is easily visualized by noting that because there is no consumption in the interior core of sea water the core will, at the steady-state, take on a uniform oxygen tension. Under this condition there is no oxygen tension gradient in the core, including at the core-interior wall boundary. (iii) The remaining boundary conditions are provided by continuity requirements at the interfaces between the various regions. The mass flux and the tension must be equal on both sides of all interfaces. For example, the tension and mass flux at the outer surface of the zoecium gut tissue must be equal to the flux and tension at the inner surface of the coelomic annulus.

Using these boundary conditions the values of A and Bin equation (4) can be obtained for the different regions (remembering that Q=0 for all coelomic sections).

The concentration profiles for the zoecium and tentacle sheath obtained from these solutions are shown in Figs. 2 and 3, using the values of D, Q and p_0 shown in Table 1.

It can be seen in the figures that the minimum oxygen tension never drops below 93 per cent of the bulk value. This value is well above the minimum required for the health of the animal. The profile for the tentacles never drops below 99.9 per cent of bulk value, so it is not shown.

There are two principal differences between our approach and that of Mangum and Schopf: (i) they did not take the individual regions into account but instead averaged the physical properties and consumption rates over each part of the animal, thereby eliminating any meaningful concentration profiles; (ii) the relations developed by

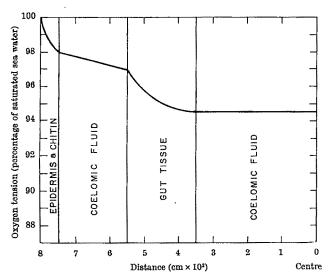


Fig. 2. Oxygen tension profile through the zoecium.

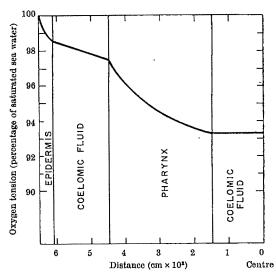


Fig. 3. Oxygen tension profile through the tentacle sheath.

Gerard', which they used for their calculations, result from incorrect applications of a boundary condition.

These differences in approach lead to the difference between their conclusion and ours. We conclude that the ectoproct is completely supplied with oxygen by a diffusion process.

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Effect of 5Bs in suppressing the Expression of Altered Dosage of 5B^L on Meiotic Chromosome Pairing in Triticum destivum

CHROMOSOME 5B of the allohexaploid wheat, Triticum aestivum (2n=42), carries out a genetic activity that results in the suppression of synapsis at meiosis between homoeologous chromosomes of different genomes1,2. Synapsis, however, still occurs between fully homologous partner chromosomes despite the 5B activity, which therefore narrows the specificity of meiotic chromosome pairing.

Chromosome 5B is markedly heterobrachial and the suppression of homoeologous pairing depends on an activity of the long arm $(5B^L)$ alone². In the presence of the short arm $(5B^{8})$, but in the absence of the long arms, homoeologous synapsis occurs as it does in the absence of the complete chromosome. By contrast there is no homoeologous synapsis if $5B^L$ is present but $5B^S$ absent³.

Initially it was thought that the activity could only be recognized when genotypes were compared in which chromosome 5B was either present or absent. Homoeologous pairing was, for example, observed to occur in 40-chromosome 5B nullisomics but not in 41-chromosome 5B monosomics or in 42-chromosome euploids. Increases in dosage of the chromosome above the monosomic level apparently did not give rise to any change in the nature or extent of synapsis once homoeologous synapsis was

suppressed.

Feldman, however, demonstrated that increased dosage of $5B^L$ —in the absence of $5B^S$ —in plants with two and three $5B^L$ isochromosomes, greatly reduced the frequencies of chiasmata formed between homologues, and also caused other abnormalities of synapsis4. This was attributed to the expression in increased dosages of the gene on $5B^L$ responsible for the prevention of homoeologous synapsis in monosomics and euploids. Riley et al. confirmed the lower chiasma frequencies of plants disomic for the $5B^L$ isochromosome and lacking $5B^{\frac{1}{2}}$, but directed attention to the contrast between these plants and those tetrasomic for the complete chromosome 5B in which there was normal synapsis. The chief distinction between these genotypes was in the presence or absence of $5B^{\circ}$; one possible explanation for its cause was that $5B^{\circ}$ has a genetic activity that influences synapsis in the reverse direction from that of $5B^{L}$. The work described here was intended to test this possibility.

All the observations were carried out on derivatives of the variety 'Chinese Spring' of T. aestivum. The forms used had a range of doses of $5B^{L}$ in the presence and in the absence of $5B^{S}$. They were: (a) 42- and 44-chromosome plants with the complete chromosome 5B in the disomic (euploid) or tetrasomic (tetra 5B) conditions, respectively; (b) 41- and 42-chromosome plants, respectively mono- or disomic for the $5B^{L}$ isochromosome and lacking $5B^{S}$ (mono- and di-iso $5B^{L}$); (c) 42-, 43- and 44-chromosome plants respectively, di-, tri- and tetrasomic for the $5B^{L}$ telocentric (di-, tri- and tetra-telo $5B^{L}$). Tri- and tetra-telo $5B^{L}$ forms were extracted by Selection from the standard di-telo $5B^{L}$ line; (d) 43-chromosome plants simultaneously disomic for complete chromosome 5B and mono-iso $5B^{L}$.

Plants of all these genotypes were grown in controlled environment rooms at 15° C and at 20° C under continuous light. Preparations were made of anthers with pollen mother cells at first metaphase of meiosis, and the numbers of chiasmata were scored in twenty cells of every plant in the experiment. The means of these scores are recorded in Table 1, expressed as chiasmata/chromosome. This form of presentation is used to avoid the effects of differences of chromosome number between some genotypes. In arriving at the means each isochromosome was regarded as two chromosomes because it was capable of forming chiasmata interbrachially with itself and where such chiasmata occurred they were included in the total for the cell.

From these observations it is clear that increases in the dosage of $5B^L$, over the range from two to four, lead to marked reductions in chiasma frequencies irrespective of whether $5B^{\nu}$ is represented by telocentrics or by isochromosomes. By contrast, there is little difference between the chiasma frequencies of plants with two or four doses of $5B^{\nu}$ when 5B is also present, for example,

Table 1. Mean chiasmata/ohromosome at 15° c and 20° c in plants with various doses of the long arm $(5B^s)$ and the short arm $(5B^s)$ of chromosome 5B of T. activum (twenty cells per plant)

a ,	5B de		Chiasmata/chromosome		
Genotype	Long arm	Short arm	15° C	20° C	
Di-telo 5B ^L	2	0	0.967 ± 0.012	0.907 ± 0.016	
Mono-iso 5BL	2	0	0.875 ± 0.012	0.988 ± 0.011	
Tri-telo 5BL	3	0	0.823 ± 0.013	0.837 ± 0.013	
Tetra-telo 5B ^L	4	0	0.415 ± 0.031	0.534 ± 0.038	
Di-iso 5B ^L	4	0	0.413 ± 0.035	0.625 ± 0.021	
Euploid Disome complete 5B	2	2	$1 \cdot 137 \pm 0 \cdot 018$	1·101 ± 0·009	
+ mono-iso 5BL	4	2	1.025 ± 0.009	0.987 ± 0.014	
Tetra 5B	4	4	1.051 ± 0.009	0.974 ± 0.009	

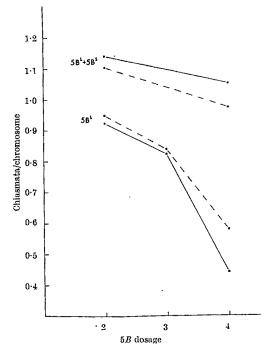


Fig. 1. The mean chiasmata/chromosome at each dose level of the entire chromosome 5B ($5B^L + 5B^S$) and of the long arm alone ($5B^L$) at 15° C (——) and 20° C (———).

when euploid and tetra 5B genotypes are compared. In addition, plants disomic for the complete chromosome and simultaneously mono-iso $5B^L$, that is, with four $5B^L$ and two $5B^S$, had frequencies like those of tetra 5B plants at the same temperature.

These data, which are summarized in Fig. 1, confirm that $5B^s$ carries out a genetic activity that influences meiotic chromosome pairing and that its effect on pairing is the reverse of that of $5B^t$. This activity of $5B^s$ therefore increases the extent of synapsis, and is capable of suppressing the expression of increased dosage of $5B^t$ because only in its absence is chiasma frequency modified by changed dosage of $5B^t$. There is also a suggestion that a $5B^s$ activity modifies the response of chiasma frequency to temperature differences. The lower frequencies occurred at the lower temperature in the absence, but at the higher temperature in the presence, of $5B^s$.

On the assumption that the $5B^{L}$ dose effect on chiasma frequency is a manifestation of modified synapsis and that it results from the operation of the same system by which homoeologous synapsis is also inhibited, the present results are consistent with the causal hypothesis proposed by Feldman4. This asserts that the 5BL activity affects the presynaptic spatial distribution, and relative orientation, of homologous and homoeologous chromosomes. The presence of $5B^L$ in a single dose leads to the greater separation of homoeologues than homologues and so to the prevention of homoeologous synapsis. increases in the dosage of $5B^{t}$ lead to progressively greater separation even between homologues and so to overall reduction on the amount of synapsis. If these ideas about $5B^L$ effects are correct, then it must be proposed $5B^s$ also influences the relative positions and orientations of chromosomes.

As with other recent work, these interpretations of the present results on a spatial model demand that the nucleus is regarded as having a quite precise internal organization in which the relative positions of chromosomes and of unlinked chromosomal segments are specifically ordered. From the 5B system of wheat it appears that there may be gene products which determine the spatial relationships of chromosomes.

Finally, it is worth while considering the practical implications of the recognition of the $5B^s$ activity. Knowledge of the effect of $5B^{L}$ on synapsis has been used in practical breeding work aimed at producing improved genotypes in the wheat crop by homoeologous recombination. The present observations suggest that the maximum amount of homoeologous pairing and recombination will occur when the $5B^L$ activity is removed or suppressed but the $5B^3$ activity is unimpaired.

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CYTOLOGY

Transformation of Polyploid Hamster Cells by Polyoma Virus

THERE is a degree of genetic heterogeneity for susceptibility to transformation by polyoma virus in the hamster cell line BHK21-13 (refs. 1 and 2) and we have shown³ variations in susceptibility to transformation related to the position of the cell in its life cycle. Our results are compatible with the hypothesis that increased susceptibility is related to the doubling of the cell's genome before division, and so a difference in ploidy could account for the genetic heterogeneity as well. In fact, chromosome preparations of BHK21 cells, although predominantly diploid (forty-four chromosomes), show a minor proportion of metaphases in the tetraploid range (up to 10 per cent), and pure lines with a higher proportion (up to 100 per cent) of such "tetraploid" cells can be isolated. (The terms "diploid" and "tetraploid" will be used here to define cell lines showing frequency distributions of chromosome numbers with a mode = 44 ± 2 or 88 ± 4 , respectively.)

We have described elsewhere³ the general culture methods and techniques related to the assay of polyoma virus infection and transformation. The transformation frequency was calculated as the percentage of infected cells that would develop into macroscopic colonies in a soft agar medium4. No correction was made for colonies that would develop from uninfected controls, because all sub-lines in which background colony growth would exceed 2-3 per cent of infected were discarded.

Clonal isolation of individual sub-lines was carried out according to the method of Puck et al.5. Chromosome preparations were made according to the technique of Moorhead et al.8 with minor modifications. Chromosome counts were performed on a random sample of 50-100 metaphase plates/cell line.

Three lines yielding high percentages of viable polyploid cells were isolated after repeated cloning, and will be referred to as 2P3, 2P4 and P108. From each of these lines, a number of almost pure "diploid" and "tetraploid" clones was selected. Infection with polyoma virus showed all these clones to be transformable. Transformation tests were carried out as soon as each clone had developed into a sizable cell population, to keep any acquired variability among sister clones to a minimum.

Table 1 summarizes the data from four independent cell lineages. Transformation tests for each individual sub-line belonging to the same family were carried out in

Table 1. Transformation frequency of "diploid" and "tetraploid" BHK21 sub-lines

Exp. No.	Sub-line	cells i	quenc n diff ly cla	ferent isses	efficiency	Transformation † frequency (b) (per cent)	Transforma- tion frequency (per cent) corrected $(b/a \times 100)$
	BH K21	46	4	- 119	27.9	6·5	23.3
1	2P4-1	84	16		15.1	3.7	24·5
•	$\tilde{2P4}$ $-\hat{4}$	0.	97	3	$\hat{1}\hat{1}\cdot\hat{9}$	3.7	31.1
	BHK21	48	2		35-8	7.8	21.8
	2P3-3	89	11		22.8	4.5	19.8
	2P3-5	90	10		22.6	4.8	21.2
2	2 <i>P3</i> -18	86	14		36.0	6.5	18-1
	2P3-4		96	4	8.6	1.7	19.7
	2P3-11		100		15.6	1.3	7.7
	2P3-13		96	4	8.2	0.66	8.0
	2P3-15	2	98		19.2	$2 \cdot 1$	10.8
	BHK21	46	4		33.8	7.4	21.9
	P108-2	96	4 4 1		26.7	1.6	5.9
	P108-8	99	1		25.2	1.5	5.8
3	P108-1		92	8 3	$32 \cdot 1$	2.4	$7 \cdot 4$
	P108-6	2	95		$32 \cdot 1$	1.7	5.2
	P108-13	_	89	11	38-8	2-4	6-2
	P108-17	1	96	3	$24 \cdot 3$	$1 \cdot 2$	4.9
	.B1	98	8		31.2	2.5	8.0
4	T6	90	10		16.8	0.68	4.0
	Hy3		95	5	38-3	1-3	3-4

* Two hundred cells/6 mm Petri dish were plated in ordinary fluid medium (eight dishes per point).
† Multiplicity of infection: 400 PFU/cell. Five or ten thousand infected cells/6 mm Petri dish were plated in soft agar medium (five dishes per point).

the same experiment. In some experiments, the parental BHK21 cell line was included as a control. It can be seen that transformation frequencies, as well as plating efficiencies in ordinary fluid medium, vary widely. Within each family a positive correlation was found between plating efficiency and transformation frequency (Fig. 1), which indicates that polyoma infection cannot induce multiplication in cells that would not otherwise develop into macroscopic colonies. For this reason transformation frequencies were corrected for plating efficiency for comparison with each other.

Experiments 1, 2 and 3 refer to three groups of sister sub-lines. Each sub-line was isolated by single-cell cloning from the high-polyploid lines 2P3, 2P4 and P108. Transformation frequencies in clones of line P108 are consistently lower than those in line 2P3. Experiment 4 refers to a polyploid clone (Hy3) obtained by growing in the presence of 10^{-5} molar aminopterin, 10^{-5} molar glycine, 4×10^{-5} molar thymidine and 10^{-4} molar hypoxanthine a

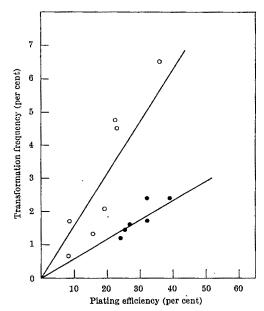


Fig. 1. Relationship between plating efficiency in fluid medium, and transformation frequency, determined as the percentage of infected cells (400 FFU/cell) that would give rise to visible colonies in soft agar medium. O, 2P3 sub-lines; •• , P108 sub-lines. Same data as those reported in Table 1.

mixed culture of two variant BHK21 sub-lines, B1 and T6, respectively, lacking thymidine kinase and inosinic acid pyrophosphorylase. (The hybrid line was kindly provided by J. W. Littlefield.) Hy3 was therefore selected for its ability to grow in a medium that required TDRkinase as well as IMP-pyrophosphorylase activity for a cell to survive. This method has been devised for the selection of somatic cell hybrids?. The modal chromosome number of Hy3 is approximately the sum of the modal values of B1 and T6. Although no chromosome markers were available to substantiate its hybrid nature, both its phenotype and its karyotype strongly suggest that Hy3 arose by fusion of B1 and T6. For this reason it was interesting to compare the transformation rate of Hy3 with that of its "parental" lines B1 and T6.

From the tabulated data it appears that in experiments 1 and 3 no consistent difference is observed between "tetraploids" and "diploids". In experiment 2, some of the "tetraploid" sub-lines show transformation frequencies lower than those of closely related "diploid" sub-lines. There is thus variation in the transforma-

tion frequencies of various "tetraploids" in comparison with "diploids", but this variation is not consistent and cannot explain the genetic heterogeneity for susceptibility to transformation which others have found. Furthermore, in no case is the difference high enough to suggest a "two-hit" phenomenon, which would be expected if each of the parental chromosome complements had to be "transformed" independently. For example, in the case of the hybrid line Hy3 (Table 1, experiment 4), if both parental genotypes had to be "transformed" for the hybrid to show a transformed phenotype, the expected frequency of transformation would be approximately $0.08 \times 0.04 = 0.0032$ —that is, about ten times lower than the frequency observed. The fact that the tumour-specific antigens and the polyoma-specific transplantation antigens present in transformed mouse cells can still be demonstrated in somatic hybrids between these cells and normal mouse cells8,9 also suggests that transformation need have occurred in but one partner of the hybrid.

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Twin Spots as Evidence for Mitotic Crossing-over in Aspergillus induced by Ultra-violet Light

SOMATIC crossing-over was first described by Stern¹, who showed that twin spots of mutant cells occurring on the abdomen of a Drosophila were produced by crossing-over at the four strand stage of mitosis.

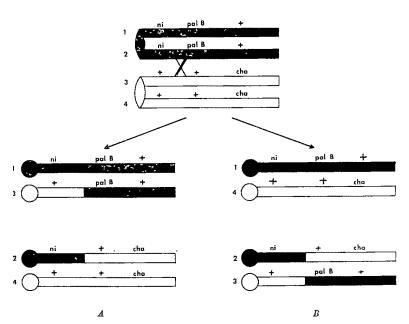


Fig. 1. Diploid 1: ni(50), nitrite requiring; palB(7), lacking alkaline phosphatase; cha(1), chartreuse conidial colour.

After the artificial production of diploids of the fungus Aspergillus, it was found that mitotic segregation resulted in the formation of homozygous, diploid segregants of mutant colour, seen as a single or a group of conidial heads in an otherwise heterozygous, parental, green colony². These segregants were believed to arise as a consequence of mitotic crossing-over3, and supporting evidence was obtained by Roper and Pritchards. Using selective techniques they reported the recovery, in a single nucleus, of the two reciprocal products of mitotic crossing-over within the ad8 cistron. The spontaneous occurrence of "twin colonies" consisting solely of the two segregants expected in twin spots was reported by Käfer⁷, thus providing evidence for a reciprocal four strand mechanism of intergenic mitotic crossing-over, as in Drosophila. Only one such twin colony, resulting from mitotic crossing-over in chromosome VIII, was observed after plating about 10,000 spores on a non-selective complete medium, indicating that spontaneous twin colonies are rare.

We have developed two diploid strains of Aspergillus, where the arrangement of markers is such that both reciprocal products of mitotic crossing-over in chromosome VIII may be phenotypically distinguished in the twin colonies produced. One of these diploids is shown in Fig. 1 together with the two possible results of normal mitotic segregation following a crossing-over between the ni and palB loci. In case A one parental and one cross-over strand segregate into each of the two daughter nuclei, producing two reciprocal segregants, each homozygous for all markers distal to the point of crossing over. The occurrence of such an event in an initial division of a germinating spore will produce a twin colony. In case B one parental nucleus and one recombinant nucleus are produced, the latter containing both cross-over strands and being phenotypically indistinguishable from the parental diploid, for it remains heterozygous. The previously mentioned segregant4 was of this type.

Both diploids have been used to study mitotic recombination induced by ultra-violet light. Up to 5 per cent of surviving conidia produce twin colonies (Figs. 2 and 3) when germinating conidia are irradiated with ultraviolet light (10-15 per cent survival). The recovery of both reciprocal products in twin colonies provides conclusive evidence that ultra-violet irradiation induces mitotic crossing-over in Aspergillus. Because analogous twin colonies have previously been observed following ultra-

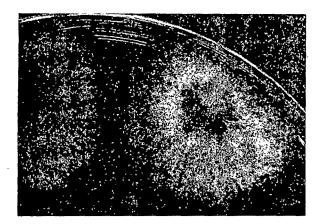


Fig. 2. Parental colony and twin colony induced in diploid 1. The parental is dark green while the twin colony is partly chartreuse and partly lacking alkaline phosphatase (these cells form a small dark brown area in the centre of the chartreuse colony due to their slower growth rate).



Fig. 3. Parental colony and twin colony induced in a bge-cha repulsion diploid. The parental colony is dark green while the twin colony is half chartreuse (light green) and half beige (bge is located proximal to ni, on the same chromosome arm)—probably allelic to fawn (Clutterbuck, personal communication).

violet irradiation of yeast⁸ and ustilago⁹, this effect of ultra-violet light on recombination may be a general phenomenon. These diploids provide a simple visual screening system for agents that may induce crossing-over.

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Growth of Cell-strains and Primary Cells on Micro-carriers in Homogeneous Culture

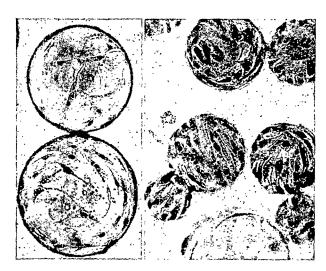
THERE are obvious advantages in culturing tissue cells in suspension instead of in monolayers and in using the suspension as a substrate for virus multiplication. Culture conditions for cell growth and virus multiplica-

tion can be studied and large scale production can be achieved more easily. The growth of cells from celllines, in suspension culture, as separate units or small clumps, has already been demonstrated. In contrast, nobody has yet succeeded in growing diploid and primary cells by this method^{1,2}, for the cells have become aneuploid or did not grow at all in suspension culture3.

Our own experiments with human diploid cells and with primary cells have confirmed earlier findings. essential difference between suspension and monolayer culture seems to be in the attachment of the cells to a solid surface in the latter method. If the wall of the monolayer vessel could be replaced by microscopic particles to serve as a surface for attachment, the advantages of homogeneous culture could be afforded to diploid cells. The suspended particles with cells need be no larger than the small clumps found in suspension culture of some cell-lines.

When looking for a suitable material to function as what we have termed "micro-carriers", we had to discount glass beads because these are too heavy to be kept in suspension. Dextran particles of the 'Sephadex' type do not have this disadvantage, but cells appeared to adhere and grow on these only if positively charged 'DEAE-Sephadex' is used. This was not unexpected, for tissue cells are negatively charged4.

Fully covered micro-carriers were obtained. About 20 h after inoculation the cells adhered to the 'Sephadex', a few on each particle, and gradually a confluent monolayer was formed. When the 'Sephadex' particles were fully covered some particles were joined together by a bridge of cells (Fig. 1).



Adhesion and growth of H cells on 'DEAE-Sephadex A-50' (\times 60). Left, after \pm 24 h; and right, after \pm 96 h.

By this method we successfully cultivated different cell-lines and also human diploid cells and primary rabbit kidney cells. Most experiments were carried out using fibroblast-like cells derived from embryonic rabbit skin (H cells) and diploid human embryonic lung cells (HEL cells). Neither grew in normal homogeneous culture.

The medium used for monolayer and homogeneous

culture was Eagle's minimum essential medium⁵, supplemented with 10 per cent newborn calf serum, 0.12 per cent 'Methocel' (15 c.p.s., Dow) and streptomycin and neomycin. To the medium for homogeneous culture 1 g/l. of sterile 'DEAE-Sephadex A-50' was added as a micro-carrier for the cells. Before being sterilized the 'Sephadex' was washed according to the standard washing procedure and equilibrated with phosphate buffered saline.

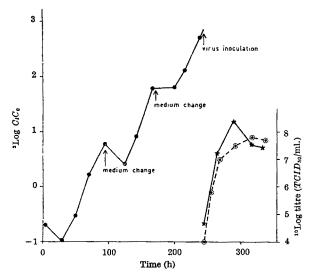


Fig. 2. Growth of HEL cells on micro-carriers in homogeneous culture and polio virus multiplication in these cells in comparison with that in cells from monolayers. The culture volume was at the start 1·5 l. and after changes in the medium 3·01.; the speed of the stirrer was 100 r.p.m., and the arbitrary C_0 is 1×10^3 cells/ml. \bullet —0, Cell concentration (calculated on 1·5 l. volume); \times — \times , virus titre homogeneous culture; \bigcirc --- \bigcirc , virus titre monolayer culture.

The Bilthoven unit⁶ was used for homogeneous culture. Culture conditions were the same as for the suspension cultures of cells from cell-lines, except that the speed of stirring was reduced. The homogeneous cultures were inoculated with trypsinized cells from monolayer cultures at an initial count of 50-100 × 103 cells/ml. The cell concentration was determined by counting nuclei in a Fuchs-Rosenthal counting chamber after staining with 0.5 per cent crystal violet in 0.1 molar citric acid. In this way the nuclei were freed from the 'Sephadex'.

The maximum growth rate of the cells in the microcarrier cultures was about the same as in monolayer cultures, but higher cell concentrations could be achieved by changing the medium. This was done by siphoning off the culture fluid after allowing the 'Sephadex' to settle. Fig. 2 also shows that as far as virus multiplication is concerned there was no essential difference between monolaver and micro-carrier culture. The optimal conditions for culturing cells and viruses by this culture method have still to be found.

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Haematopoietic Stem Cells: Evidence for Competing Proliferative Demands

A HAEMATOPOIETIC stem cell must be able both to replicate itself and to differentiate into a formed blood element. The presence of a pool of multipotent, functionally important, common stem cells for the various haematopoietic formed elements has not as yet been established. There is evidence to suggest that there is a population of bone

marrow cells which when transplanted into irradiated recipients is capable of differentiating into more than one mature cellular form1-3. We are interested in whether this class of cells is functionally important, and specifically whether demand for one blood element limits the ability of the animal to produce other cell types. Such a relationship would suggest competitive demands on a common stem cell pool. There is some evidence to suggest that this may be the case. Harris et al.4 have shown that after large acute haemorrhage the number of granulocyte precursors in the guinea-pig bone marrow is reduced. The present experiment demonstrates that an increased demand for red blood cell production reduces the ability of transplanted syngeneic bone marrow to make granulocytic progeny.

A technique for assaying the granulocytic progeny of transplanted bone marrow has recently been developed. In this technique, syngeneic bone marrow is injected into lethally irradiated mice. The bone marrow is allowed to proliferate for 7-9 days in the recipient animal and its granulocytic progeny is then determined by measuring the peripheral granulocyte response to endotoxin. response has been shown to be a measure of the marrow granulocyte reserve. The greatest response to endotoxin is proportional to the number of marrow cells injected 7 days earlier and can be used to assay the proliferative capacity of such injected cells to produce mature granulo-

cytic progeny.

In this experiment, male C3H/HEJ mice 8-12 weeks old were used. They were divided into two groups, and one group was bled of 0.6 ml. from the orbital sinus 3 days and again 1 day before irradiation. Both groups of animals then received 700 r. whole body irradiation delivered with a 250 kV X-ray machine (HVL 1.65 mm³, FSD 50 cm) at 119 r./min. Each group was then subdivided and graded doses of syngeneic normal bone marrow were injected intravenously. One week later the animals were intravenously injected with 10-2 μg of 'Pyrexal', a purified lipopolysaccharide of Salmonella abortus equi, and the white blood cell counts recorded immediately before, and at 2, 4 and 6 h after, endotoxin. This response is essentially a granulocyte response and thus white blood counts are used, which obviates the need for differential counts. Haematocrits were measured before radiation and again on the day before administration of endotoxin. All blood samples were taken and injections were made by way of the mouse tail vein.

Table 1

Group	Haematocrit before X-ray	Haematocrit on day 6, 1 day before endotoxin
1 (700 r., no bleeding) (a) 0 bone marrow cells (b) 1.0×10^6 bone marrow cells (c) 20×10^6 bone marrow cells (d) 4.0×10^6 bone marrow cells	46·0 (±1·1) 45·8 (±1·2) 44·0 (±0·0) 43·3 (±0·6)	37·2 (±1·1) 41·2 (±1·6) 37·6 (±0·7) 40·2 (±1·0)
2 (700 τ ., bled twice of 0.6 ml.) (a) 0 bone marrow cells (b) 1.0×10^4 bone marrow cells (c) 2.0×10^4 bone marrow cells (d) 4.0×10^4 bone marrow cells	27·4 (±0·6) 24·6 (±1·2) 25·6 (±1·3) 25·6 (±0·5)	25·4 (±1·9) 21·2 (±0·7) 23·4 (±0·9) 24·4 (±0·2)

Each subgroup contains five animals. The number in parenthesis is the standar l error of the mean.

Table 1 shows the groups used and their haematocrits before injection of bone marrow and on day 6 (the day before endotoxin). Fig. 1 shows the maximum white blood cell responses to endotoxin in both groups plotted against the number of bone marrow cells injected 7 days before. The anaemic recipient animals are less able to respond to endotoxin, which indicates that the same number of transplanted marrow cells give rise to fewer granulocytic progeny in the bled recipients.

We attempted to confirm this in a somewhat different manner. In this second type of experiment, erythropoietin derived from sheep treated with phenylhydrazine (supplied by Connaught Medical Research Laboratories,

Toronto) was used instead of bleeding to stimulate erythropoiesis. This agent is produced by anaemic animals and has been shown to increase production of erythrocytic precursors by the stem cell compartment⁷⁻⁹. Mice were given 700 r. whole body irradiation and then divided into two groups. Both groups were given varying doses of bone marrow cells, and 7 days later the progeny of the transplanted bone marrow was evaluated by the response to endotoxin. This is identical to the technique used with bled recipients. The animals, however, were not bled; instead, one group was given three units of erythropoietin daily on days 2, 3 and 4 after bone marrow transplantation. The response to endotoxin in these two groups of animals is shown in Fig. 2. The ability of the transplanted marrow to make granulocytes is reduced in those recipient animals receiving erythropoietin.

A number of investigators have studied the effects of bleeding and hypertransfusion on stem cells as measured by the spleen colony technique¹¹⁻¹⁴. One group indicates that plethora reduces the number of macroscopically visible erythropoietic colonies without an accompanying increase in granulocytic colonies. Microscopic examination, however, reveals that the number of erythrocytic colonies is not diminished but rather they are reduced in size¹³. Other investigators^{12,14} indicate some increase in the number of granulocytic colonies with plethoric animals. Results with the spleen colony system therefore differ with different investigators. The spleen colony technique is dissimilar to the present experiment, which measures the production of end cells and thus is related not only to colony number but also to colony size and the proportion of mature cells of the various types produced. The second difference is that this experiment measures the production of all the haemopoietic tissue while the colony method is concerned only with growth in the spleen.

In the present experiment using bleeding or exogenous erythropoietin, the transplanted bone marrow proliferates under conditions of increased stimulus for red blood cells. Under these conditions the production of granulocytic progeny is reduced. We take this as evidence of competition by the granulocytic and erythrocytic series for a common factor. It is conceivable that a small pool of a necessary nutrient is the limiting factor, but no such

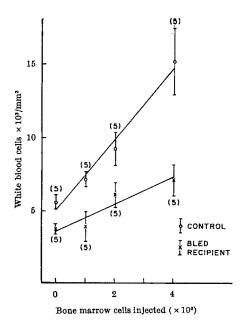


Fig. 1. Maximum white blood cell response to endotoxin plotted against bone marrow cells injected intravenously 7 days earlier. The mean and its standard error are plotted. The number in parenthesis is the number of animals used at each point. The statistical significance of the difference of the groups has a P>0.001 (ref. 10).

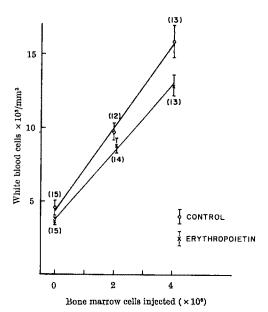


Fig. 2. Maximum white blood cell response to endotoxin plotted against bone marrow cells injected intravenously 7 days earlier. The mean and its standard error are plotted. The number in parenthesis is the number of animals used at each point. The statistical significance of the difference of the groups is 0.05 > P > 0.02 (ref. 10).

It seems more likely that these substance is known. experiments indicate competing proliferative demands on a pool of common stem cells. This implies a multipotent stem cell able to produce either erythrocytic or granulocytic progeny.

The experimental situation is such that there is a great demand for formed blood elements. The irradiated animals have a markedly reduced number of progenitor cells and the unirradiated, transfused stem cells are probably proliferating at maximum rate, because they must both repopulate the irradiated recipient with stem cells as well as differentiate to maintain a necessary level of peripheral blood cells. It is in this highly stressful situation that the interaction of proliferative demands can be demonstrated.

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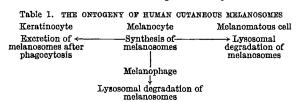
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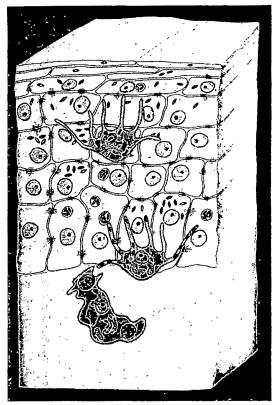
Lysosomes in Melanin Phagocytosis and Synthesis

THERE are two types of pigmented cells in normal human skin, the junctional dendritic melanocyte and the intradermal melanosome-phagocytosing melanophage (Fig. 1). In contrast to the melanocyte and its contained melanosome synthesizing process which have been thoroughly investigated, the cellular physiology of the melanophage remains unknown despite recent advances in electron microscopy and gradient biochemistry. The two cells containing melanin appear similar under the light microscope but electron microscopy reveals definite structural differences. The normal melanocyte contains various stages of melanosome synthesis occurring individually in close association with the Golgi apparatus. On the other hand, the actively phagocytosing melanophage concentrates its melanosomes 1-3 as degradative conglomerates into specially developed phagocytic vacuoles structurally resembling the lysosomes found in such tissues as kidney, liver and pancreas^{4,5}. Lysosomes were found to be rich in acid phosphatase^{4,5}, which predicted the presence of this hydrolytic enzyme in the phagocytic vacuoles of the dermal melanophage⁶. We found subsequently that acid phosphatase activity is concentrated in the melanophages and that there is little or no such activity in the melanocytes at the epidermal-dermal junction. On the other hand, the melanophages contain little if any tyrosinase while the junctional melanocytes are shown to contain an abundance of this oxidative enzyme, primarily within the premelanosomes. It is further observed with the electron microscope, using the modified Gomori reaction, that the electron opaque accumulation of lead sulphide resulting from acid phosphatase activity occurs primarily in the melanosome-concentrating phagocytic vacuoles of the melanophages7.

Although normal melanocytes show little phagocytic activity, when the melanocyte assumes melanomatous growth it can develop autophagic vacuoles, concentrate melanosomes in these vacuoles and consequently exhibit an increase in acid phosphatase activity⁹. It has also been shown during a study of Fortner's melanoma that this increase in lysosomal activity appears to be related to clinically observable necrosis and ulceration. As the acid phosphatase activity begins to appear in melanomatous growth the tyrosinase activity also increases, leaving a constant quantitative differential between these enzyme systems. The two enzymes are localized in different organelle systems. Thus a procedure for the simultaneous demonstration of these lytic and oxidative enzymes without abolition or cross contamination or both was developed 10 and shown to distinguish more precisely melanomatous cells as well as melanocytes from melanophages. In addition to these two lysosomal pathways in melanophages and melanomatous cells, the melanosome can follow a third route to complete its ontogeny. Another component of the epidermis, the keratinocyte, can phagocytose melanosomes from the melanocyte and ascend to the keratin layer rich in acid phosphatase where it completes its own ontogeny as well as that of its contained melanosomes (Table 1).

The lysosomal activities which we have found in the ontogeny of melanosomes have the following applications. (a) Melanophages can be clearly differentiated not only from normal junctional melanocytes but also from premalignant and malignant melanoma cells, the distinction between which is often critical, particularly after invasion





Ontogeny of melanosomes and melanocyte in the epidermaldermal structure. A junctional melanocyte actively synthesizing melano-somes shows individual occurrence of premelanosomes and melanosomes in its cytoplasm and transfers these melanosomes to neighbouring kera-tinocytes and melanophages with or without lysosomal activity. A high level dendritic Langerhans cell synthesizes its characteristic granules within its cytoplasm.

into the dermis. (b) Recent findings that the activity of lysosomes can be labilized and stabilized by various chemicals in vitro and in vivo 11 suggest a future therapeutic approach to such dermal hyperpigmentations as Riehl's melanosis and poikiloderma Civatte, for these disorders are the result of lysosomal accumulation of melanosomes. (c) It is known that malignant melanoma cells often undergo spontaneous ulceration and necrosis, and spontaneous regression has been observed12. We have found that these degradative processes are related to the ability of the melanoma cells to form lysosomes and to degrade themselves, and so the death of melanoma cells can be approached by the hormonal or chemical enhancement of lysosomal activity.

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Silica in the Wall of Pediastrum

In a study of the differentiation and development of pattern and cell form in the green alga Pediastrum, difficulty in extracting pigments with organic solvents led us to examine the chemical nature of the cell wall as a preliminary to a study of wall development. Structural patterns in the cell wall of Pediastrum have been described by Moner¹, and formation of the wall by Moner and Chapman². These electron microscope studies described a network of interconnected spheres, which imparts a pattern in the continuous wall phase. Although the chemical nature of the wall was not reported, it was noted that neither the wall nor the "globular network" was noticeably affected by treatment with 17.5 per cent sodium hydroxide for 48 h, by similar treatment with ethanol-ether, or by treatment for 5 h with 70 per cent sulphuric acid. Moner had previously noted that little is known of the chemical nature of the wall. Early literature describes the wall of *Pediastrum* as two-layered³. Chodat and Huber4, on the basis of staining with chloriodide of zinc and of ammoniacal congo red, reported that there is cellulose in the inner wall layer but not the outer layer. Our attempts to identify cell wall com-

ponents by routine histochemical procedures, however, failed to give characteristic results, which suggested a possible masking of the expected wall components. We present evidence here for the presence of silica in relatively high concentration in the wall of Pediastrum. In the review on silicification by Lewin⁶, Pediastrum is not included among the algae which deposit silica, and to the best of our knowledge this occurrence has not been previously reported. It should be noted, however, that Pediastrum is recorded in the fossil record7, which indicates a very resistant cell wall.

We used cultures of Pediastrum boryanum obtained from J. S. Davis⁸ and maintained in his medium "C". Colonies heterogeneous in age were centrifuged out of suspension and subjected to oxidation in several changes of concentrated chromic and sulphuric acid for several hours at high temperatures or in concentrated nitric acid for several days. The walls of young and old colonies remained intact although the cytoplasm was removed from the cells.

Other colonies were suspended in 31 per cent perchloric acid and 34 per cent

nitric acid for 20 h to remove organic matter⁸. colonies were then washed to neutrality with water, centrifuged, and suspended in ethanol-ether (1:1) for 3 h. The extracted colonies were transferred to water and placed on grids coated with 'Formvar' for examination in the electron microscope. A young colony (Fig. 1), and an older colony which had released the inner wall and the zoospores it contained (Fig. 2), were treated in this way and revealed the retention of the characteristic wall pattern in the outer wall. To examine further the resistant nature of the wall, colonies were incinerated in a 'Pyrex' crucible under a bunsen flame. The residue was extracted for 3 days with concentrated nitric acid and washed to neutrality. Insoluble material remained, but when it was dried and exposed to hydrofluoric acid the residue dissolved. Cell walls which remained after other extraction procedures as shown in Fig. 1 also dissolved in hydrofluoric acid.

For a more positive check on the presence of silicon, indicated by solubility of the residues in hydrofluoric acid, colorimetric analysis was performed on colony suspensions after oxidation with nitric and sulphuric acids in a platinum crucible, followed by ignition and fusion with anhydrous sodium carbonate. An oxalic acid-ferrous

ammonium sulphate procedure using molybdate, which minimizes interference from iron and also from phosphate usually present as polyphosphate in certain Hydrodictyaceae10, was used to detect silicon. The blue colour characteristic of the silicon-molybdenum complex developed in the wall preparation and also in a solution of sodium metasilicate. A water control remained colour-

Silicon was also found and quantitatively estimated by the colorimetric procedure of Kilmer¹¹ in which tartaric acid reduces interference from phosphate and iron. The amount of the blue complex was determined by comparison with a standard curve established from a series of sodium metasilicate standards read at 650 mµ. The silica content of a 0.5 g sample of colonies heterogeneous in age was 0.8 per cent on an oven-dry basis.

On the basis of these analyses we conclude that the cell wall of Pediastrum boryanum is relatively high in silica. Electron micrographic studies of colonies before and after release of the inner wall containing the zoospores suggest that the silica is concentrated in the narrow outer wall of the cell. The retention of an electron-dense reticulate pattern in the wall after exhaustive extraction (Fig. 2) indicates that the pattern is also cast in silicate.

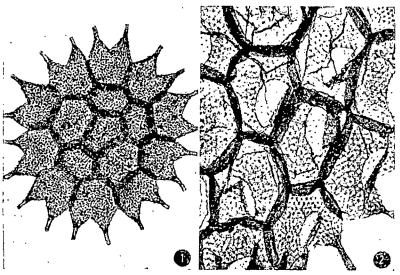


Fig. 1. Electron micrograph of young colony of *P. boryanum* after acid and ether-ethanol extraction. (×1,320.)

Fig. 2. Electron micrograph of mature colony after release of vesicle containing zoospores through semicircular pore in each cell. Colony extracted as in Fig. 1. $(\times 2,100.)$

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MICROBIOLOGY

Aleutian Disease Gammopathy of Mink induced with an Ultra-filtrable Agent

Among the lymphoproliferative diseases, murine leukaemia has interested many investigators since Gross succeeded in transmitting it with a filtrable agent¹. Another lymphoproliferative disease, Aleutian disease of mink², has received considerable attention because there is a filtrable causative agent^{3,4}. Aleutian disease can be characterized as a transmissible disease of mink and possibly other mustelids⁵, in which a hypergammaglobulinaemia⁸ occurs concomitant with a plasma cell proliferation. Unlike murine leukaemia, where the ambiguity of a "filtrable agent" has been resolved by the demonstration of virus particles⁷, the Aleutian disease agent has remained undetected by electron microscopy (personal communication from R. C. Williams, jun.).

The size of the Aleutian disease agent has not been determined, and so we have carried out a series of filtration experiments. The starting material in each case was a 10 per cent spleen suspension from infected mink, prepared by blending 40 g of spleen in phosphate buffered saline, pH 7.6 (0.85 per cent sodium chloride in 0.04 molar sodium phosphate adjusted to pH 7.6 with hydrochloric acid), followed by four freeze-thaw cycles, then centrifuged at 18,000g for 30 min at 4° C. The resulting supernatant was kept frozen as a stock material. In the first experiment, part of the stock suspension was filtered through a series of 'Millipore' filters (220, 100, 50 and 10 mµ), supported on a 47 mm fritted glass grid 'Pyrex' analytical filter holder operated under vacuum at a pressure of 70 cm mercury. Previous experiments indicated that the agent of Aleutian disease could be filtered through the 100 mu filter, and so retention efficiency of the 50 m μ filter was determined with T_1 phage and the 10 m μ filter with ϕX 174 phage for E. coli by filtering the phage in an aliquot of stock tissue homogenate and then titrating turbidimetrically in a standard culture of E. coli.

A bioassay for infectivity (made in 1 yr old mink, heterozygous for the Aleutian gene) revealed filtrates derived from all of the 'Millipore' filters to be infective. The consideration that the agent for Aleutian disease is smaller than 50 m μ seemed valid, because the T_1 phage (60 m μ , ref. 8) was completely retained on the filter. The fact that the phage $\phi X174$ (25 m μ , ref. 8) was not completely retained in the conditions used for filtration reduces the significance of infectivity observed in the 10 mu filtrate. This being the case, we tried to establish the smallest size which would retain the agent for the purposes of preparation. For this, dialysis tubing was used on an LKB ultra-filter (Fig. 1). The filtration apparatus was inserted in a cylindrical flask which contained 30-35 ml. of a 10 mµ filtrate with the entire unit supported in an ice bath. Contrary to expectation, the ultra-filtrate was infective when tested undiluted (one mink/log dilution). No evidence of serum components could be demonstrated in the ultra-filtrate with rabbit antisera prepared to whole mink sera from mink infected with Aleutian disease.

As a result of this observation, ultra-filtrates were prepared directly from the stock tissue extract without previous 'Millipore' filtration. A total of three additional ultra-filtration experiments have been performed and in each instance when mink were inoculated directly with undiluted ultra-filtrates, 88 per cent of the inoculated animals developed Aleutian disease before 100 days incubation (Table 1). With the last of these ultra-filtrate preparations, radio-iodinated (iodine-125) human albumin was incorporated in the stock extract to establish the continuity of casing. For this, 'Albumatope' from Squibb and Sons, Inc., New Brunswick, was used as a marker. Before incorporation with tissue extracts, 6.4 ml. of a 1 per cent solution of 'Albumatope' was dialysed for 4 days

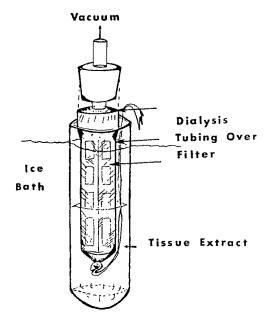


Fig. 1. Liagram showing dialysis tubing stretched over ultra-filter support. The tissue extract containing lodinated (lodine-125) albumin was placed in the cylindrical vessel.

to remove readily dissociated dialysable iodine-125. The dialysed albumin added to infective tissue extract yielded a final volume of 120 ml. (0.53 mg/ml. with 46,950 c.p.m./ml. of tissue extract). The filter was evacuated initially to 5μ pressure, then disconnected from the pump and held in an ice bath. In 4 h, 3–12 ml. of filtrate accumulated within the dialysis tubing from which it was then removed and the vacuum was reapplied. Of the total radioactivity, only 1 per cent was recovered in 35 ml. of ultra-filtrate, which indicated the system was impermeable to albumin. All estimates of radio-disintegration were made with a Nuclear Chicago model 138B scaling unit.

In instances where mink inoculated with ultra-filtrate survived for more than 30 days, pronounced hypergammaglobulinaemia developed (Fig. 2). The lesions observed in

Table 1. CONCENTRATIONS OF GAMMA GLOBULIN OF MINK INOCULATED WITH ULTRA-FILTRATE

ULTRA-FILTRATE				
Experiment	Mink No.	Per cent gamı Before	ma globulin After	
1	11 12 13 14 15	15 10 6 11 16	53 (day 93) 54 43 68 48	
Uninoc	ulated controls G2090 G2091 G2092	15 16 17	17 17 13	
2	J53 J54 J55 J56 J57 J58 J59 J60	9 10 7 7 15 10 13	18 (day 93) 43 33	
Uninoc	ulated controls J61 J62 J63 J64	9 13 14 11	12 14 12 18	
3	J4057 J4058 J4059 J4060	17 11 8 11	41 (day 64) 40 44	
Uninoc	ulated controls J4065 J4066 J4067 J4068	14 9 14 14	14 9 19 12	

* Died 3 weeks after inoculation with lesions characteristic of Aleutian disease.
† Died 3 weeks after inoculation with no signs of Aleutian disease.

these animals after death were identical to those seen in mink infected with tissue suspensions and 'Millipore'

There is evidence that the infective constituent of serum[®] is associated with the immunoglobulin fraction¹⁰ resulting in the complexes previously described¹¹. A

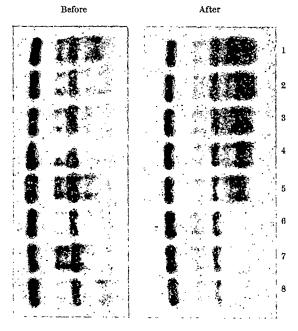


Fig. 2. Cellulose acetate electrophoresis of mink sera from animals inoculated with an ultra-filtrate. Samples 1-3 were from mink inoculated with an ultra-filtrate prepared with iodine-125 albumin marker (experiment 3) and samples 4 and 5 were from mink inoculated with an ultra-filtrate not tested with iodine-125 marker (experiment 2). Analyses were conducted on a Spinco 'Microzone' cell using the procedure recommended by the manufacturer. by the manufacturer.

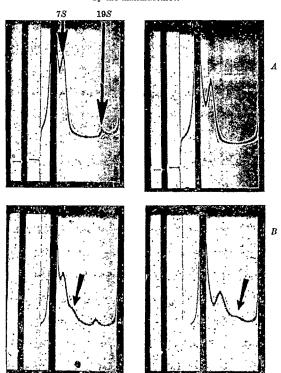


Fig. 3. Ultracentrifuge patterns (direction of sedimentation left to right) showing aggregation induced in serum diluted with an ultra-filtrate (B). A decrease in 7S components is apparent with marked increase in intermediate (arrow) and heavier components (bottom of cell). The ultra-filtrate alone produced only a salt boundary. Sedimentation was conducted at 59,780 r.p.m. at 20° C with 2-5 per cent protein.

64 min

40 min

kidney tissue ultra-filtrate prepared as already mentioned with a laboratory-constructed ultra-filter was added to hypergammaglobulinaemic serum from an infected mink (one part serum: two parts ultra-filtrate). incubation at 4° C produced aggregates which reduced the amount of material with a 7S sedimentation coefficient (Fig. 3). It is difficult to say yet whether this is a specific reaction of immunoglobulin with a renal antigen or simply the formation of strong ionic complexes with what may be the infective agent. Previous attempts to isolate the infective principle from serum by gel filtration on 'Sephadex G-200' indicated that the agent may have filtration characteristics similar to 4S components, but the possibility of ionic hold-up accounting for apparent low molecular weight components in a gel filtration pattern cannot be excluded.

The fact that infectivity of Aleutian disease was demonstrated in a fraction of tissue extract containing substances no larger than albumin makes it extremely difficult to formulate the mechanism for induction of plasmacellular proliferation in infected mink. Considering the small size of the scrapie agent 12,13 and the evidence presented here for the Aleutian disease agent, one of the few tenable explanations is that these agents are analogous to the factors responsible for induction and repression of protein synthesis and cell metabolism as described by Jacob and Monod^{14,15}. In Aleutian disease an alteration at the level of messenger synthesis, whereby a relatively small fragment might interfere with a repressor would explain the uncontrolled protein synthesis at the plasma cell level. The fact that in these animals replication of the agent takes place cannot, however, be readily explained by this theory.

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Unusual Requirement for a Vitamin by Strains of Fusarium oxysporum f. sp. melonis

MANY phytopathogenic fungi do not grow in a minimal medium containing essential elements and a source of organic carbon but, in some cases, such a medium supplemented with biotin will support growth. Although nutritionally deficient mutants of Fusarium oxysporum Shlect. emend. Snyder and Hansen which require biotin have been obtained by exposing conidia to ultra-violet light2, fresh isolated strains of this phytopathogen have not been found to need biotin for growth. We now have evidence of strains which require biotin in recently isolated F. oxysporum f. sp. melonis.

Table 1. AMOUNT OF DRY MYCELIUM/100 ML. OF CULTURE MEDIUM

Strain 15a (non-deficient)	mg/100 ml.
Medium F without supplement	229
Medium $F + \text{biotin 10 } \mu g/l$.	223
Medium F + biotin 100 μ g/ml.	218
Medium F +yeast extract 1 g/l.	373
Strain 14a (deficient)	
Medium F without supplement	55
Medium F + pimelic acid 5 $\mu g/l$.	90
Medium F + desthiobiotin 5 μ g/l.	156
Medium $F + \text{biotin } 0.1 \mu \text{g/l}$.	125
Medium F + biotin 1.0 μ g/1.	185
Medium $F + \text{biotin } 10.0 \ \mu\text{g/l}$.	207
Medium F + biotin 100 0 μ g/l.	191
Medium F + yeast extract 1 g/l.	550

During a study of genetic variability in F. oxysporum f. sp. melonis, four of six isolates did not grow on the defined F medium². Using auxanographic tests, the nutritionally deficient strains grew in F medium supplemented with biotin and failed to grow when this medium contained a number of amino-acids, pyridoxine, thiamine, riboflavine, p-aminobenzoic acid or pantothenic acid.

Four Erlenmeyer flasks (500 ml.) containing F medium (100 ml.) supplemented with pimelic acid, desthiobiotin, yeast extract or graded concentrations of biotin were inoculated with about 5 x 104 conidia of one prototrophic and one auxotrophic strain. The static cultures were incubated at 26° C for 13 days and the dry mycelial weight was recorded. The average dry weights for the quadruplicated cultures are presented in Table 1.

The auxotrophic strain grew well in medium containing biotin, desthiobiotin or yeast extract, but poorly in medium supplemented with pimelic acid. All four auxotrophic strains gave the same results

The addition of extract from healthy melon plants to the F medium gave good growth for the strains deficient in biotin. The slight mycelial growth of these strains on F medium solidified with agar may have resulted from trace amounts of biotin in the agar.

Complementation tests using all possible pairs of the four auxotrophic strains were negative, which implies that the mutation leading to the need for biotin probably occurred at the same locus in these strains.

Although one nutritionally deficient strain did not lose its requirement during eight transfers at intervals of 1 month, other strains became prototrophic after being cultured for 2 yr on a yeast extract medium. Isolates requiring biotin have been obtained three times at intervals of 1 yr from the soil in the greenhouse with at least one and sometimes two crops of tomato and lettuce between crops of melon. The nutritionally deficient strains seemed to be able to compete with prototrophic strains in the greenhouse soil with a high content of organic matter. It would be interesting to determine whether other nutritionally deficient phytopathogens are favoured in such soils.

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BIOCHEMISTRY

Polyacrylamide Gel Electrophoresis of Highly Purified Chick Interferon

EARLIER results1,2 suggested that chick interferon, purified by the methods of Fantes et al.1,3, was wholly or at least predominantly homogeneous, when judged by its behaviour on electrophoresis in polyacrylamide gel or in The alkaline polyacrylamide gel a sucrose gradient. electrophoresis method of Davis4 also suggested to us that the interferon was homogeneous2.

When chick interferon of even higher specific activity became available, polyacrylamide gel electrophoresis was repeated, but with slight modifications. (a) Because the overall recovery of interferon after electrophoresis was not very good and because some activity was usually found in the sample and spacer gels, these were omitted and the samples in 20 per cent sucrose were applied directly to the small-pore gel, thus raising the overall recovery. (b) There are slight variations of the rate of passage of bands in different columns, even in the same electrophoresis experiment, and so the same column (not two separate ones) was used to demonstrate the presence of both stained protein and of antiviral activity.

The technique which we used for alkaline electrophoresis was briefly as follows: interferon (in 1 ml. of 0.01 molar phosphate buffer, pH 7.5) mixed with an equal volume of 40 per cent sucrose was applied to a gel column (40×6.5 mm). The gel was prepared by polymerizing in aqueous solution acrylamide (7 per cent w/v), N,N'-methylene-bisacrylamide (0·18 per cent w/v) and N,N,N',N'-tetramethylethylene-diamine (0.056 per cent v/v), in the presence of ammonium persulphate (0.006 molar). The gel was buffered with 0.38 molar tris hydrochloric acid and the electrode buffer was as described by Davis'. After electrophoresis (6 m.amp/column) the gel was cut lengthwise, half was stained with amido black and the other was cut into twenty 2 mm pieces, each of which was placed in 1 ml. of phosphate buffer (0.5 molar, pH 7.5) containing bovine plasma albumin (500 μ g/ml.) and 'Tween 80' (20 µg/ml.). After 24 h at 4° C, the supernatant fluids were assayed for interferon content.

After alkaline electrophoresis of highly purified interferon (1.6 × 10° v/mg of protein), staining with amido black revealed two very sharp bands of protein close together; these coincided exactly with the peak of the antiviral activity (Fig. 1A). Because, however, the total activity spread well beyond the stained zones we suspected that staining and activity were not necessarily To investigate this possibility, we attempted electrophoresis at an acid pH employing essentially the method of Reisfeld et al.6, but again omitting sample and spacer gels, and applying interferon in 20 per cent sucrose to the small-pore gel, buffered with 0.38 molar sodium acetate/acetic acid. In these conditions, all or nearly all activity was invariably lost. This destruction was traced to the presence of persulphate, a substance used by both Davis and Reisfeld for catalysing their polymerizatione of acrylamide. Incubation of interferon with acid, but not with alkaline, persulphate led to complete loss of activity7,8. When gels were polymerized with riboflavine (0.0005 per cent) instead of persulphate, acid electrophoresis was successful. (We now use gels catalysed by riboflavine also for alkaline electrophoresis and the results shown in Fig. 1A were obtained in this way.)

When the same material as was used for alkaline electrophoresis was subjected to acid electrophoresis (Fig. 1B) two stained protein zones—this time well separated were again revealed, but the antiviral activity lay between them and had probably no connexion with either. The specific activity of the pre-electrophoresis sample was 1.6×10^6 U/mg of protein, and most of this protein does not seem to be part of the interferon molecule, so we assume that pure interferon will have an extremely high specific potency. Nagano et al.9-11 have suggested that the active portion of interferon is oligo- or polysaccharide. Gels, after electrophoresis of another sample of highly purified interferon (12,000 v and 8 µg of protein/ml.), were therefore stained with toluidine blue, 'Alcian blue' or carmine, dyes that reveal the presence of neutral or acidic polysaccharides. So far all results have been inconclusive. but this could be because there was too low a concentration of interferon.

The "glucose equivalent" of a sample of the same preparation of interferon was determined by means of the anthrone reagent12, and a value of 3-4 µg/ml. of

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² Tuveson R., and Garber, E. D., Bot. Gaz., 121, 69 (1959).

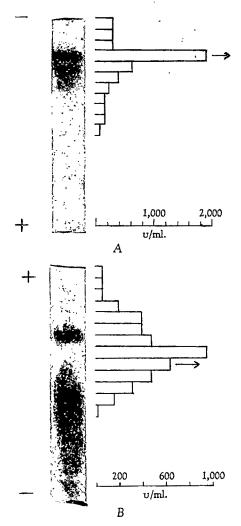


Fig. 1. Samples of 1 ml. of interferon (38,400 v/ml.; $1.6 \times 10^8 v/mg$ of protein) were subjected to alkaline and acid electrophoresis as described in the text. A, Results obtained at pH 8-9; B, those obtained at pH 3. The arrows indicate fractions the actual interferon contents of which are higher than shown, for the assay samples had not been diluted sufficiently to give endpoints.

glucose was obtained. Whether the sugar was part of interferon or of an impurity is not known.

Treatment of similar interferon samples with periodate (5 mmolar, 22° C, 5 h) at pH 7 and 4, and with trypsin (500 μ g/ml., 37° C, 2 h) at pH 7, completely destroyed activity. The result obtained with trypsin again confirmed that protein was an essential part of the interferon molecule. The results from the periodate treatment, especially that at pH 4, suggest that a sugar is also needed for antiviral activity, but this is not certain, for many amino-acids are also known to be sensitive to this re-We found that the activity of ribonuclease (100 µg/ml.), a sugar-free molecule, was completely abolished by similar treatment with periodate at pH 4, and reduced to 30 per cent at pH 7.

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Chemical Inactivation in vitro of Carbonic Anhydrase by Carcinogenic Substances

The most spectacular manifestation of malign tumours is the wild proliferation of tumour cells. Besides this, there is less zinc in tissues, blood and other fluids than in healthy people¹⁻¹⁶.

In collaboration with L. J. P. Frank, we have measured the zinc content of arterial blood of 173 patients considered on clinical grounds to be suffering from various forms of carcinoma (1,900 samples were analysed by X-ray fluorescence) with the results shown in Table 1.

> Table 1 No. of patients Analytical results in agreement with diagnosis of physician 173 (100%) 144 (~83%) 2 (~1%) 27 (-16%) Uncertainty Conflicting opinions

We have found repeatedly that the concentration of zinc in the blood parallels the progress of the illness and that for diagnostic purposes it is useful to monitor the zinc concentration of the blood of its components14.

The concentration of zinc has previously^{3,7,8,18,17} been related to that of metallo-protein carbonic anhydrase, an enzyme which regulates the transport of carbon dioxide. This regulation depends on a catalytic process, which takes place between carbon dioxide and water adsorbed (chemisorption) by chelate-bound zinc25.

Human carbonic anhydrase (HCA) can be split by paper electrophoresis into three principal fractions (Fig. 1): a small fraction of the isoenzyme A with low activity; a large fraction of the isoenzyme B, also with low activity (this fraction contains "loosely bound" zinc, which can easily be removed by extraction with dithizone at pH 6.8); and a smaller fraction of the isoenzyme C with very high activity23,24. (For the three-dimensional structure of $HCA-\tilde{C}$ see ref. 26.) The third fraction contains "firmly bound" zinc, which can be removed by extraction with

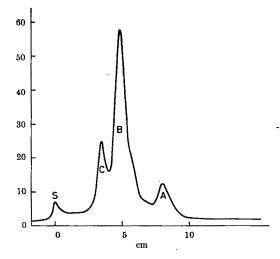


Fig. 1. Filter paper electrophoretic pattern of human carbonic an-hydrase (veronal buffer pH 8-6, for details see text). Abscissa: distance from site of application (S) of sample; ordinate: measured absorption of light on arbitrary scale.

o-phenanthroline (a compound with a strong affinity for zine), but not by extraction with dithizone at pH 6.8.

It is possible¹⁷ to remove firmly bound zinc by irradiation with soft X-rays (irradiation specifically into the zinc absorption band loosens the bond between the zinc and the organic molecule), and as this removal proceeds, the activity of the carbonic anhydrase becomes reduced17,18 in proportion. Thus it seems that the amount of "firmly bound" zinc determines the activity of carbonic anhydrase.

The purpose of the present investigation was to see whether it is possible to loosen the firmly bound zinc from carbonic anhydrase by contact with carcinogenic agents. As far as carcinogenic derivatives of anthracene are concerned, they are characteristic in their binding of osmium tetroxide and in possessing the "K-region" in the molecule which is strongly unsaturated. This unsaturated part of the molecule might be able, like the X-rays, to disrupt the chelate bond by which the zinc is held in the enzyme. It might then be possible for covalent linkages to be formed, as between DNA and butter yellow22.

Carbonic anhydrase is only soluble in water, anthracene derivatives form stable suspensions in organic solvents like olive oil, and so carbonic anhydrase and anthracene derivatives can only be brought together at the oil-water interface. At the interface the enzyme will react in the denatured form.

A preliminary experiment showed that shaking together a solution of carbonic anhydrase (1 mg in 5 ml. of water) and a suspension of 1 mg of anthracene derivative in 150 mg of olive oil produced an almost stable emulsion. If this emulsion was kept at 37° C, shaking once a day was sufficient.

We found that a reaction time of 5-10 weeks was sufficient to produce a significant effect. Table 2 shows the results after 10 weeks. (All handling was performed in sterile conditions. Neither sedimentation nor a smell of hydrogen sulphide could be observed after 5 weeks storage at 37° C, and there was no development of bacteria.) Sometimes, however, a creamy discoloration was seen, and the results of these experiments were unreliable.

Two kinds of known carcinogen were chosen: 1,2-benzanthracene, a weak carcinogen: and 1,2,5,6-dibenzanthracene, a potent one¹⁸. The carbonic anhydrase was a bovine preparation from Koch-Light Laboratories. It contained 0.10 per cent loosely bound zinc; after destruction with oxidizing acids we found 0.17 per cent total zinc; this batch therefore contained 0.07 per cent firmly bound zinc.

Table 2. Change in the concentration of firmly bound zinc in carbonic anhydrase after storage for 10 weeks at 37° c with carcinogens

	Loosely bound	Total	Firmly bound
Original solution Storage with suspension of pure oil The same, but 1,2-benzanthracene added The same but 1.2.5.6-dibenzanthracene	0·10% 0·09*% 0·10%	0·17% 0·16*% 0·16%	0·07% 0·07% 0·06%
The same but 1,2,5,6-dibenzanthracene added	0.12%	0.16%	0.045%

The decrease in firmly bound zinc, shown in the last column of Table 2, was statistically significant. The stronger the activity of the carcinogenic agent, the less firmly bound the zinc remains, which can lead to lower activity of the carbonic anhydrase17,18.

Next we established that after storage for 18 and 34 weeks, respectively, there was no further change. The deionized water which we used may have contained some organic substances; in the next experiment, therefore, we used distilled deionized water, for it was possible that organic substances have an inhibitory effect. The results are shown in Table 3, where we have also included results obtained with anthracene, which is not carcino-

Eleven weeks later there had been almost no further change. All measurements were made at least in triplicate; the differences in the triplicate measurements were negligible (± 0.005 per cent). Full details of the chemical analysis will be described elsewhere. The determination of zinc with dithizone reference has already been discussed²¹. The preparations of HCA were made up chiefly according to Keilin and Mann²⁰. (Electrophoresis at pH 8.6 (veronal sodium/veronal buffer); voltage 150 V was carried out for 23 h.) The electrophoretic work was performed in the biochemical department of this labor-

Table 3

	bound zine	zinc	bound zinc
Storage, only oil added The same, but anthracene added The same, but 1,2-benzanthracene added The same, but 1,2,5,6-dibenzanthracen	0·11 ⁴ % 0·11 ⁴ % 0·13%	0·18*% 0·18*% 0·18%	0·07% 0·07% 0·05%
added	0.15*%	0.18%	0.025%

As for Table 2, but with distilled deionized water after storage for 5 weeks at 37° C.

To sum up, it can be said that an important side effect in cases of carcinoma is the decrease in the content of zinc (compared with that in healthy people) in tissues, blood and so on. This element occurs with two different types of bonding in the metallo-protein carbonic anhydrase, an enzyme controlling the transport of carbon dioxide. The difference between firmly bound and loosely bound can be determined with dithizone. The amount of firmly bound zinc can be related to fraction C (Fig. 1) into which carbonic anhydrase can be separated, in addition to the fractions B and A, by paper electrophoresis.

The amount of firmly bound zine is the principal correlative of the activity of the enzyme. This amount can be reduced either by irradiation with X-rays of a frequency corresponding to the zine absorption band or in vitro by chemical reaction with carcinogenic agents. The greater the carcinogenic effect of these substances, the more of the firmly bound zinc is loosened and the smaller the activity of the enzyme.

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Effect of Valinomycin on Net Sodium and Potassium Transport in Ehrlich Ascites Tumour Cells

Gordon et al.¹ speculated that in the mitochondria of intact tumour cells there is a mechanism dependent on valinomycin for the uptake of potassium directly from the cell environment. This mechanism is thought to act independently of the plasma membrane potassium ion transport system. It is well known that in the presence of valinomycin isolated mitochondria accumulate large amounts of potassium ions². Thus the demonstration of potassium ion uptake which is independent of the plasma membrane localized mechanism in the intact cell would be very interesting.

To test the hypothesis that valinomycin could stimulate the mitochondria of intact cells to accumulate potassium ions from the cell environment, cellular respiration, net flux of sodium and potassium ions, and ATP content were measured with the plasma membrane transport system operative or inhibited. The results reported here show that in cells depleted of potassium ions, valinomycin was incapable of stimulating their uptake or the extrusion of sodium ions. Rather, in the presence of this antibiotic, the cellular ATP decreased rapidly with a concomitant loss of potassium ions and gain of sodium ions. The data therefore indicate that only the transport system of the plasma membrane is able to move external potassium ions into the intact cell.

Experiments were performed with Ehrlich-Lettré ascites tumour cells that were maintained in HA/ICR Swiss mice by weekly transplantation. Methods for the preparation of cell suspensions have been described previously³. In some experiments, cell suspensions $(2-3 \times 10^7)$ cells/ml.) were maintained in potassium-sodium Ringer solution³ under 100 per cent oxygen, to maintain normal sodium and potassium ion content. In other experiments, cell suspensions were maintained at 4° C in potassium ion free Ringer solution for 5 h to permit the loss of potassium ions and gain of sodium ions. For net flux experiments, cells incubated at 4° C were washed once with cold potassium-sodium Ringer solution, and then quickly warmed to 25° C. Samples of 1 ml. (2-3×107 cells/ml.) were removed sequentially and the cells were washed quickly in cold isosmotic choline chloride. Sodium and potassium ions in the cell were measured by flame photometry, and ATP was measured fluorometrically4. Oxygen consumption was measured with a Clark oxygen electrode and a chart recorder. (107 cells corresponded to 24 mg wet weight, 4.7 mg dry weight and 3.3 mg protein.)

The effect of valinomycin on oxygen consumption is shown in Table 1. The addition of valinomycin to cells with a normal content of potassium and sodium ions,

Table 1. INFLUENCE OF VALINOMYOIN ON THE RESPIRATION OF NORMAL CELLS AND CELLS DEPLETED OF POTASSIUM IONS

Normal cells: Control rate	oxygen consumption Additions	(μmoles/10 [†] cells/h) Rate
1·07 1·01	Val. KCl	1.62 0.990
1.10	Then Val. Oligo. Then Val.	1.58 0.297 1.05
Depleted cells: Control rate	oxygen consumption Additions	(μmoles/10 ⁷ cells/h) Rate
0-490	Val.	0.520
0.450	Then KCl KCl	1·20 0·890
0.475	Then Val. KCl	1·10 0·890
(Ouabain present)	Then Oligo. Then Val.	0·210 0·900
0·500	KCl Then Val.	0·550 0·880

Normal medium was potassium-sodium Ringer solution; that of depleted cells was Ringer without potassium ions. Potassium was added to a final concentration of 5 mmolar. Valinomycin was added to $3\times10^{-3}~\mu g/10^{7}$ cells, oligomycin to $0.8~\mu g/10^{7}$ cells, ouabain to 5×10^{-4} molar. A volume of 1 mi. of cell suspension (5×10^{7} cells) was added to 4 ml. of medium. The temperature was 25° C.

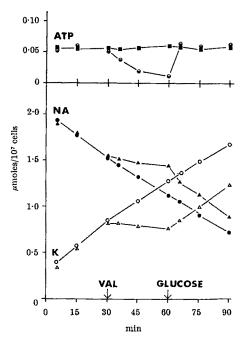


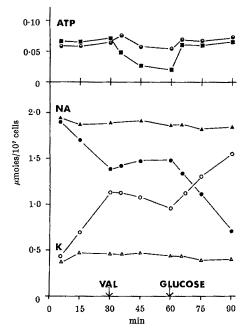
Fig. 1. Effect of valinomycin on net transport of sodium and potassium ions and on ATP content. Two flasks of cells depleted of potassium ions (2·5 × 10⁷/ml.) in potassium-sodium Ringer solution were warmed from 4° to 25° C at zero time. At the time indicated valinomycin (3 × 10⁻³ μ g/10⁷ cells) was added to the experimental flask, and glucose (final concentration 6 mmolar) was added to both flasks. Control: (K) — O — O, (Na) — • (ATP) — Experimental: (K) — \triangle — \triangle , (Na) — • (ATP) — • (ATP) — • (Na) — • (Na) — • (ATP) —

which oxidized endogenous substrate, was a prompt stimulation of respiration. Furthermore, valinomycin when added to oligomycin-inhibited cells caused an immediate release of inhibition. Addition of valinomycin to cells containing few potassium ions and many sodium ions (temperature changed from 4° to 25° C) also resulted in a stimulation of respiration and a release of oligomycin inhibition. In addition, the inhibition of respiration by ouabain was relieved by valinomycin. The effect of valinomycin on respiration and its dependence on external potassium ions confirm earlier observations¹.

In the presence of valinomycin isolated mitochondria can accumulate many potassium ions from the environment, and so experiments were carried out to determine whether valinomycin could stimulate the accumulation of potassium ions in intact depleted cells of these ions.

Fig. 1 shows that when cells depleted of potassium ions were warmed from 4° to 25° C, they accumulated these ions and extruded sodium ions. When, however, valinomycin was added the cellular ATP content declined and the net cation transport was inhibited. Addition of glucose to this system restored cellular ATP (by glycolysis) to the control value which then supplied energy for the accumulation of potassium ions and extrusion of This finding is consistent with other work sodium ions. which showed ATP to be essential for cation transport in these cells. Fig. 2 shows the inhibitory effect of ouabain on net sodium and potassium ion transport, and the inability of valinomycin to relieve the inhibition. Ouabain alone had no effect on the ATP content, but when valinomycin was added there was a significant decrease. Glucose restored ATP to the control value, but this did not result in the accumulation of potassium ions or the extrusion of sodium ions. It follows that even in the presence of adequate ATP valinomycin was unable to induce mitochondrial uptake of potassium ions from the medium or extrusion of sodium ions from the cells.

We suspected that oligomycin specifically inhibited the plasma membrane cation transport system (similar to ouabain), and so we have investigated its effect on net transport and ATP content. The data in Fig. 3 demonstrate that potassium uptake and extrusion of sodium were inhibited because of reduced ATP content. The addition of valinomycin, sufficient to relieve respiratory inhibition, resulted in further loss of potassium ions and gain of sodium ions, but had little effect on the residual ATP content. The addition of glucose to this system restored the ATP content and initiated



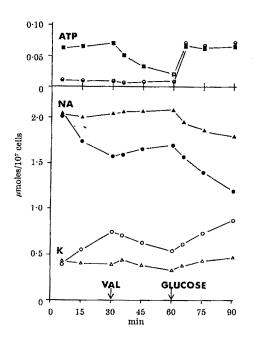


Fig. 3. Effect of valinomycin in the presence of oligomycin on net transport of potassium and sodium ions and ATP content. See Fig. 2 for a description of the experimental procedure and an explanation of the symbols. Oligomycin $(0.8~\mu g/10^7~{\rm cell}_3)$ was added to the experimental flasks.

the extrusion of sodium ions and the accumulation of potassium ions. This suggests that oligomycin does not act exclusively at the level of the plasma membrane, but rather this antibiotic acts at a mitochondrial site to inhibit ATP synthesis.

The results reported here are consistent with the suggestion that valinomycin stimulates the activity of mitochondrial ATPase2, which results in increased respiration. This enhanced activity is thought to utilize rapidly the available ATP and thus inhibit the plasma membrane cation transport system. The restoration of net fluxes after the addition of glucose indicates that valinomycin did not directly interfere with the transport mechanism, but that this antibiotic caused a rapid dissipation of cellular energy reserves. Ouabain, however, specifically inhibited net transport of sodium and potassium ions. Valinomycin was unable to overcome this inhibition even when adequate cellular ATP was present, and so we conclude that there is no path for the uptake of potassium ions from the medium directly to the mitochondria and independent of the plasma membrane transport system in the ascites tumour cell.

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Gel Permeation Chromatography: Gel Preparation and Packing Technique

Since the original publication by Moore¹, gel permeation chromatography has been widely recognized as a rapid means of assessing the molecular weight distributions of polymeric materials soluble in organic solvents. While the use of this technique, principally based on the reliable commercial instrument², is well established, reference in published work to the preparation of the cross-linked polystyrene gel, and its packing into columns with high plate count, is very brief. Some information is to be found in the writings of Moore¹ and Altgelt³ and in the review of Johnson, Porter and Cantow⁴. This communication describes how gels of varying porosity may be prepared, and packed into columns having a plate count within the range 700–1,300 theoretical plates/ft.

The gels were prepared using a suspension polymerization of a mixture of styrene, divinyl benzene and diluents. Trial experiments quickly showed that a workable pressure drop of less than 40 p.s.i. across a 4 ft. column was achieved with gel particles lying within the range 15 to 90µ. A partially hydrolysed polyvinyl acetate ('Gelvatol' 30-60) was used as a suspension stabilizer, as suggested by Winslow and Matreyek⁵. A typical preparation was as follows: a solution of 20 g of 'Gelvatol' in 1,200 c.c. of water was placed in a 2 l. flask fitted with a collapsible paddle measuring 2.5 in. by 0.5 in. The flask was heated to 70° C and a mixture of styrene, divinyl benzene (55 per cent divinyl benzene plus 45 per cent ethyl vinyl benzene) and diluents, having a total volume of about 300 c.c., were added. The monomers had been previously freed from stabilizer by washing in alkali, and 1 g of benzoyl peroxide was used as initiator. Polymerization was allowed to proceed at 80° C for 7 h using a stirring

rate of about 500 r.p.m. After cooling and decanting, the gel particles were washed with hot water and acetone in order to remove stabilizer and organic materials.

In spite of the experimental conditions the particles were found to be agglomerates and these were broken down by ball milling for 3 h in a polyethylene bottle containing 0.25 in. porcelain balls. The milled particles were suspended in acetone and the suspension sieved through a 63 or 90 μ sieve. The suspension passing through the sieve was degassed by boiling and cooling in a stoppered vessel. The fine particles (less than 15 μ) were removed by sedimentation in acetone. A 2 l. beaker, filled to a depth of about 5 in., was used to disperse the gel, and after standing for about 30 min, the finer particles were decanted away. This separation process was repeated three times.

Microscopic examination of these particles showed them to be irregular in shape, not spherical as neight be expected. Because they could be packed into columns having high plate counts, however, the lack of clearly defined shape appears to be of no consequence.

The columns were constructed from lengths (4 ft.) of stainless steel tubing with a $\frac{3}{4}$ in. external diameter and 20 s.w.g. walls. End-fittings of the type illustrated in Fig. 2 were fashioned from modified Wade couplings, which incorporate a 'Porosint' sintered bronze disk of $\frac{3}{4}$ in. diameter and 1/16 in. thickness, of grade D, with a maximum pore size of 25μ . A brass plug A, through which passes a stainless steel hypodermic tube (1/16 in. external diameter), rests on top of the sinter B. The plug A has both radial and concentric grooves in an attempt to maintain plug flow, and is slightly dished with respect to the sinter. The connecting tubing is brazed into part C, which on tightening presses A into intimate contact with B, and seals on the shoulder D. All parts are a good tight fit.

The pressure packing apparatus is shown in Fig. 3, where all the necessary details are given. It is based on the design first given by Waters². The micropump (D.C.L. type M) is capable of delivering zero to 3,100 c.c. per hour. The apparatus may be clipped to a rigid back board during use.

A typical packing procedure was first to degas 2 l. of tetrahydrofuran (THF) by boiling and cooling, 0·1 per cent of 2:6-ditertiarybutyl-p-cresol being added as

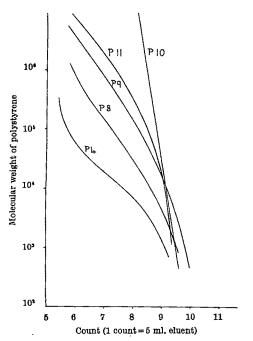


Fig. 1. The permeability curves for the gels prepared as described.

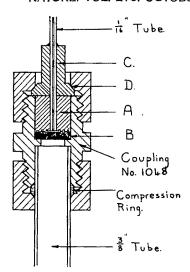


Fig. 2. Details of the end-fittings designed for the chromatographic columns.

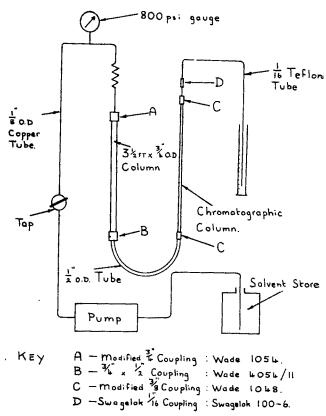


Fig. 3. Details of the column packing assembly used for the packing of the chromatographic columns with crosslinked polystyrene gels.

stabilizer, and placed in the solvent store. About 100 c.c. of dry gel was placed in a balanced solvent mix (a mixture of acetone and perchloroethylene in approximate ratio 6:5 by volume). The resultant slurry was degassed by boiling and cooling, a small sample centrifuged, and thus the solvent mix was accurately balanced against the density of the gel. This accurately balanced mix was then added to the supply tube, and the remaining space filled with balanced mix. The pump was started so as to deliver 1 c.c. per minute, and when the THF emerging from the supply line was free from air, the connexion was made to the supply tube. Pumping was maintained at 1 c.c./min until about 10 c.c. had been collected, and then the pumping rate increased until the gauge indicated

550 p.s.i. During the subsequent packing operation the pump stroke was decreased in order to maintain the recorded pressure at the same level. After passing 400 c.c. of solvent pumping was stopped and a 10 c.c. hypodermic syringe, filled with THF, attached to the 'Teflon' tubing at the exit. This syringe was held at a higher level than the column, and the supply tube was disconnected from the U-tube. The column and U-tube were then inverted, and the U-tube removed. A little gel was taken from the U-tube and placed on the porous disk of the end-fitting finally used to close the column. The hypodermic syringe was then depressed to pass THF through the end-fitting before finally sealing the column with bellows containing THF.

Table 1. Permeability of Gels prepared using 6.7 per cent styrene, 33.3 per cent divinyl benzene, and 60 per cent diluent

Gel	P4	P 8	P9	P10	P11
Per cent styrene	6.7	6.7	6-7	6.7	6-7
Per cent divinyl benzene	33-3	33.3	33-3	33.3	33.3
Per cent toluene	15				
Per cent n-dodecane	45		_	*****	_
Per cent diethyl benzene		16	10	5	7.3
Per cent isoamyl alcohol		44	50	55	52-7*
Porosity limit A	2×10^{3}	3×10^{4}	2×10^{5}	1012	5 × 10 ⁶

All percentages relate to composition by volume.

* A mixture of 78 per cent 3-methyl butanol and 22 per cent 2-methyl * A mi butanol.

The permeability curves for these gels packed into a 4 ft. column are given in Fig. 1.

The estimated porosity of the gels prepared as described here is given in Table I. The plate counts, determined by injecting 1 per cent trichlorobenzene in THF, for 15 sec at a flow rate of 1 c.c./min, with the refractometer at normal sensitivity, fell within the range 700-1,300 theoretical plates/ft.

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Variations dependent on Age and Diet in the Metabolism of 7,12-Dimethylbenz[a]-anthracene by Rat Liver Homogenates

7,12-DIMETHYLBENZ[A]ANTHRACENE (DMBA) severe adrenocortical necrosis when administered intragastrically to 50 day old rats1, but it has no effect on the adrenal glands of 25 day old animals2. The activity of the hydrocarbon seems to depend on the formation of an active metabolite, probably 7-hydroxymethyl-12-methylbenz[a]anthracene (7-OHM-12-MBA)^{3,4}; the isomeric 12hydroxymethyl-7-methylbenz[a]anthracene (12-OHM-7-MBA) is a metabolic product of DMBA but has no effect on the adrenal³. Damage to the adrenal can be prevented by pretreating animals with compounds that enhance hepatic detoxicating enzymes^{5,8}, apparently by increasing the yields of inactive ring-hydroxylated products, such as 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene, at the expense of those hydroxylated on the methyl groups and also by inactivating 7-OHM-12-MBA itself by ring hydroxylation, to yield products such as 8,9-dihydro-8,9-dihydroxy-7-hydroxymethyl-12-methylbenz[a]anthra-Treatments which reduce hepatic detoxicating enzymes also prevent DMBA-induced adrenal necrosis8, probably by reducing the overall metabolism of the hydrocarbon. It seemed possible that the differing susceptibilities to DMBA-induced adrenal necrosis of the 25 and 50 day old rats developed because animals of the two age groups have different activities of hepatic enzymes, and so we have investigated the in vitro metabolism of DMBA by liver homogenates prepared from rats of different ages.

Livers from at least two rats of the same age and sex were pooled and homogenates prepared by methods previously described. DMBA (generally labelled with tritium, specific activity 400 mc./mmole) was added in ethanol (3.8 × 106 c.p.m. in 0.1 ml.) to duplicate portions of homogenate (each equivalent to 1 g of liver) and the mixture was incubated at 37° C for 30 min. Unchanged hydrocarbon and the metabolites formed were extracted with ethyl acetate and separated by thin-layer chromatography on plates coated with silica gel G and developed for 15 min in benzene-ethanol (9:1, v/v)7. The 8,9-dihydro-8,9-dihydroxy derivative of DMBA showed as a violet fluorescent band of R_F 0.30 when examined in ultra-violet light. The 8,9-dihydro-8,9-dihydroxy derivatives of 7-OHM-12-MBA and 12-OHM-7-MBA could not be detected by this means. These derivatives had R_F 0.21 in the solvent system used, and a band in this region of the chromatogram and also the band at R_F 0.30 were removed. The radioactivity of the silica gel was measured by liquid scintillation counting.

The results show that the ability of rats to metabolize DMBA, as measured by the formation of 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene (Fig. and to metabolize the 7-OHM-12-MBA and 12-OHM-7-MBA, as measured by the formation of the corresponding 8,9-dihydro-8,9-dihydroxy compounds (Fig. 2), increases sharply in rats between 15 and 25 days old and then falls to a basal value in adults. Compounds known to increase DMBA and 7-OHM-12-MBA metabolism⁷ also protect against the adrenocorticolytic effect of the hydrocarbon10,11, probably by reducing the effective concentration of 7-OHM-12-MBA, and it seems likely that the absence of adrenal damage in 25 day old rats treated with DMBA is the result of an increase in DMBA metabolism to yield

ring-hydroxylated products.

No increase in the ability of rats to metabolize DMBA as compared with control animals was found when liver homogenates were prepared from litters of 25 day old unweaned rats whose only source of food was their mothers' milk from 15 days old (Table 1). Similar results were obtained from litters of 25 day old rats that had been fed sweetened condensed milk from 15 days old. When, however, liver homogenates from unweaned or milk-fed 25 day old rats, subsequently transferred to a diet of rat cubes (Diet 86, Plowco Feeds, Ltd.) for 10 days, were used, DMBA metabolism increased (Table 1).

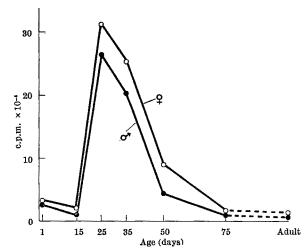


Fig. 1. Formation of 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[aj-anthracene following incubation of DMBA with liver homogenates prepared from rats of different ages. Each point represents the mean of two determinations.

Table 1. FORMATION OF THE 8,9-DIHYDRO-8,9-DIHYDROXY METABOLITES OF 7,12-DIMETHYLBENZ[A]ANTHRACENE AND OF ITS MONOHYDROXYMETHYL DERIVATIVES WITH RAT LIVER HOMOGENATES

Group	Age* (days)	Sex	Formation of 8,9-dihydro-8,9-dihydroxy-7,12- dimethylbenz[a]anthracene (c.p.m. × 10 ⁻⁸)	Formation of 8,9-dihydro- 8,9-dihydroxy derivatives of the monohydroxy- methyl compounds (c.p.m. × 10-3)
 Rats from litters weaned to cube diet at 15 days 	25	M	313 ± 2	88 ± 9
old	35	F M F	$\begin{array}{c} 265 \pm 5 \\ 272 \pm 6 \\ 205 \pm 6 \end{array}$	85±4 98±8
2. (a) Rats from litters denied access to solid food	25	M	15±2	$\begin{array}{c} 92 \pm 6 \\ 4 \pm 1 \end{array}$
from 15 days old		\mathbf{F}	37 ± 2	$\overset{\bullet}{2}\overset{\circ}{\pm}\overset{\circ}{1}$
(b) Rats from same litters weaned to solid cube	35	M	265 ± 3	$9\overline{5}\pm\overline{5}$
diet at 25 days old	0.5	F	248 ± 6	55 ± 6
3. (a) Rats from litters fed condensed milk from 15	25	M	9 ± 1	4±1
days old	or	F	8±1	3 ± 1
(b) Rats from same litters weaned to solid cube	35	M	84 ± 3	17 ± 2
diet at 25 days old		Ŧ.	98 ± 9	22 + 3

^{*}Age of rats when liver homogenates were prepared.

In adult animals, both DMBA and 7-OHM-12-MBA are metabolized by hepatic microsomal hydroxylating systems that are enhanced by pretreatment with some foreign compounds7. One interpretation of these results is that the increase in the metabolism of DMBA by liver from 25 day old rats is caused by an induction of microsomal enzymes by dietary components first ingested during weaning. The fact that this increase in DMBA metabolism can be delayed in rats restricted to milk diets supports this view. Further support is provided by the results of recent studies of hexobarbital metabolism in mice where variations with age have been examined12. Hexobarbital sleeping time was minimal in 21 day old animals and this was found to coincide with maximum liver metabolism: in some earlier work with this drug these effects were not noted13. There is some evidence that, in rats, the toxicity of DMBA increases between 25 and 50 days1: this also supports the concept of enhanced microsomal enzyme activity in weanling animals.

The apparently transient nature of the increase in DMBA hydroxylating activity of rat liver at weaning is also of interest in view of the report that the chronic administration of DDT to rats causes only a temporary increase in the aniline hydroxylating capacity of the liver: in these same animals DDT caused a continuous increase in the liver enzymes metabolizing hexobarbital, aminopyrine and p-nitrobenzoic acid14.

The increases in the hepatic metabolism of DMBA in rats and of hexobarbital in mice found to coincide with weaning indicate that the possibility of diet-dependent variations in microsomal enzyme systems should not be overlooked, particularly where experiments cover the normal weaning period for the species used.

We thank Professor E. Boyland for his interest and Miss J. Brangwin and Mr A. Hewer for technical assist-

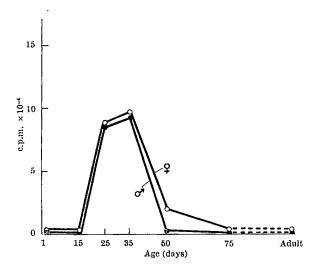


Fig. 2. Formation of the 8,9-dihydro-8,9-dihydroxy derivatives of 7-hydroxy-12-methylbenz[a]anthracene and 12-hydroxy-7-methylbenz-[a]anthracene from DMBA as in Fig. 1.

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Multiple Analyses on a Single Gel **Electrophoresis Preparation**

GEL electrophoresis has been shown to be a rapid means of producing high resolution separations of proteins1,2, although some of the resolved components may be artefacts when the so-called disc technique is used3. It is difficult to correlate the results of replicate disc separations because of variations from one gel cylinder to another4, but by applying multiple samples to a single acrylamide gel slab⁵, using the vertical acrylamide gel slab technique originally described by Raymond⁶, individual components of many samples processed together on the same gel slab can be traced across sample boundaries with high reliability, using the principle of continuity8.

The equipment used is the vertical gel electrophoresis cell of Raymond⁶ which provides a gel slab 125 × 175 mm between two parallel water-cooled plates 6 mm apart (Fig. 1). Direct water-cooling of the gel significantly improves the reproducibility from run to run, and makes possible the use of low temperatures (for example, for use with thermally sensitive proteins) or high temperatures (for example, when it is desirable to keep 12 molar urea in solution). The design of the cell makes it unnecessary to rely on gaskets or dialysis membranes to retain the gel during polymerization. The cell is easily dismantled to remove the gel slab at the end of the electrophoresis run. When both running and spacer gels are to be used1,2, the bottom of the column is occluded by polymerizing a plug of running gel in place, with the cell supported at an angle of 45°. The column is then placed

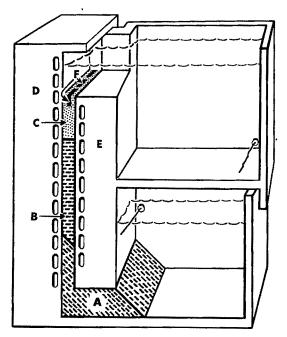


Fig. 1. Vertical gel electrophoresis cell, cross-section showing position of gel slab. A, Gel plug; B, running gel; C, spacer gel; D, outer cooling plate; E, inner cooling plate; F, sample slots.

vertically and filled with running gel solution to the desired level. Water is overlaid to ensure a planar interface between running gel and spacer gel. After polymerization of the running gel, the column is rinsed out and filled with spacer gel solution, which is polymerized in the horizontal position, using a slot-form as described. After polymerization, the gel column is placed vertically, the excess gel and slot form removed, and the electrode chambers filled with buffer. At this point, catalyst residues can be removed from the gel, if necessary, by pre-running with no samples in place. The column is now ready to receive the samples into the sample slots. The use of slots permits up to sixteen samples to be run in parallel under identical conditions without intermixing, and prevents edge effects from affecting the uniformity of the patterns.

Several points deserve particular mention.

- (a) The sample is applied in solution, avoiding the artefacts and loss of sample components which occur when the sample is polymerized into a sample gel. The density of the sample solution must be greater than that of the electrode buffer.
- (b) In order to maintain a straight buffer front, the sample slots must be filled to the top of the gel with a solution of approximately the same buffer composition and pH, and with nearly the same viscosity effect, as the spacer gel. These requirements, and also the density requirement, are conveniently realized by diluting the sample solutions with polymerized acrylamide solution (omitting cross-linking agent) dialysed against the appropriate buffer until free of catalyst residues. A sample is diluted with this buffered acrylamide solution so that a volume sufficient to fill the sample slot contains the appropriate amount of protein. The subsequent electrophoretic separation is performed by the use of standard techniques.
- (c) In order to preserve direct intercomparability of pattern, especially when individual components are to be identified in several different samples using the principle of continuity, staining techniques must be used which do not require cutting the gel slab apart.
- (d) For application of different stains, it is usually possible to immerse selected portions of the slab in separate staining solutions without cutting it apart.

Alternatively, an agar layer containing the appropriate reagents can be applied to selected portions of the slab. In many systems it is possible to apply different staining solutions in sequence to the entire slab. Fig. 2A shows the appearance of one such slab successively stained with



Fig. 2. A, Serum protein patterns, successively stained for lattic dehydrogenase (tetrazolium reaction), haptoglobins (peroxidase reaction), and finally general protein (light green SF). B, Diagrammatic representation of A. The hatched areas correspond to lactic dehydrogenase, solid black bands to haptoglobins, and outlined areas to material staining only with light green SF.

E

a lactic dehydrogenase, a peroxidase and a general protein stain.

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Observations on the Metabolism of Fluoride in Acacia georginae and Some Other Plants

When working with homogenates of Acacia georginae in an attempt to trace the pathway of fluoroacetate synthesis, we found that there was a loss of fluoride, and we extended our experiments to some other plants to study this effect. This communication describes experiments which have led us to believe that plants can convert fluoride, possibly in part, to a volatile form.

A plant of Acacia georginae, 15 months old and about 9 in. high, grown from seed, was used. A homogenate was made from 4.6 g of mixed leaves and small roots in the mortar as previously described. The homogenate and washings were reinforced with specially prepared sodium pyruvate (1.0 mmole), manganese chloride (0.1 mmole), potassium dihydrogen phosphate (0.01 mmole brought to pH 7.0 with sodium hydroxide), and ATP (0.1 mmole) to make a total volume of 10.8 ml. Three millilitres was kept as a control, and sufficient fluoride was added to the remainder to make a concentration of 40·0 μg/g of plant. The latter sample was then incubated at 30° C with a slow passage of 95 per cent oxygen with 5 per cent carbon dioxide over it for 2 h, after which the two samples were deep frozen. After standing overnight, the homogenates were allowed to thaw and centrifuged at about 50,000g for 20 min. The separating solid was washed once with water. Determinations of fluoride were made on both the control and the sample with added fluoride: estimations of inorganic F- by diffusion and total F- by diffusion after combustion in the supernatants were made by the technique of Hall² and the total fluoride in the "solid" was also determined. The results are given in Table 1.

Table 1. METABOLISM OF FLUORIDE BY A REINFORCED HOMOGENATE OF A. georginae

Fluoride ($\mu g/g$ wet weight)						
Fluoride added	Inorg. F-	Total F	Solid F-			
Nil	2.4	2.3	2.3			
40.0	24.0	24.0	7.2			
Loss of fluoride adde	d-33.8 per cen	ıt.				

Making allowance for the fluoride originally present, the fluoride recovered in the total F-+solid was 26.5 µg/g, making a loss of fluoride of 33.8 per cent. It is to be noted that this older plant showed no synthesis of organically combined fluoride. Similar and even greater losses of fluoride have been observed in other experiments with homogenates of the Australian plant.

The phenomenon observed with A. georginae led to a similar trial with homogenates of other plants taken at random. The results are given in Table 2 and are calculated in the same way as Table 1. Losses of fluoride varied from 15 to 52 per cent.

There seems to be no doubt that the phenomenon is not confined to the Acacia, and may be rather general. An attempt to identify the volatile constituent is in progress.

We realize that our conclusions depend entirely on the accuracy of our methods, so we have spent much time in deciding that these losses are not due to experimental error. Errors caused by the fluoride combining with glass were eliminated by using 'Pyrex'. We proved that silicates and various metals combusted with F- did not interfere and also that our colorimetric stage in Hall's technique² was insensitive to acetaldehyde (1.0 mg) and formaldehyde (0·1 mg).

Our final figures are a summation of four separate estimations—that for total fluoride, and for fluoride in the separated solids, except in Asclepias, where no solid separated (see footnote to Table 2). Each figure is the average of satisfactory duplicates. Even if we assume that there is an underestimation of 1.0 per cent for the homogenate plus added fluoride and a similar overestimation for the residual fluoride in the control, this would only add up to a total of 8 per cent. Allowing a figure of 5 per cent for possible losses by adsorption on the glass, errors of 15 per cent could be reached by supposing that the errors are all additive. Even so, an experimental error of 15 per cent still leaves a substantial loss in many experiments. We have not yet related the losses to different stages of growth, so the difference between the two experiments on Asclepias, taken at different times, is not significant.

We can find no reference in the literature to the conversion of added fluoride to a volatile form by plants, and conclude that the observations are new. We have made some attempt to prove volatility of fluoride by using the radioactive isotope fluoride-18, supplied by the MRC Cyclotron Unit. The short life of this element of about 110 min precludes investigations for longer than 6-7 h. There is also the handicap that we do not yet know the nature of the volatile fluorine compound. Nevertheless, we have passed the mixture of oxygen and carbon dioxide from our homogenates of both A. georginae and Asclepias successively through water, ethanol and mercury per-chlorate, and at the end of 1 and 1.5 h, respectively, a significant amount of radioactivity has been detected in these solutions. The amount is small, but we know from other experiments that, for example, the absorption of methyl fluoride is very inefficient, so that the experiments again show the presence of volatile fluoride.

Our experiments have been made on homogenates in vitro, so that it has still to be proved that any effect occurs in vivo, or that the losses are all explained by volatility. It is natural to associate the loss of F- with the formation of ethylene which has been proved to be formed in our homogenates³. The aim in our research on the Acacia plant has been to find the biochemical path for the synthesis of fluoroacetate. We think it possible that

Table 2. LOSSES OF FLUORIDE FROM HOMOGENATES OF VARIOUS PLANTS

Plant	Part taken	Weight (g)	Volume (ml.)	F- added (µg/g) wet weight	Per cent fluoride disap- pearing
Asclepias curassavica	Leaves	3.1	7.0	55.3	31.0
(6 months)	Leaves	2.9	5.3	26.2	52.5
Pea: 'Laxton Superb	,	-			
(1 month)	Seedlings	16.0	12.0	90-5	28.0
Early 'Gradus'	Seeds	12.6	9.0	11-65	26.0
Poa annua	Whole seedling	11.0	8.0	10.42	43.5
•	Seedling	5.64	8.0	33.6	14.5
Felicia pappei	Whole	7.0	8.5	15.9	41.5
variety Gracilie	Not in flower	25.8	25.0	40.5	36-0
Aquilegia canadensis	Whole	$2 \cdot 2$	5.0	31.8	46.0
(8 weeks)	Whole	3.35	8.0	40.0	15.0
Acacia armata	Leaves and				
	fine roots	3.0	6.2	20.2	33.0
Thea chinensis	New leaves and				
	fine roots	4.3	8.0	35-4	35.0
Doronicum	Flowers	7.6	11.0	35.1	39.5

In the case of Asclepias, it was possible to sample the treated homogenate without centrifuging and so to eliminate the extra error due to separate estimation of the solid.

the conversion to a volatile step may be the first stage in the metabolism of fluoride and that it may be a general one for plants. The formation of fluoroacetate would then be caused by a special mechanism in the plants which carry out this step.

If plants in general can contribute to the air around them in vivo one or possibly more volatile compounds containing fluorine, it is fortunate that such compounds. though anaesthetic in high concentration, are on the whole non-toxic. This has been proved for gases like vinyl fluoride4,6.

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IMMUNOLOGY

Increased IgG Synthesis during the Induction of Immunological Paralysis in Adult Guinea-pigs

It is well known that after immunological stimulation, animals produce non-specific gamma globulins as well as specific antibody1. Quantitative studies have shown that injection into guinea-pigs of an antigen mixed with Freund's adjuvant causes a marked increase in the serum concentrations of γ_1 and γ_2 globulins, only part of which can precipitate with the antigen2. On the other hand, the induction of immune paralysis in adult guinea-pigs is accompanied by a transient immunological unresponsiveness to unrelated antigens. A possible explanation for this phenomenon could be that immunological paralysis may start by a defective commitment of immunologically competent cells, which does not result in the production of specific antibody. The aim of the present work was to find out whether the induction of paralysis also results in an increase of γ_1 and γ_2 globulins which might account for some kind of commitment of the competent cells.

Random bred adult guinea-pigs weighing 350-500 g were divided into groups and given daily intraperitoneal injections of bovine serum albumin (BSA) according to the following schedules; group 1, 200 µg/day for 12 days; group 2, 100 mg/day for 20 days; and group 3, 1 g/day for 25 days. Blood samples were collected before the beginning of the treatment (day 0) and then at regular intervals. In all groups, including the control group, an immunizing injection of BSA (I mg) mixed with complete Freund's adjuvant was given to both hind foot pads on Serum anti-BSA titres were determined by passive haemagglutination using bisdiazotized benzidine BSA conjugated to rabbit red blood cells4. The γ1 and γ₂ globulin content in the serum samples was determined by the antibody-agar plate technique⁵ as described before2. The results reported are the average values of the analysis of mixed sera (5 to 10) of each group of animals.

In normal adult guinea-pigs the serum content of γ_1 globulin is roughly one-tenth of that of γ_2 globulin, that is 1 mg/ml. and 10 mg/ml., respectively (Fig. 1, day 0). Under the influence of the daily treatment with various amounts of BSA, the serum content of both γ_1

and γ_2 globulins increases progressively until day 30 and then declines. Although the percentage increase of Y1 globulins is much more important than that of γ_2 globulins, the absolute production of γ_1 is about half that of γ_2 globulins: (1) 2.5 mg/ml. versus 5.5 mg/ml.; (2) 4.5 mg/ml. versus 8 mg/ml.; and (3) 7.5 mg/ml. versus 10.5 mg/ml. in the various schedules of treatment. It is apparent from these figures and from Fig. 1 that the total increase of IgG in the treated animals was proportional to the total quantity of BSA used in the treatment.

Irrespective of the amount of BSA injected, in no case did the treated guinea-pigs produce anti-BSA antibody which could be detected either by Ouchterlony plates or

by passive haemagglutination.

On day 45 all the treated animals as well as a control group were again injected with BSA (1 mg) mixed with Freund's complete adjuvant. This injection resulted in a moderate increase of both γ_1 and γ_2 globulins in the control group (Fig. 1d), and also in the appearance of high titres of haemagglutinating antibody. This secondary injection of BSA produced much lower passive haemagglutination titres in the animals of groups I and 2, showing that a degree of partial paralysis had been induced in these animals by pretreatment with the same antigen. Only in group 3, which had been treated with 1 g of BSA each day for 25 days, was a complete and long lasting state of immune paralysis achieved. In the partially tolerant animals, the challenging injection of BSA produced a fresh increase of γ_1 and γ_2 globulins, comparable with that produced in the control animals. In the completely tolerant animals, however, no such increase was produced and the concentration of γ_1 and γ_2 globulins continued to diminish down to the physiological level (Fig. 1c). It seems likely therefore that "non-specific" γ_1 and γ_2 globulin production is a regular sequel of specific antigenic stimulation and is only suppressed if the animal is fully tolerant to that antigen.

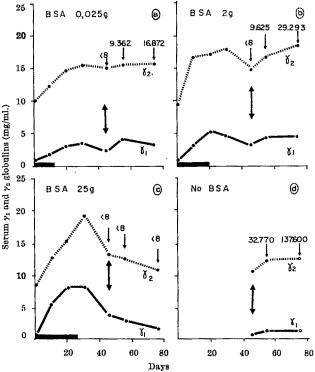


Fig. 1. Effect of treating guinea-pigs with various quantities of BSA on serum γ_1 and γ_2 globulins. \blacksquare , Duration of treatment; \spadesuit , injection of BSA (1 mg) mixed with Freund's adjuvant; 1. passive anti-BSA haemagglutination titre (for further explanations see text).

It is unlikely that the gamma globulins produced during primary treatment and which lack anti-BSA specificity are antibodies directed against impurities contained in the BSA solution. Immunoelectrophoretic analysis of experimental sera tested against whole bovine serum detected only two weak lines of precipitation in the region of alpha globulins. On the other hand, precipitin tests carried out with these serum samples and bovine serum remained negative or very weakly positive. It is known that the limiting sensitivity of these techniques is about 15-30 μg/ml. of antibody protein. This quantity, compared with the 10-20 mg/ml. of new gamma globulin production, leaves little doubt that the main quantity of new-formed gamma globulin is not antibody to the antigen(s) injected. It is also improbable that the administration of BSA provoked an increase in the bulk of natural antibodies, because a secondary type response elicited by unrelated antigens has never been clearly demonstrated.

Injections of Freund's complete adjuvant without added antigen in rabbits have caused a very large increase of 7S gamma globulin. Tests carried out on these sera and a large number of antigens showed that only a small part of the newly formed gamma globulins could be characterized as antibody. Although this gamma globulin may not bear any specific combining site, this must be "virtually unprovable".

Several authors have reported that the injection of sensitizing amounts of soluble proteins often results in induction of immune tolerance rather than in antibody The present findings, although they production8,9. confirm these observations, clearly show that animals regularly respond to these injections with a new production of gamma globulins. Furthermore, this production is proportional to the total antigen given during the primary treatment. It seems therefore that whether the injection of soluble antigens results in antibody production or in immune paralysis, it regularly stimulates lymphoid cells to the hyperproduction of gamma globulin molecules. Perhaps what is decisive in specific antibody production is the form in which the antigen is presented to the lymphoid cells. Recent work indeed suggests that the passage of protein antigen through macrophages greatly enhances the extent of antibody production in a primary response¹⁰, whereas antigen presented in non-phagocytosable form directly produces immune paralysis¹¹. results do not contradict this assumption, but suggest that non-immunogenic antigens can still stimulate animals as evidenced by the production of non-specific gamma globulins, although optimal conditions for antibody production are not met.

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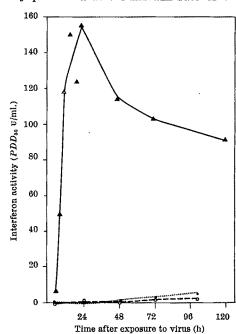
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Loss of Interferon Activity from the Medium of Cell Cultures producing Interferon

THE time course for the production of interferon in various types of cell cultures has been reported before¹⁻⁴. For example, Heller³ found that chick embryo fibroblast-like (CEF) cell cultures infected with chikungunya virus began producing detectable amounts of interferon 3 h after infection. Thereafter, the concentration of interferon increased linearly for the next 21 h, reaching maximum yield 24 h after infection. We have followed the time course of interferon production in CEF cell cultures infected with chikungunya virus, but were more interested in the ultimate fate of the newly synthesized interferon after the maximum yield had been reached.

CEF cell cultures were prepared by the method of Lindenmann and Giffords slightly modified. The production of interferon was induced by infecting the cell cultures with 2 ml. of a 1:100 dilution of stock chikungunya virus produced in mouse brains and subjecting the cultures to a temperature of 37° C. The medium used consisted of Gey's balanced salt solution without serum, 0.11 per cent (w/v) sodium bicarbonate, 0.1 per cent (w/v) yeast extract (Difco), 0.1 per cent (w/v) proteose peptone and 0.1 per cent (w/v) lactalbumin hydrolysate. At various times after infection, the medium from two cell cultures was removed, pooled and stored at 3° C until interferon could be assayed. Before assay, the fluids were kept at 65° C for 30 min to inactivate the virus which was present in the preparation. Interferon was assayed by using the vaccinia virus plaque-reduction method described by Lindenmann and Giffords. One unit of interferon (PDD_{50}) is described as that amount of interferon needed to reduce the number of vaccinia virus plaques to 50 per cent of the number found on control cultures which had not received any interferon.

Production of interferon could be detected as early as 2 h and continued to be produced until 20-24 h after viral infection (Fig. 1). Thereafter, there was a rapid loss of interferon activity from the medium during the next 24 h, followed by a much slower loss for the remainder of the experiment. The initial rapid loss of interferon activity proceeded at a minimum rate of 0.340 U/h/10⁶



cells followed by further loss during the next 72 h at a rate of 0.066 U/h/106 cells. The overall rate of loss of interferon activity was 0·150 U/h/10° cells. Fig. 1 also shows that interferon was not produced when the CEF cultures were inoculated with chikungunya virus that had been heat-inactivated at 65°C for 30 min. No significant interferon activity was detected in the culture medium from uninfected cell cultures.

Several experiments were conducted to determine if this loss of interferon activity was caused by proteolytic enzymes present in the culture fluid. Fluids containing interferon were collected from cell cultures infected with chikungunya virus for 62 and 120 h. Each sample was divided into two aliquots. One aliquot from each sample was heated at 65° C for 30 min and assayed immediately for interferon activity. The remaining aliquots were placed in a waterbath at 37° C for 9 h before heating to 65° C and assaying for interferon. The results of the assays for each of the two aliquots for a given sample were comparable within 4 per cent, which demonstrated the absence of degradative enzymes in the culture medium.

It is interesting to note that the initial rate of loss of interferon activity from the cell culture medium (0.340 U/h/106 cells) is comparable with the rate of uptake of interferon by cells in culture (0.330 U/h/106 cells) (our unpublished work). Thus it seems reasonable to assume that the loss of interferon activity from the culture medium of cells producing interferon is caused by the adsorption of this same interferon by some of the cells in the monolayer, if not by all of them. This loss of interferon would not become apparent until production of interferon had either ceased or slowed down to such an extent that the rate of adsorption exceeded the rate of production.

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PATHOLOGY

Hormonal Pregnancy Tests and Congenital Malformation

DURING a survey of babies born with meningomyelocele or hydrocephalus, one hundred mothers of such children provided histories of the relevant pregnancy. other things they were questioned by one of us (I. G.) as to the drugs which they had taken. The same information was obtained from a matched control group of one hundred mothers recently delivered of healthy Data obtained from the mothers about drugs during pregnancy were checked with the medical advisers. As part of the survey the mothers were asked how their pregnancy was diagnosed. Nineteen mothers in the survey group and four of the control mothers reported having received oral tablets for the diagnosis of pregnancy (Table 1). There was no significant difference between the two groups with respect to any other drugs taken in the first trimester. The average interval between conception and test was 5.6 weeks in the survey group, and 6.2 weeks in the control group.

	Table 1		
	Pregnancy test	No test	
Survey cases	19	81	
Controls	4	96	
	$2^2 > 6.635$	P < 0.01	
	(1 degree of freedom)		

The age of the mothers is shown in Table 2. The survey mothers were on average some 2 yr older than the controls, and there were more older and younger mothers among those who had been given the pregnancy test. Mothers over 35 years of age are more liable to have infants with a malformed nervous system1, and so the tests of significance were repeated leaving out those cases over 35. A high level of significance remained (P < 0.01).

Defects such as meningomyelocele originate before the closure of the neural canal and fusion of vertebral laminae during the second to eleventh week of intra-uterine life. The tests were administered at this time, the tablets most commonly used being 'Primodos' and 'Amenorone Forte'. The recommended dose is one tablet daily for 2 successive days in the case of 'Primodos' and for 3 successive days in the case of 'Amenorone Forte'. If the patient is not pregnant withdrawal bleeding should occur 3 days after the last tablet is given. The test depends on imitating the normal menstrual cycle during which there is an elevation of circulating progesterone and oestrogen followed by a fall which results in menstrual bleeding. Each tablet of 'Primodos' contains 10 mg of norethisterone acetate and 0.02 mg of ethinyloestradiol. 'Amenorone Forte' contains 50 mg of ethisterone and 0.05 mg of ethinyloestradiol. These doses are claimed to be safe and the test, which was introduced in 1953 by the intramuscular route and in 1956 by the oral route, has been considered free of danger if used on women who prove to be pregnant. It is used very frequently. Contraceptive pills. which have been subject to a very careful scrutiny, have a similar composition, but the dose and balance of the constituents are different; for example. 'Norlestrin' contains 2.5 mg of norethisterone acetate and 0.05 mg of ethinyloestradiol. Any effect of these pills on the foetus comparable with that suggested for the pregnancy test would be limited to cases in which they were taken by already pregnant women.

		Table 2		
Maternal	age (yr)	< 20	20-35	> 35
Survey	Test	2	13	4
	No test	5	66	10
Control	Test	1	2	1
	Ma toot	0	88	6

The possibility cannot be excluded that the difference significant at the 1 per cent level between the two groups of mothers whom we questioned might emerge purely by chance. Nevertheless, our observations indicate the need for a more detailed scrutiny of the role of hormonal preparations in the causation of congenital malformations. particularly when taken in the organogenic stages of pregnancy. Full details of this work will be published elsewhere.

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Induction of Tumour Immunity with Tumour Cells treated with Extract of Garlic (Allium sativum)

It is generally believed that in some conditions tumour cells act antigenically, and that immunization of the host animal prevents tumour growth on subsequent challenge with virulent tumour cells. One of the most important problems in this field is to find a suitable and effective method to obtain attenuated tumour cells which still Recently, two effective possess their antigenicity. techniques were reported—the use of irradiated tumour cells1 and the use of tumour cells treated with iodoacetate²—and Ishidate³ has used nitrogen mustard N-oxide for this purpose.

We have found that pretreatment of tumour cells with an extract of garlic (Allium satirum) is effective and that mice injected with these cells developed strong immunity to the same type of tumour cells. One volume of fresh, unwashed Ehrlich ascites tumour cells was mixed with the same volume of extract of freshly ground garlic (5 g of ground garlic extracted with 25 ml. of distilled water) and incubated for 1 h at 37° C. The mixture was centrifuged at 800g for 5 min and the sediment was washed once with two volumes of physiological saline and then suspended in one volume of the same solvent. suspension (0·1 ml.) containing $5 \times 10^6 \sim 10^7$ cells was injected intraperitoneally into mice (strain DDD).

In the initial experiment, we found that tumour cells which were pretreated with an extract of fresh garlic did not produce ascites tumours and no mouse died during the observation period of 10 weeks. In contrast, both tumour cells pretreated with an extract of garlic that had been boiled before grinding and tumour cells incubated in physiological saline alone produced ascites tumours in all mice, which died 2 or 4 weeks after intraperitoneal injection.

Table 1. Effect of garlio extract on immunogenic capacity of ehrlich ASCITES TUMOUR CELLS

Materials	No. of attenuating treatments	Time of challenge after the last treatment (days)	Mortality after Challenge
Physiological	meannemo	or casmons (days)	
saline (control)	1 (i.p.)	7	10/10
Tumour cells treated with	2 ,,	14	10/10
fresh garlic	1 ,,	7	9/10
,,	1	14	8/10
**	2 .,	7	8/10
,,	2 ,,	14	0/10
1.0	2 (s.c.)	14	0/10

In another experiment, we found that when mice were treated twice at intervals of 7 days with freshly prepared tumour cells exposed to the extract of fresh garlic, they acquired resistance against a challenge with Ehrlich ascites tumour cells. None of the mice developed ascites tumours or died within a period of 100 days even though 10⁵ live fresh Ehrlich ascites tumour cells were injected 14 days after the second treatment with cells exposed to garlic. All control mice challenged with the same number of tumour cells developed ascites tumours and died within 2 or 4 weeks. Subcutaneous injection of the attenuated tumour cells gave similar results. The results are summarized in Table 1.

Although the mechanism by which immunity is achieved when cells are attenuated with extract of fresh garlic is not yet clear, it is known that garlic contains allicin which derives from alliin by the action of alliinase, as elucidated by Stoll and Seebeck⁴. Because allicin reacts with sulphydryl groups of tumour cell proteins^{5,6} it is not improbable that our technique is based on similar actions of garlic extracts on ascites cells.

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Chromosomal Constitution of Meningiomas

NUMERICAL and structural chromosome anomalies have often been reported in human tumours. It is not known whether the change from normal to tumoral cells is induced by or followed by a disturbance of the chromosome complement. Neither is it known whether all chromosome aberrations develop at the same time, or whether any special type of aberration induces further disturbances. Cytological and cytogenetical studies of benign tumours are thus very important; they may well give more information about the mechanism of cell transformation.

The tumours were cultured by the method of Kersting¹. The medium used for 600 brain tumour cultures did not allow excessive growth of connective tissue. Chromosome analysis was carried out on primary particle cultures in roller tubes. Cultures grown for 5-9 days were subjected to a hypotonic treatment in citrate solution. The cells were fixed in glacial acetic acid and methanol (1:3), air dried and stained with aceto-orcein. Well spread metaphases from between five and fifteen cover slips were photographed under phase contrast.

Table 1 shows the cytogenetic observations and their correlation with the morphology of the meningiomas. A striking similarity can be seen in the basic type chromosomal aberrations; in all eight tumours a G-group chromosome is missing. In four tumours additional chromosomes are missing, and in one case a C-group chromosome is supernumerary, resulting in a final chromosome count of forty-six. The pathological chromosome complement is uniform for every tumour. As well as the aberrant stemline there is a stemline with a normal number of chromosomes. One of the eight meningiomas has three different stemlines. Hyperploid cells were absent except for a few tetraploid cells deriving from the pathological as well as the normal stemlines. Structural anomalies like breakages, translocations or other reunion figures were very rare. Only a very tight end to end association of different chromosomes was often found. In 7-15 per cent of the mitoses there was a varying hypoploidy and in most of these cells the membrane was broken. The basic karyotype of forty-five, XX (or XY), G-, corresponded to an isomorphic endotheliomatous psammomatous meningioma with many whorls and onion skin-like structures and psammomatous bodies. The two tumours with only forty-two chromosomes can be classified as fibromatous meningiomas. The prolifera-tion pattern in tissue culture was not clearly different from that of the other tumours. After 10-12 days an accessory fibroblast-like cell type used to appear in most of the cultures, particularly in the marginal zone.

Benign tumours other than meningiomas usually have either a normal diploid chromosome complement or a non-systematic loss or gain of chromosomes which varies from cell to cell, with a tendency to hyperploidy. Most of the meningiomas which we studied were a mosaic of normal and abnormal cells. The abnormal cell strain was hypoploid and homogeneous in one tumour. This homogeneity suggests that these cells had a common origin.

The basic chromosome aberration in our meningiomas is the loss of a small acrocentric chromosome, accompanied in four out of eight tumours by further numerical aberrations. There were no essential structural abnormalities in any chromosomes. A similar situation is well known in chronic myeloic leukaemia, where the only aberration is the partial loss of a G-group chromosome. Atkin and Taylor² have reported a case of chronic myeloic leukaemia with a Ph^1 -chromosome and another missing G-chromosome in about 60 per cent of the cells. Ishihara³ found in a case of gastric cancer the partial loss of a Ggroup chromosome as an isolated chromosome aberration. Van Steenis⁴ and Simons⁵ calculated on the basis of the karyotypes published by Makino⁶ and Ishihara^{7,8} that in hyperploid tumours only the G-chromosomes are not

Table 1. COMPARISON OF HISTOLOGICAL, CYTOLOGICAL AND CYTOGENETICAL FINDINGS ON EIGHT MENINGIOMAS

No. of tumour	Sex	No. of cultures	Day of collec- tion	Total kary- otypes	No.	Karyotype	Morphology in sections	Morphology in culture	Growing speed
T562	F	7	8	60	52 2 6	45, XX.G —, 46, XX,	Endotheliomatous meningi- oma of psammomatous type, many whorls and onion skin-like structures	Isomorphic endotheliomatous cells predominate after primary spindle cell outgrowth	+++
T582	F	5	9	54	46 8	45, XX, G—,	Endotheliomatous meningioma of psammomatous type, many whorls and onion skin-like structures	Isomorphic endotheliomatous cells predominate after primary spindle cell outgrowth	+++
T'543	F	11	4+8	29	17 8 3	45, XX, G—, 46, XX,	Endotheliomatous meningioma of psammomatous type, many whorls and onion skin-like structures	Isomorphic endotheliomatous cells predominate after primary spindle cell outgrowth	+++
T559	F	9	7	56	4 47 5	45, XX, G—, 46, XX,	Endotheliomatous meningi- oma with few whorls and onlon skin-like structures, single psammomatous bodi	Isomorphic endothelial-like cells without primary spindle cell outgrowth	+ +
T465	M	15	5	57	49 8	44, XY, G, D,	Endotheliomatous meningi- oma of psammomatous type	Isomorphic endotheliomatous cells predominate after primary spindle cell outgrowth	++
T579	F	15	7	91	68 5 10	45, XX, G—, 46, XX, G—, C+, 46, XX,	Endotheliomatous meningioma of arachnothelious type	Fibrous spindle cells together with excessive macrophages, in the marginal zone fibro- blast-like cells	+
T585	F	11	8	57	48 1 8	42, XX, G—, D—, C—, E—, 46, XX,	Fibromatous meningioma, heavy collagenization, rich whorl and onion skin-like structures	Isomorphic endothelial-like cells without primary spindle cell outgrowth	++++
T584	М	7	6	72	67 5	minute) 42, XY, G—, 2D—, C— (In 32 mitoses 1 fragment)	Fibromatous meningioma, rare whorl and onion skin- shaped structures	Isomorphic elongated triangular cells with a tendency to grow in files	+++

·-·-, Hypoploid chromosome complement; cell membrane probably broken (see text).

usually augmented, which results in a relative reduction of G-chromosome material. Kiseleva $^{\circ}$ suggested that loss of G-chromosome material could be related to uncontrolled cell multiplication. This does not agree with our own findings, for our meningiomas showed increased but not uncontrolled cell multiplication.

The occurrence of a varying percentage of cells with a normal karyotype suggests that cells with an aberrant chromosome complement's could stimulate the surrounding normal cells to an excessive proliferation and to produce different histological structures in relation to the karyotype of the inducing cell. This would mean that the cytogenetically identifiable "tumour cells" in a meningioma may only be a part of such a tumour.

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Peroxidation of Lipid from Paroxysmal Nocturnal Haemoglobinuria-like Erythrocytes

ERYTHROCYTES from patients with paroxysmal nocturnal haemoglobinuria (PNH) are lysed excessively by agents which peroxidize lipid, and their lipid forms more lipid peroxides than that of normal erythrocytes^{1,2}. Recently we noted that incubation of normal red blood cells in reduced glutathione results in cells with all the usual in vitro lytic features of "natural" PNH cells. In the work reported here we have compared lipid extracts from red blood cells of normal subjects and patients with various haemolytic disorders with those of "natural" and "induced" PNH cells, with respect to peroxidation.

Blood samples drawn by venipuncture from six normal laboratory personnel, from patients with various types of haemolytic anaemia and from four patients with established PNH, were defibrinated in sterile containers using glass beads. After centrifugation at 3,000 r.p.m. for 10 min and removal of serum and buffy coat, the red blood cells were washed three times with physiological saline. Washed normal erythrocytes (14 ml.) were incubated at 37° C in 56 ml. of a solution of reduced glutathione (210 mg/ml. at pH 8·0) by shaking in a 50 ml. Erlenmeyer flask at 60 oscillations/min for 15 min. Control samples of washed normal red cells incubated in saline solutions of comparable ionic strength and pH were simultaneously carried through all procedures. After incubation, the red cells were centrifuged at 3,000 r.p.m. for 10 min, the supernatant removed, and the cells washed three times with physiological saline.

Selected in vitro lytic features of PNH erythrocytes are shown in Table 1. Normal red cells incubated with reduced glutathione were indistinguishable from red cells of patients with PNH. The optimum pH for acid-serum lysis was the same for "natural" and "induced" PNH red cells—6.0

Lipids of control red blood cells (including samples from normal subjects and patients with haemoglobinopathies, immune haemolytic anaemia, spherocytosis and glucose-6-phosphate dehydrogenase deficiency) and "natural" and "induced" PNH red cells were then extracted as described by DeGier et al.3. Washed cells (4 ml.) were haemolysed to a final volume of 25 ml. with water. The final chloroform layer was evaporated to dryness at 37° C under vacuum and the residue collected in a final total volume of 9 ml. of physiological saline. The lipid extract was analysed for total phosphorus and used immediately4. Lipid extracts so obtained were white and opalescent and no gross differences were observed between the groups studied Quantities of lipid extracted from each batch of cells (as lipid phosphorus) did not vary, and thorough mixing of the lipid extract "suspen-

Table 1. In vitro LYTIC EFFECTS OF PNH ERYTHROCYTES

	Percentage of haemolysis*					
	No acid	Serum Acid	Acid and thrombin	Sucrose		
Control red blood cells	0 `	0	0	0		
"Natural" PNH red blood cells	0	15-40	25-60	6-30		
Normal red blood cells with reduced glutathione	0	20-60	40-80	5-25		

Values given represent the ranges obtained with twelve separate determinations.

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Table 2. CONCENTRATIONS OF LIPID PEROXIDE IN RED BLOOD ORLL LIPID*

	malonylaldehyde/ 10^{-2} µgmP)					
	Before ultra- violet light		After ultra- violet light			
		Mean	Range			
Control red blood cells	0	87	78- 92			
"Natural" PNH red blood cells Normal red blood cells with	Ó	126	122-130			
reduced glutathione	0	145	111-193			

^{*} Values given represent six separate studies.

sion" before sampling resulted in reproducible concentrations of lipid.

Portions of lipid extract (2-4 ml.) were shaken at 100 oscillations/min in 100 ml. quartz Erlenmeyer flasks 11 cm below an ultra-violet source (General Electric G15-T8) for 2 h. After incubation, 2 ml. of the extract was mixed with 2 ml. of 10 per cent trichloroacetic acid and filtered through Whatman No. 3 paper. A sample of the filtrate (1 ml.) was then mixed with 1.2 ml. of 0.67 per cent thiobarbituric acid, heated in a boiling water bath for 15 min, allowed to cool to room temperature and the absorbance read at 535 mu. Tetraexthypropane (which hydrolyses to ethanol and malonylaldehyde) was used in the thiobarbituric acid reaction as a standard and lipid peroxides were expressed as mμmoles malonylaldehyde/μg lipid phosphorus.

As Table 2 shows, the lipid of "induced" PNH cells formed greater quantities of lipid peroxides more rapidly than lipid from control cells and at concentrations comparable with those of "natural" PNH red blood cells. All patients with PNH had normal serum tocopherol concentrations and normal red blood cell glucose-6-phosphate dehydrogenase, catalase and glutathione peroxidase activities.

These studies show that normal red cells incubated with reduced glutathione acquire the in vitro lytic features which are used to classify "natural" PNH red cells. In addition, the lipid of these cells behaves like the lipid of "natural" PNH cells with respect to lipid peroxidation. The fact that lipid of "natural" and "induced" PNH cells formed greater quantities of lipid peroxides more rapidly than that of control cells during exposure to ultra-violet light provides additional evidence that lipid peroxidation is involved in the basic intracorpuscular defect of PNH.

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RADIOBIOLOGY

Protection of Canine Pancreatic Ultrastructure against Radiation Damage by Post-treatment with Alloxan

In a previous study we demonstrated that pretreatment of dogs with a single small, non-diabetogenic dose of alloxan monohydrate (15 mg/kg) intravenously protects the pancreatic ultrastructure against severe irradiation damage.

In non-alloxanized dogs the irradiation by a single, large dose of roentgen rays (5,000-9,000 r.) of the exteriorized pancreatic remnant after partial surgical pancreatectomy produced extensive cellular damage characterized by widespread focal cytoplasmic degradation, alterations of endoplasmic reticulum and mitochondria, and reduction in size and number of mature zymogen granules2-4. Pancreatic amylase, leucine aminopeptidase and lipase activities showed a marked decrease in these animals. After alloxan, however, ultrastructural alterations induced by irradiation were not observed apart from occasional minor lesions¹. On the other hand, the marked reduction in the activities of pancreatic tissue enzymes noted after irradiation alone was also found in the dogs pretreated with alloxan.

In addition to the series of twenty-nine irradiated dogs pretreated with alloxan1, we have also examined five dogs which were given alloxan several days after radiation treatment, and three more dogs which received alloxan both before and after irradiation. Table 1 lists the radiation and alloxan doses, the various time intervals and the pancreatic tissue enzyme changes in these dogs; a summary of the observed ultrastructural alterations is given in Table 2. The methods for partial pancreatectomy and radiation treatment were identical with those previously described2,3.

Table 1. RADIATION DOSES, ALLOXAN DOSES AND TISSUE ENZYME ACTIVITIES OF EIGHT IRRADIATED, ALLOXANIZED DOGS

							Pancre	atic tiss	ue en-
						2	ymes a	t time o	f killing
						Time	(units/	mg wet	tissue)
		Alloxa	n before	Alloxa	n after	of killing		Leucine	
\mathbf{R}	idiatio	n irrac	liation	irrad	liation	(after		amino-	
Dog	dose	Dose	Interval	Dose	Interval	irradi-	Amy-	pepti-	
No.	(r.)	(mg/kg)	(days)	(mg/kg)	(days)	ation)	lase	dase	Lipase
1	10.000) —		15	2	7 days	421	98	13.4
$\bar{2}$	10,000		2	15	4	11 days	890	45	28.6
	7,000			15	7	4 weeks	2.110	51	21.6
3 4 5	7,000			15	14	4 weeks	2,297	43	12.7
5	10,000		5	15	5	8 weeks	745	35	3.5
6	7.00) —	*******	25	7	10 weeks	2,500	30	26-6
6 7 8	9.00	Ď —		15	1	17 weeks	5,000	37	46.0
8	9,00	0 15	1	15	2	26 weeks	725	28	3.3
	,		Norm	nal (mea	n calcul	ated from	3,360	178	57.0
					pancre		± 169	± 15	± 3·0

Table 2. ESTIMATION OF ULTRASTRUCTURAL CHANGES IN THE PANCREASES OF EIGHT IRRADIATED DOGS POST-TREATED WITH ALLOXAN

Dog No.	Sequer cytopl bod	asmic	Zymogen granule		Mito- chondrial	Centro- acinar cell	Islet cell	
110.	Large	Small		reticulum		changes	changes	
	Transfe		OHESTIGOS	ronomani	omerigos	CIMETERS	OHOTHECO	
1	+	+	+	+	+	-		
2	_	_	_		-	+		
3	++	++	++	++	++		+	
4	-	+	++	+	-	++	+	
5	_	+		+	-	_		
в	+	+		+	++	_		
7	_	+			++	_	***	
8	-	_		-	_	_		

, No changes; +, minimal focal changes; ++, focal to moderately extensive changes.

Severe changes, similar to those observed in irradiated but not alloxanized animals, were not seen

When they were examined with an electron microscope no severe or extensive cytological alterations were found in any of the eight dogs (Table 2), especially when compared with two large series of similarly irradiated animals which had not been pretreated with alloxan²⁻⁴. observed lesions, however, though neither severe nor widespread, were somewhat more prominent that those seen in the pretreated irradiated dogs. At any rate, it is evident from these data that alloxan arrests many otherwise observable post-irradiation changes of the cytoplasm of the canine pancreas, even when administered as late as 1 or 2 weeks after irradiation.

These observations are in keeping with a recent com-munication by Doolittle and Watson⁵, who reported that alloxan, when administered to mice within a few hours after exposure to X-rays, reduced the level of mortality induced by radiation below that of mice which received saline solution instead of alloxan. There was a decrease in the tissue activities of three important pancreatic enzymes (Table 1), however, which was in keeping with a similar decline of enzyme activity after irradiation alone²⁻⁴, alloxan pretreatment plus irradiation¹ or alloxan treatment alone1

The rationale for the radioprotective action of alloxan, whether administered before or after irradiation, as well

as for its depressive effect on pancreatic tissue enzyme activities, is not known. It might be explained by the hypothesis that its interaction with amino-acids modifies the structure and reduces the synthesis of proteins, particularly those with free amino-acid groups or radicals 6-8.

The post-treatment of dogs with a single, small nondiabetogenic dose of 15 mg/kg of alloxan monohydrate protects the pancreatic ultrastructure against severe irradiation damage. A decline of pancreatic activity of amylase, lipase and leucine aminopeptidase, however, occurs. We suggest that the radioprotective action of alloxan on the pancreas may be due to the reduction of protein synthesis induced by alloxan and to its known effect on amino-acids and —SH radicals⁶⁻⁸.

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PHYSIOLOGY

Molecular Sieving by Glomerular **Basement Membrane**

Or the three layers of the glomerular capillary wallendothelial cells, basement membrane and epithelial cells—the basement membrane appears to be the only complete barrier between the plasma and the glomerular filtrate1,2. Support for the view that the basement membrane is the most important filtering mechanism was provided by the studies on ferritin transfer across the glomerular capillary wall²⁻⁵. Graham and Karnovsky⁶ have concluded that the epithelial slits are the primary

filtration barrier and suggested that glomerular basement membrane acts only as a "coarse" filter. It is thus still not established whether the basement membrane acts as a filter.

The nephrotic syndrome is characterized by increased permeability of the glomeruli to serum proteins and consequent proteinuria. Paradoxically, the permeability is associated with an increase in thickness of the glomerular basement membrane⁷⁻⁸. Movat, Steiner and Huhn¹⁰ suggested that in acute glomerulonephritis a decrease in density of the basement membrane presumably associated with hydration and separation of the constituents could account for the increased permeability. Similarly, on the basis of chemical composition and X-ray diffraction patterns11,12, it was concluded that the membrane thickening in experimental nephrosis might be caused by molecular rearrangement of the membrane components so that the interstices between molecules become larger and permit passage of large molecules such as plasma proteins. If this explanation is correct, the normal and nephrotic membranes are analogous to dextrans of greater or lesser degree of cross linkage, and should show similar molecular sieving effects.

Rat glomerular basement membrane was prepared by a modification of the procedure of Krakower and Greenspon¹³. Nephrosis was produced by three daily injections of rabbit antiserum to whole rat glomeruli. The animals which showed high proteinuria (> 200 mg/24h) 2 weeks after the first injection were used for experiments.

Suspensions of basement membrane particles in 0.9 per cent saline were allowed to settle by gravity in small chromatographic tubes to give columns 1.5×60 mm. Columns packed with 'Sephadex G-25' were used as controls. A solution containing albumin- ^{131}I and mannose-¹⁴C (10 μl.) was applied to each column, the column was washed with 0.9 per cent saline and the cluate was collected in fractions of 10 μl. with capillary glass tubes. Carrier albumin and mannose were added to each fraction. The protein was precipitated with 10 per cent trichloroacetic acid, dissolved in 2.5 normal sodium hydroxide and assayed for iodine-131 in a well scintillation counter. The supernatant containing mannose-14C was assayed for carbon-14 in a liquid scintillation counter.

The elution patterns of protein and mannose from the columns are given in Fig. 1. As anticipated, albumin, being excluded from the 'Sephadex', was eluted well ahead of the mannose. The column of normal basement membrane produced a small but definite retention of the mannose relative to the albumin. Thus basement membrane from normal glomeruli produced a molecular sieving effect.

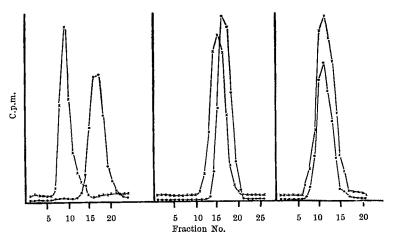


Fig. 1. Elution patterns of albumin-¹³¹I (×) and mannose-¹¹C (♠) from microcolumns. From left to right: 'Sephadex G-25', glomerular basement membrane from normal rats and glomerular basement membrane from nephrotic rats.

When basement membrane from nephrotic rats was used, there was complete overlap of the albumin and mannose peaks, indicating that albumin was retarded to the same extent as mannose. The elution volume of albumin from the column of nephrotic membrane was smaller than that from the column of normal membrane. Attempts to determine the void volume of membrane columns using 'Blue Dextran 2000' were unsuccessful because the dextran was adsorbed by the membrane. It is known that the void volume of 'Sephadex' columns varies with the degree of cross-linkage and that the void volume of a column of 'Sephadex G-25' may be equal to or even greater than the elution volume of a material which is retained on a column of less highly cross-linked 'Sephadex'. This phenomenon probably explains the small elution volume from the column of nephrotic membrane.

These results are compatible with the hypothesis that renal glomerular basement membrane acts like 'Sephadex' in separating large and small molecular species and that in nephrosis the basement membrane behaves like a more porous, less highly cross-linked 'Sephadex'. It seems likely therefore that basement membrane acts in vivo as a differential filter and that in nephrosis the basement membrane is more permeable because of a more open molecular structure.

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Suppression of Oestrous Behaviour in the Immature Male Rat

THE neonatal testis of the rat is responsible for suppressing the capacity of the nervous system to mediate female sexual behaviour. Male rats castrated on the day of birth and treated with ovarian hormones as adults display oestrous behaviour; if castration is performed after the first post-natal week, female sexual behaviour is greatly

Neonatally castrated rats have not been tested for oestrous behaviour before 80 days of age1-4. The possibility therefore remains that rats castrated at the end of the first week of life and treated with ovarian hormones at the time of testing display greater sexual receptivity before puberty than post-pubertally. This hypothesis is supported by evidence that action of neonatal androgens on sexual differentiation of the brain does not take complete effect until after 60 days of age. For example, ovaries transplanted into male rats at the time of castration-5-6 days after birth-contained fresh corpora lutea and ripe follicles when the animals were killed between 39 and 61 days of age; ovaries removed from

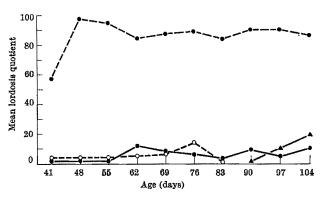


Fig. 1. Female sexual behaviour in rats treated with ovarian hormones.

. Males castrated on day 7 (group 1); A—A, males castrated on day 27 (group 2); O - - - O, males castrated on day 27 (group 3);
. - - O, females ovariectomized on day 27 (group 4).

similarly prepared males killed about 120 days of age contained follicles but no corpora lutea⁵. Similarly, female rats treated with androgens shortly after birth exhibit ovarian cycles for a few weeks beginning at 70 days of age and only subsequently develop the syndrome of anovulatory sterility6.

Four groups of rats were used: groups 1 and 2 contained, respectively, twelve and eight males castrated 7 days after birth; group 3, eight males castrated 27 days after birth; and group 4, ten females ovariectomized at 27 days of age. Groups 1, 3 and 4 were tested for oestrous behaviour beginning at 41 days of age and group 2 beginning at 90 days of age.

To induce receptivity each rat was injected subcutaneously with 6 µg of oestradiol benzoate and 40 h later with 0.4 mg of progesterone. Seven hours after the progesterone injection, each animal was placed in a cage with three stimulus males for 3 min or until it had been mounted six times^{7,8}. The number of times each test animal was mounted and the number of lordoses elicited by these mounts were recorded. The ratio of lordoses to mounts ×100 (lordosis quotient) was used as the index of sexual receptivity. Successive tests were separated by one week.

Male rats castrated at 7 days of age do not display lordosis frequently, either before or after puberty (Fig. 1). Animals in group I generated uniformly low lordosis quotients regardless of their age at the time of testing. Action of the neonatal testis in suppressing lordosis is complete by the seventh post-natal day; males in group 1 were not more receptive at any age than males castrated on day 27 (group 3). All male castrates displayed lordosis less frequently than the female castrates (P < 0.01) on each of the weekly tests. This confirms and extends earlier findings1.

Female rats first develop full behavioural responsiveness to ovarian hormones at 30 days of age. The failure of males to display lordosis may have been due to their advanced age (41 days) at the time of the first test. To test this possibility six male rats were castrated 5 days after birth and an equal number on the seventh day of life. Tests for oestrous behaviour were given at 26 days of age and again at 33 days. These young rats were mounted only infrequently; in no case was lordosis observed. Oestrous behaviour is therefore observed only rarely in male rats castrated later than the fifth day of life, regardless of the age at which tests are initiated.

In conclusion, suppression of the neural substrate for oestrous behaviour in the male rat is induced by the fifth day of life and does not depend on a post-pubertal maturational process. Suppression of female sexual behaviour follows a different time course from inhibition of the female pattern of gonadotrophin release; the intact male hypothalamus cannot mediate oestrous behaviour at a time when it can apparently support the cyclic female pattern of gonadotrophin release. For behaviour, then, the male nervous system functions in the stable manner of adulthood beginning very early in life, whereas, for regulation of gonadotrophin secretion, "all hypothalami are of the female type during a significant part of the life span and . . . action of androgen . . . in the neonatal period does not take effect until some time after puberty"5.

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Voltage Clamp Studies on Snail (Helix aspersa) Neurones

Investigations of snail neurones have shown that some cells can maintain their action potentials in solutions free of sodium1-4 and are not affected by tetrodotoxin3. These nerves may have a calcium carrying current system similar to that described in barnacle muscle⁵.

We have used a system where the whole membrane of the snail neurone perikaryon can be clamped at a specified voltage without showing any notching or oscillations in current⁶, and the preparation showed a current waveform similar to that described by Hodgkin and Huxley' for the squid axon.

In Fig. 1, curve A shows the maximum inward current plotted against membrane potential and curve a shows the steady-state outward current, when the cell is in

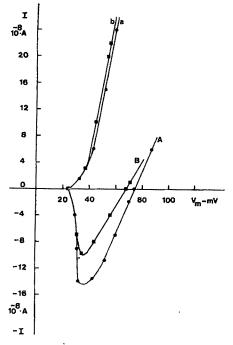


Fig. 1. Current-voltage relations of the membrane of a neurone of Helix aspersa. A. Maximum inward current in normal Ringer; B. maximum inward current in sodium-free Ringer; a. steady-state current in normal Ringer; b, steady-state current in sodium-free Ringer.

normal Ringer (sodium, 80 mmolar; calcium, 7 mmolar). When the external solution was replaced by a sodium-free Ringer there was still an inward current (curve B) although this was less than that seen in normal Ringer. inward current was found even in neurones washed in sodium-free solution for 20 min. When the solution was replaced by normal Ringer, the full inward current was restored after about 100 sec.

The reduced inward current seen in sodium-free solution disappeared if the cell was placed in a solution containing neither sodium nor calcium. Although the reduced inward current seen in sodium-free solution could be increased by replacement of sodium, the reduced current could not be restored by an increase of calcium up to seven times the normal value. Cells injected with EDTA showed a normal inward current in standard Ringer but not in sodium-free Ringer containing calcium.

The inward current of cells in normal Ringer was not reduced by tetrodotoxin in concentrations between 10-9 and 10-5 g/ml. The inward current in sodium-free solution could be abolished by charging the membrane at 30 mV or higher and maintaining this potential for more than 20 min. The current was re-established when the cells were replaced in normal Ringer.

There may be a small store of sodium ions closely associated with the nerve membrane (either trapped in the glia or in the folds of the membrane, or even actually in the membrane structure) which cannot be easily removed by washing in sodium-free Ringer but which is affected by the calcium ion concentration.

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Amnesia induced by Scopolamine and its Temporal Variations

An anticholinesterase drug, diisopropylfluorophosphate (DFP), has been reported to produce weak amnesia for habits learned 30 min before its intracerebral injection, no amnesia for habits learned 3 days before and considerable amnesia for habits 5 and 14 days old1. There is also no amnesia for habits learned I day before injection of this drug (unpublished results of Deutsch and Stone). One of the effects of DFP is to slow down the destruction of the transmitter acetylcholine, thus permitting it to accumulate, and so DFP may block conduction at a synapse. This action would particularly affect a synapse where the amount of acetylcholine ejected during transmission is high, because accumulation sufficient to cause a block would in this case be quickly reached.

The temporal variations in amnesia induced by anticholinesterase have therefore been interpreted2,3 as showing that after the learning experience there is a gradual change in the level of transmitter ejected by a synapse during transmission. This level would be high immediately after learning (during temporary storage) and would drop rapidly to a low point, from which it would slowly increase to a new high level (underlying the long-term memory of a habit).

This hypothesis was tested in an experiment paralleling the one using DFP already reported, but injecting the

anticholinergic drug, scopolamine, instead, An anticholinergic agent reduces the effectiveness of acetylcholine and would therefore block transmission to the greatest extent at synapses where the concentrations of this transmitter are low. This is the opposite of the action of an anticholinesterase. If the inferences concerning transmitter output are correct, the reverse effect of the one described should be obtained using a drug with an anti-cholinergic action. The use of the anticholinergic, scopolamine, should produce greatest amnesia where the effect of DFP was least and have its smallest effects where the effects of the anticholinesterase were greatest. This would support the hypothesis that the level of chemical transmitter output varies with age of memory. Such a prediction is supported by some other preliminary

Male rats (Sprague Dawley, Rockland strain, 350 g) were trained to a criterion of ten consecutive correct choices to escape shock to their feet by running to the lighted arm of a Y-maze, the position of the light changing randomly, as described before. Five groups of rats were then injected with scopolamine intracerebrally under 'Nembutal' anaesthesia in a stereotaxic instrument. The first group was injected 30 min after learning, the second at 1 day, the third at 3 days, the fourth at 7 days and the fifth at 14 days. The animals were included in each of the groups on the basis of initial learning scores, so that the groups were matched on the basis of initial learning. The placement of the bilateral injection was anterior 3, lateral 3, vertical +2; and anterior 3, lateral 4.75, vertical -2, according to the atlas of DeGroot⁶. Peanut oil (0.01 ml.) containing 0.58 per cent of scopolamine was injected in each placement. Twenty-four hours after the injection, the rats were retrained to the same criterion. This gave a measure of the relative amount of retention of the various groups.

The results of the experiment are shown in Table 1. The maximum amnesic effects were found on habits acquired 1 day (group 1) or 3 days (group 3) before injection. A specific comparison between the mean relearning scores for groups 2 and 3 combined and all other groups was found to be statistically significant $(F=10\cdot42, df=1/35, P<0\cdot01)$ as revealed by an analysis of variance. A reduction of effect for the 3 day group (group 3) appears, compared with the 1 day group (group 2). In the case of DFP no effect is seen at 1 or 3 days, both groups having scores indistinguishable from peanut oil controls.

The design of the DFP experiment does not allow us to see whether the minimum effect of DFP on memory is at 1 or at 3 days after training, and this makes accurate comparison with the effects of scopolamine uncertain. An additional test (Mann-Whitney-U) of the difference between the median relearning scores of groups 1 and 2 was found to be significant at P < 0.01. It can be seen that the amnesic effect obtained with scopolamine shows an initial increase followed by a decline, this being the opposite of the DFP effect, although of about the same time course.

predicted, scopolamine produced its greatest amnesias for habits of the ages where the effects of DFP were least and had its smallest effects where the effects of the anticholinesterase were greatest. results therefore support the hypothesis that the effective physiological basis of memory is a change in synaptic conductance, mediated by an increase in the concentration of effective transmitter, in this instance probably Such a change in conductance, once acetylcholine.

			Table 1			
Group	Time to injection after initial training	N	Mean trials to criterion initial training	Mean trials to criterion relearning	Standard deviation	Median trials to criterion relearning
1	30 min	8	22.25	11.88	15.87	7.5
2	1 day	8	24.38	23.88	14.59	20.0
3	3 days	8	26.29	13.12	7-44	14.0
4	7 days	8	23.38	7.25	4.43	7.0
5	14 days	8	20.75	8.25	5.23	7.5

initiated by learning, seems to vary, even without further learning or "exercise", simply as a function of time.

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Secretions from the Lateral Scent Glands of the Green Vegetable Bug, Nezara viridula

GREAT interest attaches to the division of biosynthetic activities between the different types of cell in a gland complex and to the mechanisms whereby toxic secretions are prevented from causing harm during synthesis and storage. The scent gland complex (Fig. 1) of Nezara viridula var. smaragdula (F.) consists of a median ventral metathoracic scent reservoir, which is orange yellow in colour, and of paired colourless lateral glands sometimes called accessory glands. The lateral glands discharge through ducts into the reservoir, which also receives secretions from the gland cells which form its epithelium. The multicomponent secretion stored in the reservoir is used in defence and the gland complex resembles that of other Pentatomid and Coreid1,2 bugs.

The secretions from the reservoir cells cannot be obtained for separate examination uncontaminated with the stored scent, but it is possible to isolate the lateral glands and examine their composition. Table 1 shows the compounds extracted with 2-propanol from lateral glands collected in Canberra and in Hawaii. Excised glands from 20-100 insects were steeped in a few µl. of 2-propanol and the extract analysed by gas chromatography⁸ on columns of 'Apiezon L' and didecyl phthalate by comparison of retention times with those of authentic compounds. Lateral glands of bugs from Hawaii were moistened with 2-propanol during transport in sealed tubes and were several days old before analysis. Besides known scent components, these extracts had an additional unidentified component, not present in stored scent or shown in Table 1, which increased on further standing and therefore appears to be a storage artefact.

N. viridula is a cosmopolitan species. We have shown previously³ that of scent collected from the storage reservoirs of Australian specimens the principal constituents comprise n-alkanes, alk-2-enals, alk-2-enyl acetates and 4-keto-hex-2-enal. Comparison in Table 1 of the principal components of the scents from the storage reservoirs of insects in Hawaii and insects in Canberra shows that

PERCENTAGES OF COMPOUNDS IN LATERAL GLAND EXTRACTS AND IN SCENT FROM STORAGE RESERVOIRS OF Nezara viridula

	Hydro- carbons		All	Alk-2-enals			c-2-en cetate	4-keto- hex-2-enal	
	C13	C23	C_{20}	C_8	$\mathbf{C}_{\mathbf{g}}$	C_{10}	C_8	C_8	
Lateral gland extract									
Canberra*	18.1	2.4	3.3			76.2			
Hawaii†	7.5	0.3	3.8			87.5			
Scent									
Canberra ²	54.1	2.3	25.8	0.5	1	3.9	0.5	2.9	7.1
Hawaii	50-5	1.5	23.5	1	1.5	6.5		1	13.0
Ratio $\frac{[gland]}{[scent]}$									
Canberra	0.33		0.13			19-6			
Hawaii	0.15		0.16			13.5			

Identifications by gas chromatography on 'Apiezon L' and DDP columns. * Averages of determinations on seven separate batches of insects. † Unidentified decomposition product present also.

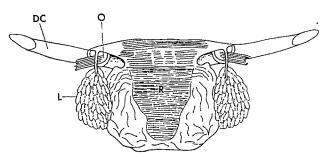


Fig. 1. Diagram of scent gland complex in Nezara. DC, Discharge canal; L, lateral gland; O, occluding mechanism; R, scent reservoir.

both these scents are very similar in composition. They contained none of the C, alk-2-enal reported4 for the scent of this species from Louisiana, USA.

The proportions of scent components are very different in the lateral glands and in the storage reservoir. The hydrocarbons, particularly n-tridecane, are much less abundant in the lateral gland extract and must therefore be assumed to be synthesized chiefly by the reservoir gland cells. Indeed, it is possible that the hydrocarbons which find their way into the gland extract may not be produced in the lateral glands because, in other experiments, tridecane and dodecane were detected in 2-propanol extracts of lateral glands of the coreid, Amorbus rubiginosus (Guerin). Hydrocarbons are not present in the stored scent of this insect2 and thus these alkanes in the lateral glands may be characteristic of the cytoplasm of the gland rather than of the contents of the discharge

The principal component of the lateral gland extract is decenyl acetate, which is present in the extract at up to twenty times its percentage in the stored scent. Decenal, on the other hand, is much less abundant than in the scent. It is possible that the small amounts of decenal and hydrocarbons are the result of their diffusion back from the scent reservoir for some distance into the larger ducts of the lateral glands. When fat body adjacent to the lateral glands was extracted and examined in the same way as the lateral glands, no scent constituents were detected.

In unpublished data on the defensive secretions of a number of stink bugs, we have observed that the presence of an aldehyde in the scent is usually associated with the presence of some of the acetate of the corresponding alcohol. We have also suggested that the biosynthetic pathways of the two series of compounds are linked. Thus Commius elegans (Donovan) which has a Nezara type scent composition (chief odour component: decenal) also has a preponderance of decenyl acetate in extracts of lateral glands, but only a trace of decenal. In the bronze orange bug Musgraveia sulciventris (Stal) the principal odour component is oct-2-enal and extracts of lateral glands contained octenyl acetate, together with tridecane and a trace of dodecane.

The present results indicate that the alkenyl acetates are produced in the lateral glands. The duct from each lateral gland branches in a racemose fashion, the finer branches being surrounded by groups of secreting cells. Because these glands lie free in the haemocoele their secretion is seemingly less well isolated from the haemolymph than are the contents of the scent reservoir. We suggest that the highly reactive and toxic aldehydes may be subsequently formed within the relatively impermeable storage sac itself.

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PSYCHOLOGY

Myers-Briggs Type Personality Scales and their Relation to Taste Acuity

SENSITIVE quinine taste responders, that is, subjects with low taste thresholds for quinine and other compounds the taste thresholds of which also follow a Gaussian distribution in a population, are more particular in their choice of food1,2, react earlier and in response to smaller doses of systemic stimulants3 or tranquillizers4 and display faster reaction times as measured by the serial There are also significantly fewer heavy seven test⁵. cigarette smokers among sensitive quinine responders as a group if contrasted with an age and sex-matched population of subjects with high quinine taste thresholds1,6.

The work reported here was conducted in an attempt to determine whether personality type is related to a The Myers-Briggs type subject's taste threshold. indicator7 was selected because it is a brief, self-report inventory which results in simple continuous scores on four scales of dichotomous Jungian personality types: extroversion-introversion (E-I), sensation-intuition (S-N), thinking-feeling (T-F) and judging-perceiving (J-P).

Fifty-five college students in the Department of Anthropology of the Ohio State University, 19-29 years old (mean age, 20), were administered the Harris-Kalmus⁸ forced-choice, double blind taste threshold test with our modification *,10. Because the cumulative effect of age and smoking on taste thresholds is not apparent at the age level of our subjects6, it was not necessary to control for smoking.

The Myers-Briggs was administered only to subjects at the extremes of the log normal distribution of taste thresholds, consisting of very sensitive tasters, with thresholds 2-3, and the very insensitive, with thresholds 6-7, assuming that in the middle range of sensitivity other factors could interfere with a relation between taste acuity and personality.

On the sensation-intuition scale, sensors (S), as a group, were found to be taste insensitive, whereas intuitors (N)belonged to the taste sensitive group (see Table 1). The means of the continuous scores were 85.2 for the sensitive tasters and 63.6 for the insensitive tasters, with higher scores in the direction of intuition. The value for t = 2.00(using Student's t test) is almost significant at the 0.025 level for a one-tailed test and at the 0.05 level for a twotailed test. Because the relation was predicted on the basis of earlier data¹⁻⁵, the one-tailed test is justified.

Table 1. RELATION BETWEEN TASTE THRESHOLDS FOR QUININE AND THE MYERS-BRIGGS PERSONALITY TYPE SCALES

MYERS-BRIGGS PERSONALITI TIPE SCALES											
Sub- jects		_	Quinine thresh- old	I	\boldsymbol{E}	s	N	res (con T	F	J	P
Taste sensitive subjects (quinine thresholds 2–3, that is, $1\cdot4648\times10^{-4}$ molar to $2\cdot930\times10^{-6}$ molar)											
A.S. D.D. T.T. S.M. T.K. J.E. L.A.	F M M M M M	23 20 19 19 21 21 19	2·0 3·0 3·0 2·0 2·0 3·0	88 86 76 56	52 26 16	48	110 84 78 82 76 94	47 33 5	59 65 57 67	31 31	93 101 97 69
K.B. B.S. D.W. A.B. G.Z. Taste i	M F M F nsen	21 19 19 28 20 sitive	2.0 3.0 3.0 3.0 3.0 e subjects -5 molar)	108 110 84 (quinir	18 18 ne thre	52 sholds	108 82 94 114 7–8, the	13 15 37 at is, 4-6	95 87 3875 ×	19 51 29 10-4	63 85 molar
K.M. M.K. F.R. K.W. K.S. E.V. S.R. J.M.	F M M F M F M	20 21 22 18 18 22 20 20	8·0 7·0 7·0 8·0 7·0 7·0 7·0	72 80 72	18 38 34 28 24 46	28 46 2 52	106 76 78 76 98	23 15 29 49	75 93 85 51 81	59 15 33	78 99 65 73
G.S. M 20 7-0 72 98 49 9 G.R. M 19 7-0 36 74 69 17 *Obtained from raw scores as follows: For E against $I = 53 + I$ For N against $S = 67 + N$ $C = 67 - S$											

For T against F C=50+FC=50-T

For J against P

The Myers-Briggs Manual describes personality patterns based on scores on all four scales, whereas we obtained a difference only on one, the S-N dimension. Because we have separated the scores for those properties which reflect this dimension, the available descriptions will be less specific than if they had been measured separately.

According to MacKinnon¹¹, in the United States three out of four show a preference for the actualities of sense perception while the remainder prefer the possibilities of intuitive perception. In contrast to an estimated 25 per cent of the general population who are intuitive, 90 per cent of the creative writers, 92 per cent of the mathematicians, 93 per cent of the research scientists and 100 per cent of the architects are intuitive as measured by this test.

On the other hand, Vandenberg12 believes, based on research performed with 27 fraternal and 40 identical twin pairs, that the distinctions measured by the Myers-Briggs scales with the exception of extroversion-introversion apparently are not based on hereditary factors but presumably reflect environmental influences. own quinine taste threshold studies with Kaplan¹³ with 70 dizygotic and 75 monozygotic twin pairs show that the within pair variance for dizygotic and monozygotic twins, combining like sex pairs, is significant for the P < 0.05 level indicating a small but definite role of heredity in taste threshold for quinine and other Gaussian compounds. These data may appear to conflict with Vandenberg's conclusion, in that a significant difference between taste sensitive and insensitive groups was not found on the extroversion-introversion scale. On the contrary, we prefer to suggest that the present day American culture tends to encourage extroversion and sensation and that this cultural influence may be particularly strong in overshadowing any predispositions to introversion and intuition in the taste sensitive subjects of our sample of mid-western "middle-class" college students. A study with very rich or very poor older subjects might prove or disprove our hypothesis.

In summary, as a group, insensitive tasters are factual down-to-earth organizers, whereas sensitive tasters are theoreticians with insight who follow their inspirations.

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Selective Action of Pentobarbital on a Multiple Schedule of Reinforcement

Many demonstrations of the sensitivity of behaviour to drugs in a variety of situations have established the importance of environmental factors in determining drug behaviour interactions1. More specifically, patterns of responding that are differentially sensitive to drugs can be obtained by arranging different schedules of reinforcement² or by manipulating the parameters of a particular contingency3.

Hearst⁴ studied the effects of pentobarbital on behaviour within a system for classifying reinforcement schedules in terms of temporally defined parameters. The system defines two basic variables: t^D and t^Δ time periods during which a reinforcement can be obtained or is never obtained, respectively. During operation of the schedule, the durations of t^D and t^Δ are kept constant, they are alternated and only the first response to occur in t^D is reinforced. When, within a 30 sec cycle, t^D is decreased progressively from 30 to 0.4 sec a transition from "interval-' to "ratio-like" behaviour is observed. Hearst, using these contingencies in a multiple schedule to generate both response rates separately in individual rats, found that "ratio-like" behaviour was relatively insensitive to pentobarbital at a dosage of 8 mg/kg, although the drug increased response rates on the component generating "interval-like" behaviour. The present experiment extends Hearst's observations and examines also the effects of pentobarbital on behaviour produced by a schedule in which low rates of responding are differentially reinforced

Three adult male albino rats, which had not previously been used in experiments, were maintained at 80 per cent of their free feeding weights and exposed to a multiple schedule of reinforcement for 40 min daily during 5 months. The soundproof test chamber was equipped with a lever and a Gerbrands feeder which dispensed 45 mg food pellets as reinforcement. Depression of the lever activated counters and a cumulative recorder. equipment and electronic timers controlled the experimental arrangements automatically.

After magazine training⁶ and several sessions of continuous reinforcement, the animals were transferred to a multiple schedule consisting of two components which alternated for a total of eight 5 min periods in each session, each component being separated from the next by 1 min in which no contingency was operative. During component 1, behaviour was maintained on a schedule consisting of 15 sec DRL and 5 sec LH, that is, each depression of the lever was reinforced only if preceded by a minimum pause of 15 sec and a maximum pause of 20 sec timed from the preceding response. On component 2, reinforcements were available according to the system employed by Hearst with the following parameters: rat 1 was exposed throughout to a cycle length of 20 sec in which $t^D = 5 \sec (\tilde{T} = t^D/t^D + t\Delta = 0.25)$; rats 2 and 3 were exposed throughout to a cycle length of 10 sec in which $t^{\bar{D}} = 0.3$ sec $(\bar{T} = 0.03)$ and $t^{\bar{D}} = 0.15$ sec $(\bar{T} = 0.03)$ 0.015), respectively, these values being attained over a period of 75 days in which tD was reduced progressively from 5 sec ($\overline{T} = 0.5$). A red light and masking noise accompanied component I; noise was absent and a white light illuminated the chamber for component 2. After the response rates on each component had become stabilized, intraperitoneal injections of pentobarbital were given at intervals of 3-4 days in dosages ranging from 1-8 mg/kg. Each injection (control injections of distilled water were also given) was made 10 min before the commencement of a given session. The pentobarbital supplied contained 60 mg/ml. of pentobarbital sodium, 10 per cent alcohol and 20 per cent propylene glycol in sterile water. Carlton' reported some insensitivity of operant behaviour in rats to a mixture of pentobarbital and these alcohols, compared with an equivalent dose of pentobarbital dissolved alone in water.

Figs. 1A, B and C show representative cumulative records of behaviour generated by the components of the schedule before the drug was given; component 1 appears on the left and component 2 on the right for each rat. During component 1, low, stable rates of responding characteristic of DRL contingencies were obtained after

between seventy-five and eighty sessions. On average, subjects made twenty-three responses and secured between nine and twelve reinforcements during 5 min although each rat showed a characteristic pattern of collateral behaviour which intervened between responses. On component 2, it is evident that rats 2 and 3 responded appreciably faster than rat 1, thus affording general confirmation of Hearst's findings that marked increases in rate were observed when t^D was reduced to a small proportion of cycle length and when, as a consequence, the number of reinforcements obtained decreased. Whereas rats 2 and 3 responded on average at corrected rates of 110.9 and 115.2 responses/min ("ratio-like" behaviour), rat I gave a corrected mean rate of 65.5 responses/min, missing hardly any reinforcements, and displaying evidence of "scalloping" in the cumulative record ("intervallike" behaviour).

When low doses of pentobarbital were given, no reliable effects of the drug were observed. For doses of 1-5 mg/kg, it was found that the first time a given dose was administered rates of responding on component I tended to increase while, with the exception of rat 3, rates of responding on component 2 decreased. observations were unreliable, however, because a second administration of the same dose of the drug 3-4 days later frequently did not affect behaviour on one or both components consistently. It is worth noting therefore that at low doses any effect of the drug was transitory for one or both components of the schedule and to this extent the findings agree with those of Hearst, who failed to obtain clear effects at a dosage of 4 mg/kg.

When the drug was given repeatedly in a dose of 8 mg/ kg the selective effects on behaviour shown in Figs. 1D, E and F were observed consistently. Pentobarbital exerted an excitatory effect on behaviour maintained by component 1, that is, the distribution of inter-response times, characteristic of efficient DRL performance, was affected markedly by an increased incidence of responses occurring in close temporal proximity to one another. The effect is in agreement with results reported by Sidman⁸ and may be partially attributable to lack of motor co-ordination which impaired specific patterns of collateral behaviour. For example, rat 1 developed a consistent tendency to rear onto its hindlegs and to move backwards and forwards in this manner between responses. Pentobarbital impaired rearing, simultaneously increasing the rate of lever-pressing.

On component 2, results inconsistent with those of Hearst⁴ were obtained, for overall rates of response were reduced by the drug as compared with control sessions, the effect being more pronounced the higher the rate engendered by T. The results are summarized in Fig. 2

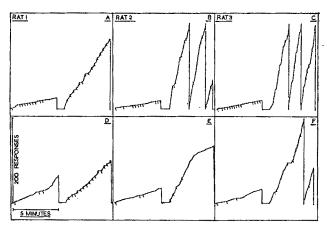


Fig. 1. Sample cumulative records showing the behaviour of each animal under control (A, B and C) and drug (8 mg/kg pentobarbital: D, E, F) conditions. Component 1 (DRL) is shown on the left and component 2 (Hearst contingency) on the right in each compartment of the figure. The control record appears above the drug record for each animal, respectively. Oblique marks denote reinforcements.

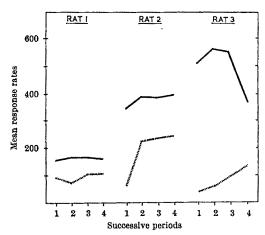


Fig. 2. Response rates for 5 min periods on component 2 of the multiple schedule under control (——) and drug (----) (8 mg/kg pentobarbital) conditions.

and do not support the contention that "ratio-like" behaviour is relatively insensitive to the drug (rat 3) as compared with "interval-like" behaviour (rat 1) at a dose of 8 mg/kg, although at doses of less than 5 mg/kg this may have been true if consistently reliable effects of the drug had been observed.

We may note, in conclusion, that, although the results obtained are at variance with those of Hearst, procedural differences distinguish the two experiments. are in agreement, however, with those of Sidman's for component 1 and are consistent with those of Waller and Morse⁹ for component 2 if the behaviour of rats 2 and 3 can be regarded as resembling fixed-ratio performances. Waller and Morse reported that low dosages of pentobarbital (2-4 mg/kg) in pigeons increased response rates engendered by a low-valued, fixed ratio schedule (FR 30); at higher dosages, however, the drug (5-6 mg/kg) produced decrements in rates of responding, indicating that insensitivity to drug effects cannot necessarily be viewed as a general property of behaviour maintained by ratio contingencies.

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Markovian Models of **Dialogic Time Patterns**

THE duration histograms of pauses and vocalizations in continuous speech have the form of negative exponentials1. For example, the shortest audible pauses are most numerous and the frequency at each longer pause interval is some fixed proportion of the frequency at the next shorter interval. This implies that the probability of prolonging a given pause is constant, regardless of the elapsed time in the pause. The same holds true for duration of vocalizations. The future of the temporal sequence is then stochastically determined by the present and is independent of its history.

Considering pause and vocalization to be the two "states" of monologue, regular sampling yields a sequence of state transitions in successive enquiries. When adjacent samples are found to be in the identical state, the process is assumed to have remained in that state during the intervening interval. The data reported here were sampled at a rate of 200/min (ref. 2). Such a time series can be described as a finite Markov chain, that is, a stochastic process which moves through a finite number of states and for which the probability of entering a certain state depends only on the last state occupied3.

Analogous treatment of the temporal pattern of dialogue yields a sequence of transitions among four dyadic (two person) states. They are: both speakers silent (state 0); A speaks and B is silent (state 1); B speaks and A is silent (state 2); and both speak (state 3). The temporal pattern of a dialogue may then be described as a sequence of the states zero, one, two, three. Because the duration histograms of these dyadic states are also exponential4, a finite Markov chain is a reasonable model for the time pattern of dialogue.

To test the fit of the model, one may compute certain necessary mathematical consequences. For example, the time series of dyadic states may be summarized by the transition matrix P of equation (1).

$$P = \begin{bmatrix} p_{00} & p_{01} & p_{02} & p_{03} \\ p_{16} & p_{11} & p_{12} & p_{13} \\ p_{20} & p_{21} & p_{22} & p_{23} \\ p_{30} & p_{31} & p_{32} & p_{33} \end{bmatrix}$$
(1)

The rows of P are the states at any time t, the columns are the states at time t+1, and the elements p_{ij} the conditional probabilities of moving from state i at t to state jat t+1. P is a stochastic matrix, the rows of which each sum to 1. One consequence of the model is that the probability of reaching any state j from any state i is given by the ijth term of the nth power of matrix P. We have shown that these higher order transition probabilities are indeed approximated by this computation. Another consequence computable from matrix P corresponds to a commonsense feature of dialogue, that is, the time between "switches" of speaker. A new switch is defined the first time a speaker vocalizes alone (states 1 or 2) following any sample in which the other speaker has been the sole The state sequence between switches correvocalizer. sponds to the intuitive phenomenon of "holding the floor". In Markovian terms, it is the mean waiting time in a set of states before absorption, and varies from 3 to 10 sec in our recorded dialogues.

The estimates under the Markovian hypothesis are then correlated with the actually observed mean switching The model predictions so computed for a series of 200 experimental interviews yielded an average product moment correlation of 0.93 (ref. 5). Such concentration of information about long range events (3-10 sec) in short range transition probabilities (300 msec) implies that the latter are quite stable, or approximately stationary.

Two criteria for preferring a particular stochastic model of behaviour are its parsimony and theoretical intelligibility. From this standpoint, the model described is not very parsimonious in that twelve parameters are necessary to account for sixteen transition probabilities: three elements of each row of matrix P define the fourth. Furthermore, the model offers no hypothesis as to the way in which the separate speakers interact to generate

this temporal sequence. Our purpose here is to test one hypothesis about the interaction of speakers which may account for the Markovian property observed. It can be characterized as an "independent decision" hypothesis, and was suggested to us by Professor Alex Heller, of the City University of New York.

Let q_i = the probability that speaker A vocalizes at time t+1 given dyadic state i at time t; r_t = the probability that speaker B vocalizes at time t+1 given dyadic state i at time t; $1-q_i$ = the probability that speaker A is silent at time t+1 given dyadic state i at time t; and $1-r_i$ = the probability that speaker B is silent at time t+1 given dyadic state i at time t. (i = 0, 1, 2, 3.)

From these assumptions a Markov chain can be generated, as summarized in matrix \tilde{P} of equation (2).

$$\widetilde{P} = \begin{bmatrix} 1-q_0 & (1-r_0) & q_0 & (1-r_0) & (1-q_0)r_0 & q_0r_0 \\ (1-q_1) & (1-r_1) & q_1 & (1-r_1) & (1-q_1)r_1 & q_1r_1 \\ (1-q_2) & (1-r_2) & q_2 & (1-r_2) & (1-q_2)r_2 & q_2r_2 \\ (1-q_3) & (1-r_3) & q_3 & (1-r_3) & (1-q_3)r_2 & q_3r_3 \end{bmatrix}$$
(2)

Note that \widetilde{P} is also a stochastic matrix, the rows of which each add up to 1; however, there are only four parameters for each speaker.

If the matrix P of equation 1 is taken to be the maximum likelihood estimate under the hypothesis that the process is a Markov chain, then it can be shown that the maximum likelihood estimates for the q_1 and r_1 are

$$q_1 = p_{i_1} + p_{i_3}$$
 and $r_1 = p_{i_2} + p_{i_3}$ $(i = 0, 1, 2, 3)$

As a test of the combinatorial hypothesis, matrix \tilde{P} was generated for sixty of the two hundred interviews of the previous study, and the Markovian predictions of mean speaker switching durations were repeated as before. The correlation of the actual with the predicted mean speaker switching times for the "independent decision" hypothesis was 0.80, thus accounting for the temporal sequence almost as well as did matrix P, albeit with eight as opposed to twelve parameters.

To the extent that this independent decision model fits, it suggests that speakers in these interviews act independently of each other at any given instant, but always with a probability which is dependent on the joint behavioural configuration of the immediately prior instant. Furthermore, it suggests that these transition probabilities are relatively stable, so that a 300 msec history is sufficient to account for the temporal pattern of the vocal interaction.

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BOOK REVIEWS

TRANSLATED WITH COMMENT

Celestial Mechanics

By Laplace. Translated with a commentary by Nathaniel Bowditch. Vol. 1: Pp. 168+xxiv+746. Vol. 2: Pp. xviii+990. Vol. 3: Pp. xxix+910. Vol. 4: Pp. xxxvi+ 1018. (Bronx, N.Y.: Chelsea Publishing Company, Inc., 1966.) \$79.50 the set of four volumes.

Among the proliferation of reprints of older works which has become quite a feature of the book market, the issue, in a facsimile of high quality, of Bowditch's translation of the Mécanique Céleste makes widely available one of the most remarkable productions in the astronomical literature.

The importance of the French original needs no emphasis. It was intended to be much more than an elaboration of the Newtonian theory of the solar system: it purported to lay the foundation for an all-embracing, purely dynamical description of the material universe, considered as an ensemble of particles interacting by central forces. In striving towards this ambitious aim, Laplace had to extend in every direction the methods of which Newton had laid the foundations, and which Euler and his followers had already considerably refined. On one hand he sharpened the mathematical tools, especially the theory of differential equations and series expansions, and on the other hand he gave the principles of dynamics a more systematic and more complete expression. Every step of his progress was a response to some challenging problem presented by the celestial phenomena, and the picture of the "system of the world" gradually emerged in conjunction with a continual deepening and widening of the powerful method of analysis which we now call theoretical This monumental treatise, conceived on an encyclopaedic scale, was rightly regarded by contemporaries, not only in France but in the whole scientific world, as the source from which every physicist had to draw. The flourishing schools of theoretical physics founded in Germany by Gauss and in Britain by W. Thomson have their origin in Laplace's inspiration. Not only his methods, but his general conception of science-Laplacian determinism—moulded the natural philosophy of the nineteenth century.

In the British and German universities, there was no great demand for a translation of a book written in French. In England, however, science was actively studied by a large number of people barred from the universities by class prejudice or religious intolerance, and who needed textbooks in their own language. To translate Laplace's treatise in its entirety was too formidable a task, but there appeared in 1816 a translation of the first book, comprising the exposition of the laws of mechanics. It was this translation that introduced the miller's son George Green of Nottingham to the Laplacian methods he so brilliantly extended. In America, the social basis of scientific studies was very similar: the colleges were primarily religious institutions, and active scientific work was carried out by practical men. Nathaniel Bowditch was one of them, by trade a sailor, learning the art of navigation the hard way, and completely autodidactic with respect to its scientific foundations. In 1802, he published The New American Practical Navigator, a manual which went through eight editions. He wrote extensive notes and comments on Laplace's treatise "at the time of reading the volumes, as they were successively published", and he undertook the full translation of the text during 1814 to 1817. With his proud sense of independence and self-reliance, he preferred to set aside the manuscript until he was able to publish it at his own expense, after he had gone into a prosperous insurance business in Boston. The four superb volumes appeared between 1829 and 1839—the last one a posthumous tribute to the indefatigable worker, who had died the year before, as the printing was nearly completed. The typographical execution is a masterpiece, which does honour to early American craftsmanship; one sympathizes with the craftsman's pride expressed in the mention "Ira Berry and Lucius A. Thomas, compositors" unobtrusively printed at the end of the first volume, and a similar one at the end of the second volume. It is an antiquarian rarity, for only 250 copies were printed.

The work itself is much more than a translation; indeed, the extent of Bowditch's own contributions equals, or perhaps exceeds, that of the translation proper. It is well known that Laplace wrote with extreme concision, and was even in the habit of disposing of intricate or unconvincing arguments by the misleading phrase "it is clear that . . .". Bowditch's commentary restores all the intermediate steps omitted by Laplace with unsparing, sometimes pedantic, explicitness. In long appendices he presents whole treatises on the elementary mathematical methods which the author had assumed to be part of the reader's general knowledge, or on special problems such as the practical computation of orbits, worked out in every detail, with all necessary tables. The notes also contain full accounts of progress subsequent to the publication of the original volumes. The fifth, supplementary, volume of the French edition, which appeared twenty years after the others, is not translated, but most of its content is embodied in the translator's

notes.

A memoir of Bowditch, written by his children, was appended to the last volume; it is now more appropriately placed at the beginning of the first. With its incredibly stilted style, often producing involuntarily caricatural effects, this memoir is a most revealing period piece. It gives a vivid portrait of the industrious commentator, who, putting Laplace with Archimedes in the class of the great creators, compared himself with Euclid, 'a second-rate mathematician"; and it evokes a striking picture of the parochial Yankee society that could foster such extraordinary personalities. L. ROSENFELD

DECIDING BY NUMBERS

Introduction to Probability and Statistical Decision

By G. Hadley. (Holden-Day Series in Industrial Engineering and Management Science.) Pp. x+580. (San Francisco and London: Holden-Day, Inc., 1967.) \$11.85. STATISTICAL decision theory depends on the idea of utility, an idea which sounds as though it could have been borrowed from the world of business. Whether or not it was, there is almost a campaign going on in America to return the loan with interest, by presenting techniques of decision making in the face of uncertainty in forms that emphasize their place in the training of business men. A technique that could determine correct decisions both about building Concord and about rejecting a large batch of radio resistors on the basis of the fraction defective would have to be pretty magical. The fact, however, that the techniques are statistical makes it clear that they will be of more use for decisions like our second example.

The work being reviewed resembles several others in this and related fields, coming from the United States, in that attractively constructed examples are described and worked out in the text, under headings like "The Tomato Soup Company's Problem". Reading the latter example we find data about prior probabilities, costs of various actions, information available about observable random variables, and so on, motivated and supported by a lively story about a production manager who has to decide how many tons of tomatoes to buy. The problem is worked out, with the accompanying story bringing in further data as required, to the conclusion that he should contract for about 375 acres of tomatoes.

Most of the characteristics of the book are exemplified in "The Tomato Soup Company's Problem". decision process described is Bayesian and the production manager in the story firmly adopts a normal prior distribution for the yield of tomatoes, having a mean of 60 tons/acre and a standard deviation of $\bar{4}$ tons. examples are thoroughly numerical, though the figures have surely been organized so that none of the calculations get messy. The text accompanying the calculations is descriptive and colourful, which accounts to some extent for the 580 pages to which the book extends. The length of the book, however, is also due to the large amount of material in it. A reader who has started with finite event sets in the first chapter will be using posterior distributions for jointly continuous distributions by the time he reaches the "Tomato Soup" problem in the eighth chapter, having been taken through a mass of technical material on the way. In connexion with continuous distributions we note that sections using the calculus are specially marked and the book is so designed that a great deal can be read without even this tool. Mathematical topics like the gamma function and $\lim_{p\to 0} (1-p)^{1/p}$ are outlined, although the level is illustrated by the fact that the latter is shown numerically to be "approaching a number whose value is 0.367", followed by a remark about proof, and a reference.

The contents of the book are divided fairly evenly between the two subjects in the title. The first, fifth, sixth and seventh chapters provide a fairly standard treatment of probability theory, from finite event sets up to a statement, without proof, of the central limit theorem. The second chapter introduces the idea of utility, and decision problems are dealt with in the third and fourth chapters. In the eighth chapter the two threads of the book are brought together to deal with "problems which have the characteristic that in determining the probabilities of the states of nature we make use of historical data or personal feelings and, in addition, make use of the results of a random experiment". In the ninth chapter the classical techniques of hypothesis testing and estimation are introduced and their relation with the decision-theoretic methods in the book is outlined. The tenth chapter treats the Poisson process and in the final chapter sequential decision problems are introduced.

This book is stimulating in many ways and in the United States it will clearly be a saleable addition to Holden-Day's series on "Industrial Engineering and Management Science". It is harder to see a place for it in a British university syllabus. It might be useful in a degree course in mathematics and statistics, but most students in such a course would not need the lengthy numerical illustrations and the partial avoidance of calculus. Postgraduate students in operational research would also, probably, have sufficient background to find the pace of this book slow. Nevertheless, instructors in such courses would find it a useful source and a good book to refer some students to after a tutorial. It could well be just the book for self-instruction by someone who decided to take up the subject on his own.

W. A. O'N. WAUGH

SOURCE BOOK OF SEPARATION

Chromatography

By Erich Heftmann. Second edition. (Reinhold Chemistry Textbook Series.) Pp. xli+851. (New York and London: Reinhold Publishing Corporation, 1967.) 220s.

This is the second edition of the book published in 1961 and reprinted in 1963. There has been much revision; obsolete matter has been eliminated while latest developments have been incorporated and several new chapters have been added. The author index has been eliminated, which I think is a mistake, especially because the authors of publications listed at the end of each chapter are not arranged alphabetically. The book is intended as a reference work and also "as a supplementary textbook for graduate school courses in analytical chemistry, biochemistry and chemical engineering". Whether this applies to students or teachers is not clear, but whatever their standard of living in the United States, very few in this country could afford to buy the book.

This book is a collective work with contributions from more than thirty authors, and is organized in two parts. The first deals with theories and techniques of all the forms of chromatography and includes electrophoresis. The second part contains chapters which cover the applications of all these techniques to particular classes of compound. Much of this part covers the literature to 1965, which is a praiseworthy achievement, but some sections do not go so far and consequently are already outdated, examples being gas chromatography of sugars and of amino-acids. Some sections of this second part tend to be highly concentrated reviews which make them rather difficult reading, and I feel that more use could have been made of tables which would have made it easier for the reader to obtain information. This has been done with good effect in the chapters on nucleic acids, phenolics, terpenes, non-hydrocarbon and hydrocarbon gases.

The first part of the book generally reaches a high standard. The two chapters on the general theory of chromatography and on the theoretical basis of partition chromatography are particularly valuable contributions. Techniques are covered adequately and the chapters are well written expositions of principles and practical aspects. This part does not suffer from the shortcomings of the second part which perhaps are inevitable, as one cannot expect a single book to cover such a vast subject fully.

Nevertheless, this volume contains a great deal of useful, comprehensive and up to date information and is probably the best general book on chromatography which exists.

R. Consden

ENVIRONMENTAL RADIOACTIVITY

Radioecological Concentration Processes

Edited by Bertil Aberg and Frank P. Hungate. (Proceedings of an International Symposium held in Stockholm on April 25-29, 1966.) Pp. xiv+1040. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 300s. net.

MAN began the spread of artificial contaminants through his environment when he lit his first smoking fire, and from this small beginning he has gone on to pollute air, land and water with a wide variety of toxic substances. Five to ten years ago, the contaminant which aroused greatest public alarm was the radioactive fallout from nuclear weapons exploded in the atmosphere. Undoubtedly, some of this alarm was an expression of general unease at the very existence of the weapons, but it did

stimulate many governments to initiate measurements of existing levels of radioactive contamination, and to encourage research into the ways in which the radioactivity reaches the human body. This book contains just over one hundred papers reporting some of the findings. There are contributions from both Europe and America, with a preponderance of papers from Russia, Scandinavia and the United States.

The reader is led to wonder whether any other form of artificial contamination has been the subject of such exhaustive research. Papers contributed to the eleven sessions of the symposium report the concentrations of radioactive isotopes in soil, fresh water and sea water, and describe their metabolism by plants and animals in all three environments. In addition to the detailed reports, there are five review papers by specialists in different branches of the subject.

The isotopes caesium-137 and strontium-90 receive most attention, because they are potentially the most dangerous constituents of fallout, but there are also accounts of work on isotopes such as zinc-65 and chromium-51, which are present in the waste discharged from nuclear reactors, and on iodine-131, which might be released in a reactor accident. The need to understand the biological properties of many radioisotopes gives practical application to some seemingly recondite studies, such as the one concerning the metabolism of chromium-51 by marine worms.

Several papers are devoted to studies on Eskimos and Lapps, for to these people belongs the distinction of having the highest known burdens of caesium-137. In 1965, the average content in each person was about 1 μc., compared with a typical content of about 0·03 μc. in those of us who live in more temperate regions. Eskimos and Lapps acquire these relatively large amounts of activity in reindeer meat, which figures prominently in their diet. The reindeer becomes contaminated with caesium because it grazes on lichens which, in comparison with more rapidly growing plants, are particularly efficient accumulators of the isotope. There are papers from Russia, America and Scandinavia describing in detail the passage of caesium-137 and other isotopes through this

unique food-chain.

In trying to assess the seriousness of artificial radioactive fallout, it is useful to compare its contribution to
the radiation background with that from the natural radioelements, and a group of papers reports work on the
radium and thorium series. The hazard from these natural
sources may not always be negligible. An extreme
example is reported by a Russian worker, who describes
an area with a natural background dose rate some three
hundred times the average, and claims that burrowing
animals show the ill effects of chronic exposure to ionizing
radiations. Another paper in the same group discusses
the observed concentration of stable lead in the environment, and serves to remind us that radioactive materials
are not the only toxic substances which are widely
distributed.

The editors and publishers have succeeded in issuing these proceedings with only moderate delay. The editors explain in their introduction that, to speed publication, the papers are printed as they were received, with only minor alterations. The most noticeable result of this is a serious lack of clarity in a few of the papers translated into English from other languages. In one paper, figure captions have been interchanged, and, in another, two graphs are printed in duplicate, but otherwise the book appears to be free from typographical errors. Its price will not recommend it to the individual, but it should find a place in the library of any institution where work of this nature is in progress. Not only does it contribute to our knowledge of biological mechanisms, but it is also a useful compendium of data on levels of radioactivity in the environment in the years 1958 to 1965.

L. BURKINSHAW.

GONADOTROPHIN CLUB MINUTES

Recent Research on Gonadotrophic Hormones Edited by E. Trevor Bell and John A. Loraine. (Proceedings of the Fifth Gonadotrophin Club Meeting, Edinburgh, 1966.) Pp. viii+345. (Edinburgh and London: E. and S. Livingstone, Ltd., 1967.) 70s. net.

THE Gonadotrophin Club, we are told, met five times during the past thirteen years, but this is the first publication of its deliberations. Thirty-one members from nineteen laboratories were selected to attend, and discussed the following topics in relation to gonadotrophins: bio-assay and immunological assay; chemistry; extraction from urine, blood and tissue; excretion; clinical applications; and biological and biochemical actions. Most space (eighty-three pages) is devoted to clinical applications; the remainder is divided almost equally among the other topics.

Three or four papers are presented on several aspects of each topic. Each group of papers is followed by a short, frank discussion. Editorial revision of the verbatim record and a logical progression through the subject have enabled an extremely readable account of the meeting to be produced.

The papers are of a high standard, as one would expect from the list of participants, and a vast amount of information is presented. It is worth noticing, however, that there are no reports of the action of follicle stimulating hormone at a biochemical level, nor of efforts to examine the control of follicular growth and atresia. Progress in these two particular areas appears to be lagging.

Unfortunately, much of the work described at the meeting had been published previously, or appeared in print before publication of this volume, and will be known to prospective readers. Consequently, one has the impression that little really new work was discussed. This rather detracts from the interest and importance of the book.

The editors achieve a more useful purpose by providing a record of the discussion of each paper. Several papers were presented on most topics, thereby illuminating the different approaches which have been made. A careful study of both papers and discussion will be found rewarding, at least by those with some familiarity with the subject.

On balance, this volume provides a useful summary of the state of research on gonadotrophins in early 1966, and illustrates the different opinions held in the various centres around the world. Those who need a concise, readable account of current thinking on this subject will do well to refer to this book.

R. E. Oakey

NEUROTIC BACKGROUND

Neurosis in the Ordinary Family

A Psychiatric Survey. By Anthony Ryle. (Mind and Medicine Monographs.) Pp. 156. (London: Tavistock Publications, Ltd.; Philadelphia and Toronto: J. B. Lippincott Company, 1967.) 32s. 6d.

One of the great problems of psychiatry is to discover the background of neurosis. Although in examining a patient we take a full history, we can rarely obtain other than a vague and tenuous picture of the environment behind the illness. Moreover, the facts we obtain are often distorted or insufficient and only severe neurosis in parents and grandparents strikes the informants as being worth mention. Slighter conditions are glossed over and frequently the whole background is blurred by prejudices, carelessness or stupidity. It is important, therefore, that not only neurotics but their families should undergo complete psychiatric examination. The person to perform this work successfully is more likely to be the general practitioner than the consultant. He knows the families, and may, indeed, have treated other relatives before the

presentation of the patient. It is not easy to mislead him or hide facts.

This excellent little book is an attempt to show the personal relationship of 112 working-class families. The clinical information regarding the patient is considered in relation to information obtained by a social worker, questionnaires filled in by the parents, and reports obtained from the children's schools.

The picture is built up by examining the social circumstances, the parents' backgrounds, neurosis in the parents themselves, their marriages, child rearing practices, psychological disturbances in the children, and the parental factors associated with them, family diagnosis and treatment.

It is impossible in a short space to describe all that has been unearthed. It is clear, however, that marital adjustment depends to a great extent on the presence of neurosis in the parents and this is later reflected in the personalities of the children. Although the author states that "the relative importance of nature and nurture remains uncertain", in spite of all his work, there is no doubt that the presence of a poor environment tends to produce fresh generations of neurotics, and so on in an endless chain.

The fact that evidence of any marked fall in the neurosis rate with age does not seem to exist contradicts the suggestion, which has been made in some quarters, that neurosis tends to recover spontaneously without treatment. The illness of civil life does not follow the recovery pattern shown in war neurosis.

This book is worth reading and obviously records a great deal of social and clinical work. It is unfortunate that the final chapter on evaluation and conclusions is not clearer, for it needs a great deal of searching to discover the implications of this research. CLIFFORD ALLEN

GENETICS OF MENTAL ILLNESS

Humangenetik

Ein Kurzes Handbuch in Fünf Bänden. Herausgegeben von P. E. Becker. Band V/2: Psychiatrische Krankheiten. Bearbeitet von H. Bickel, H. Cleve, G. Koch, W. Lenz, E. Strömgren und E. Zerbin-Rüdin. Pp. xvi+613. (Stuttgart: Georg Thieme Verlag, 1967.) 190 D.M.

This volume is in the best tradition of the German handbuch, with encyclopaedic coverage of the world literature, a detailed summary and critical analysis of former publications (including both large surveys and reports of single cases), and a synthesis of widely scattered information into a coherent whole. The neuroses are considered by Strömgren in the only contribution from outside Germany, a delightful essay but without the minute documentation found elsewhere in this book. Lenz deals with the autosomal abnormalities associated with mental deficiency; a valuable summary of many individual case reports but, perhaps inevitably, lacking the generalizations that one expects after the collection of so much material. In the chapter on inborn errors associated with mental deficiency, Bickel and Cleve are equally at home with the clinical, genetical and biochemical aspects of the various disorders. Koch reviews the epilepsies, a field where the slow identification of distinct genetical categories is only just beginning, and where genetical analysis is correspondingly difficult.

The most complex problems are those offered by schizophrenia, manic-depressive illness, and the organic dementias; here diagnosis is difficult, there is no clear segregation between affected and non-affected, and no assistance from biochemical characterization. The assessment of previous investigations is a hard task, for varying diagnostic criteria and selective factors render them hazardous to compare. Zerbin-Rüdin has risen magnificently to the challenge; previous investigations are summarized, their

findings tabulated (and occasionally, where appropriate, re-arranged) and their conclusions keenly examined. In this way a vast collection of information about these disorders has been digested, and the conclusions have been presented in a most readable fashion. The review of the literature is remarkably complete, and includes a full discussion of important papers published as recently as last year. These chapters are the highlight of a volume that will be indispensable for all students of psychiatric genetics.

R. T. C. Pratt

MILK IN THE HUNGRY COUNTRIES

Milk Production in Developing Countries By R. O. Whyte. Pp. 240. (London: Faber and Faber, Ltd., 1967.) 50s. net.

This most interesting survey of the conditions necessary for increasing the production and use of milk in the developing countries provides the first comprehensive and practical account of the problems met with in this type of endeavour to improve standards of nutrition, particularly of children and other vulnerable groups of the population.

Many of the existing milk production schemes in such countries stem from the extensive work, starting shortly after the end of the Second World War, of the UN Children's Emergency Fund (UNICEF). Rehabilitation of milk production, conservation and consumption in the devastated countries of Europe, most of which had had a dairy industry of some importance before the war, was the first objective. Later, usually in conjunction with the UN Food and Agriculture Organisation (FAO), the attempt was made to tackle the more difficult task of beginning or extending, in underdeveloped countries with a hungry population, the indigenous production and processing of milk to provide a safe, high-protein, supplementary food. UNICEF funds met the cost, and its engineers made the selection, of suitable modern processing plant and equipment; governments undertook to house these in appropriate buildings provided with the essential services and close to actual or potential sources of an adequate milk supply. While UNICEF sent in experts to erect and manage the processing dairies until indigenous staff could be trained to take over, FAO provided advisers to assist in the improvement of local fodder production and dairy husbandry. Governments also agreed that, in the distribution of the processed milk, the special needs of young children and nursing mothers should be given priority.

In most of the European countries that received aid from UNICEF, milk production and distribution were fairly quick to recover; in most of the underdeveloped countries, with a very primitive milk industry-or none at all-progress was, not unexpectedly, much slower. The basic difficulty of producing sufficient milk, within an operable distance of a processing plant of economic size, in order to keep the plant working efficiently throughout the year sometimes proved, with its associated logistics and incentives, impossible of solution. Inadequate water supplies, poor roads and transport, ignorance of dairy husbandry among the suppliers, shortage of trained farming advisers and of processing staff were among the handicaps which confronted most of these enterprises in their early days and still do today. It is surprising that, despite these difficulties, several countries in the Near, Middle and Far East, in Africa and in Latin America are making good progress with their milk nutrition schemes.

This book not only gives a detailed, penetrating and up to date account—not by any means confined to the countries benefiting from FAO and UNICEF support—of the essential requirements for improved milk production in warm and in tropical countries, but also records the various degrees of achievement already attained in several of them. The author, with his long experience in India and

the East, provides an authoritative assessment of most of the problems which have a direct bearing on milk production, whether geographical, soil and climate, grassland, fodder, animal husbandry, or economic. This assessment should be of special value to the advisers of such governments of developing countries as are genuinely interested in the nutrition of their children and of their population generally, and who are willing to do something about it.

Dr Whyte's analysis and well-documented proposals, which he supports by figures which are probably as near the mark as statistics from underdeveloped countries are likely to be, will meet with little criticism from those with experience of these countries. He gives two numerical items, however, which catch the eye: on page 167 "over 80 per cent of the milk produced in India comes from buffalces"—this is surely a misprint; the figure given recently by other authorities is between 55 and 60 per cent. In describing milk production in Tunisia on page 136, 5,000 dairy cattle and 130,000 dual purpose cattle are mentioned, but nothing is said about the 380,000 buffalces¹ which must also contribute substantially to the Tunisian milk supply.

H. D. KAY

¹ FAO Production Yearbook, 19, 190 (1965).

INSECT ECOLOGY

The Ecology of Insect Populations in Theory and Practice

By L. R. Clark, P. W. Geier, R. D. Hughes and R. F. Morris. Pp. xiii + 232. (London: Methuen and Co., Ltd.; distributed in the USA by Barnes and Noble, Inc., 1967.) 45s.

This book, written by four experienced insect ecologists from different continents, cannot and does not fail to be extremely valuable. It is recommended at some stage of their careers to all who are interested in insect ecology.

A combination of authors, however, in ironing out each other's opinions, also carries the possible hazard of underemphasis in striking a line for the uninitiated. For this reason the reader must either start with opinions which the book will undoubtedly modify, or be patiently prepared to read repetitively in order to erect a basic and biased set of opinions from which further consideration can lead to true appreciation.

The system adopted in this book of presenting, often usefully by quotation rather than mere reference, a set of diverging opinions followed by a discussion, is attractive but may in some cases be spoiled by a lack of final opinion especially if the reader is a beginner. A more extensive treatment would have been welcome in spite of the comment in the preface that principles only would be discussed.

The two introductory chapters are excellent in clarifying the idea of the "life system" approach to the greater dynamic equilibria. Similarly, the third chapter, "Current Theories to explain Insect Numbers", is a really fine précis of a voluminous literature. It is true that there is scope here for disagreement, that quotation is selective and does not bring out this or that point, but by and large a good bird's-eye view emerges.

On the other hand, the fourth chapter, "The Functioning of Life Systems" (89 pages), is at once interesting and all important, but to some people will be slightly exasperating. Certain readers would prefer a more unified approach to each case, possibly summarized by diagrams and followed by an overall discussion, whilst others, including myself, would prefer it as it is. In any case, however, the references are ample and the wide selection of types reviewed is gratifying. This chapter must constantly be reviewed because herein lies the "proof of the pudding". Never is this more true than when reading on to the "Ecology of Pest Control", the sixth chapter, and "The Further Development of Research on Insect Populations", the seventh chapter.

Having completed one's reading and started to ponder,

one inevitably becomes aware of the lack of a subject index. It is true that individual ideas can be traced if one remembers a biological name, a used term or an author, but if one only has a hazy idea, for example, "that it had something to do with DDT or an insecticide". some chasing backwards and forwards is necessary.

It now remains to state an exceedingly important characteristic of this book, which is to aid in sorting out the exceedingly numerous facets of ecology. Again and again as I read and decided that some aspect was missing, such as genetics under-emphasized, I checked and decided that it was indeed raised and relegated reasonably to certain areas, and that to go further would have required a book on the principles of biology rather than the ecology of insect populations. Similarly with the influence of behaviour and physical factors on population problems to those particularly interested in such factors. As an example, there is the reference on page 24 to the inevitability of population survival due to the average prevailing consistency of locust swarm drop in favourable wet front areas. I eventually had to concede that it is (a) mentioned and (b) perhaps does not require emphasis in this book. As the authors say on page 34, "because our interest is focused on populations, we find it useful to think of demographic occurrences as primary events and those which affect or qualify as secondary events". Ecologists whose interests are otherwise will find this attitude a corrective influence.

Finally there is much to stimulate those concerned with pest control. The approach of "protective management" favoured by most ecologists as against species eradication is realistically discussed in a stimulating manner. Although a plea for future work towards this end is made, the reader is reminded of the ever present threat to such schemes which may result from unpredictable evolutionary occurrences. This appears to be a salutary warning that to proceed slowly is to proceed wisely.

The production of this book is excellent and printing errors are minimal.

F. L. WATERHOUSE

BIOLOGICAL OCEANOGRAPHY

Aspects of Marine Zoology

Edited by N. B. Marshall. (The Proceedings of a Symposium held at the Zoological Society of London on 23 and 24 March 1966. Symposia of the Zoological Society of London, No. 19.) Pp. x+270. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967. Published for the Zoological Society of London.) 80s.; \$14.

It is to be both welcomed and expected that the advances in underwater technology that have occurred during the past decade are leading to a re-orientation of the traditional approach to the study of the organisms living in the deeper parts of the oceans. This book probably marks the beginning of a period when greater scientific effort than ever before will be devoted to the study of the biology of these organisms rather than their morphology and taxonomy.

Those who are familiar with the books by Beebe and Cousteau will have been introduced to a world where the light intensity rapidly falls with increasing depth until, at about 1,000 metres, the faint blue light from above is no longer adequate for visual purposes. C. M. H. Harrisson gives an interesting and comprehensive account of the methods used for sampling mesopelagic fish, assessing the merits of each type of gear while stressing the difficulties in obtaining a representative sample from such large volumes of water unless several methods are used. In this twilight region and below, the majority of the fish carry luminous organs of one kind or another, and the variety and function of these are reviewed by J. A. C. Nicol. As he points out, most of the specimens are dead

when they reach the surface, and consequently very little is known of the physiological mechanisms which control their function.

Although some fish may use the luminous organs as species specific and sexual recognition signals, many bathypelagic fish have macrosmatic males. Marshall suggests that in these species the nose plays an important part "in sniffing out odorous females in vast aqueous haystacks". In many cases this is combined with a well developed lateral line system which would increase the awareness of the fish to small movements in the surrounding water.

Nearer the surface, light coming from above has been shown to play an important part in controlling the vertical movement of the organisms which cause the deep or sonic scattering layers on echo sounder traces. In the two papers dealing with this topic Boden and Kampa demonstrate the strong correlation between surface irradiance and the migrations to or away from the surface, while Blaxter and Currie describe how they succeeded in forcing the scattering layer down by lowering artificial light sources into the water.

The other parameter, which fluctuates with depth and might be used by animals to regulate their vertical level, is that of the hydrostatic pressure. Sensitivity to pressure has been demonstrated in a number of shallow water species, and the mechanism used for detecting pressure changes is discussed by P. Digby.

The study of a large and complex community such as the plankton requires special methods of sampling and analysis to reveal the pattern of seasonal changes and inter-relationship of organisms in areas as large as the North Sea and the North Atlantic. R. S. Glover presents some of the results of the Continuous Plankton Survey which uses the facilities offered by commercial shipping for routine sampling of large areas of water. He stresses the necessity for estimating abundance as well as distribution in ecological analyses of this type and looks at the future developments in technology and techniques. This section is supplemented with a description of the properties of the blue carotenoid pigments which are characteristic of the neuston, a sub-division of the plankton found at the surface in tropical oceanic water.

Other articles include an account of the distribution of Pogonophora in the N. Atlantic by E. C. and A. J. Southward, a description of a deep sea squid by M. R. Clarke, and an account of the seasonal movements of sperm whales by R. Gambell.

The book provides a number of valuable and interesting articles on modern trends in biological oceanography, and with the exception of the photographs of echo traces, the illustrations are clear and easy to follow. The balance of the contents suffers from the absence of a section on underwater sounds of biological origin, which could perhaps have been remedied if the contributors had been invited from a somewhat wider circle.

DEREK A. DORSETT

LIBRARIES AND DOCUMENTATION

The Provision and Use of Library and Documentation Services

Edited by W. L. Saunders. Some Contributions from the University of Sheffield Postgraduate School of Librarianship. (International Series of Monographs in Library and Information Science, Vol. 4.) Pp. x+198. (Oxford, London and New York: Pergamon Press Ltd., 1966.) 50s. net.

Among the professions today librarians are demonstrating in the organization of their education that they are by no means unaware of the changing needs of society. The tremendous growth in education, the "information explosion", the growing acceptance of the need for higher

standards of library provision and the ever increasing amount of leisure time becoming available; all these factors underline the need for more and better qualified librarians. The growth of schools of librarianship in numbers, in size and in the influence they are having on the pattern of library education is one of the most important developments in this profession.

This book is a collection of special studies prepared as part of the requirements for the University of Sheffield's postgraduate diploma in librarianship. There are seven studies supplemented by contributions from two members of the school's staff. The title of the book and the series in which it appears do not clearly define its contents. A monograph is usually accepted as being a treatise on a particular subject whereas the contents of this book cannot really be said to be related. Nevertheless, the separate studies are valuable contributions to the literature.

The first study is Mrs Elizabeth Melrose's account of the work and life of Mr J. P. Lamb, the former Sheffield City librarian. Mr Lamb's achievements in the modern service he developed at Sheffield and his contributions to librarianship are clearly evident in this well and carefully written account.

"Trade literature: its value, organization and exploitation" is the title of the next contribution by Miss Elizabeth Smith in which she states: "There should... be a more conscious link between existing knowledge and the inventor; between industrial activity and pure research. One way in which industry plays its part in forging this link is in supplying a continuous flow of information on new products and processes in the form of 'trade literature'."

Miss Smith found little evidence of effective communication between researchers through this medium in this country, whereas abroad, universities collect trade literature to give students a balanced impression of industrial activity in relation to their own research. The standard of trade literature is improving, so with an associated improvement in the methods of collecting trade literature and retrieving required information there should be no reason to discourage the greater use Miss Smith advocates. There is, however, the question of economics, particularly for the public or university library attempting to serve a wide and disparate range of subject users. A recent survey¹ indicates that the use made of trade literature in public libraries at least might not be "economic" compared with the considerable work involved in keeping files up to date and maintaining indexes. For special libraries the value and use of trade literature cannot be questioned.

The next two studies, "A survey of the provision and use of library services in certain London prisons" by Mrs Penelope Rowlinson and Mr M. W. Moss's "Library service for undergraduates", are concerned with the library requirements of two very different sections of the community. Mrs Rowlinson has made a thorough survey of the services provided in four prison libraries, which have greatly improved since the public libraries took over responsibility for them, but as she points out, "The Prison Department is, in fact, availing itself of an excellent service at a relatively small cost to itself. It seems only reasonable to hope that the department will soon increase its grant to public libraries, so that the prison library service may become financially self supporting, instead of relying so heavily upon the generosity of the public library service". Mr Moss in his study is concerned with an aspect of university librarianship which at the time of writing had not influenced the development of British universities to any great extent. There are useful lessons to be learnt in this country from this well presented account mainly of activities in America.

The director of the Sheffield Postgraduate School of Librarianship, Mr W. L. Saunders, together with Mr E. W. Roberts and Miss Lisbeth Wickison, have presented the "Survey of borrowing from the University of Sheffield Library during one academic year". This survey report contains a considerable volume of data which have been

interpreted to reveal certain patterns of borrowing which have proved to be compatible with the results of previous similar surveys carried out at Birmingham and Leeds. The extent of "off-subject" or non-curricular borrowing is of particular interest; however, the provision of departmental libraries for some subjects, physically separate from the main library, is shown to have an inhibiting effect on undergraduate borrowing outside their own departments.

¹'A critical review of the surveys of scientists' use of libraries" by Mrs A. Stephanie Barber shows the same careful examination of her subject which occurs in all the studies in this volume. She states "...it is a pity that the data amassed...do not correlate well and therefore form no sound basis to a theory for increasing the scientist's effective use of published information". Recently a committee of the Library Association has been investigating scientific library services in this country. It has studied existing surveys and where no reports have been available has had to investigate requirements itself. It will be of particular interest in view of Mrs Barber's conclusions to see what assessment of existing and required services the committee will produce.

The last study is an evaluation of Referativnyi zhurnal: nauchnaya i tekhnicheskaya informatsiya, the Russian abstracting journal, by Mr H. Schur, a lecturer at the postgraduate school. There is an abvious need to examine this publication, and its coverage in various fields, in much greater detail than has been possible in this interesting but short report.

The value of this book lies in the quality of the individual contributions, and it deserves a place on library shelves for this reason alone. I hope we may see further collections of studies from the Sheffield School, but that they will have a closer relationship with each other to provide a treatment in depth of aspects of library and documentation services.

Derek Jones

¹ Clements, D. W. G., J. Documentation, 23, 131 (1967).

OBITUARIES

Professor Frank Ernest Hoare

THE death of F. E. Hoare on May 25, 1967, after a short period in hospital, came as a great shock to all who knew him. The basic illness which had gradually developed had been completely unsuspected, and there had been no premonition in any decrease in his energy and cheerful enthusiasm.

He was born on June 22, 1907, at Brighton, where he attended the Municipal Secondary School. He gained scholarships to Imperial College, London, and studied under H. L. Callendar, who roused in him what was to prove a lifelong interest in heat and thermodynamics.

He joined the department of physics at University College, Exeter, in 1928, and remained there, first as assistant lecturer, and then as lecturer, until he was seconded to the Admiralty Scientific Service in 1940. His relations with Professor F. H. Newman were happy, and he enjoyed all aspects of university life and work. As in so many physics departments at that time, however, the teaching duties were onerous, and the general facilities and the financial backing for research were extremely meagre. It was then that Hoare developed his skill in designing and constructing apparatus, and acquired the do it yourself philosophy which coloured so much of his later work.

Hoare's early research at Exeter was mainly concerned with the determination of the Stefan-Boltzmann constant, using the Callendar radio-balance. With the improved techniques which he developed he finally obtained what was long accepted as the "best" of the directly determined values. In 1931 Hoare published A Textbook of Thermodynamics which, clear and unpretentious, and with a fuller than usual treatment of applications, was widely appreciated. His subsequent work was in the accurate measurement of the susceptibilities of diamagnetic salts. The analysis of the results, partly in collaboration with G. W. Brindley, at Leeds, led to comprehensive and reliable sets of values for the susceptibilities of ions with inert gas configurations in diverse surroundings. These have been widely used in testing theoretical calculations of space charge distributions in atoms and ions, and in correcting for diamagnetism in determining the magnetic moments of ions in paramagnetic salts.

In the course of his war service with the Admiralty, Hoare planned and conducted two research expeditions to Iceland, and later became deputy leader of more than a hundred naval and civilian personnel concerned with the testing and trials of newly developed weapons. His scientific and practical approach was invaluable.

In 1946, Hoare joined the department of physics at Leeds, where he was to become reader (1954) and later professor (1964). Much of the theoretical work at Leeds in the past thirty years was concerned with the interpretation of magnetic and related properties of metals and alloys on a collective electron basis. This led to an early appreciation of the need for much more extensive measurements in the low temperature range. Hoare was anxious to embark on experimental work in this field. This was the beginning of the building up at Leeds of the low temperature laboratories which were to provide basic facilities for a very wide range of research in solid state physics. The undertaking was a formidable one, for very little assistance by grants was obtainable for work in this field. A hydrogen liquefier was designed and built in the department, and later a helium liquefier-cryostat. It then became possible, through the efforts of a small group of which Hoare was a member, to buy liquid helium from a central source of supply; but it was not until 1963 that a modern hydrogen-helium liquefier could be installed in the department through grants which had at last been obtained.

Hoare took a helpful interest in all the low temperature work, but his more personal research, with a succession of colleagues and research students, was mainly on the determination of the low temperature specific heats of metals and alloys. The lattice and electronic contributions can be sorted out, and by measurements on, for example, palladium and its alloys with the neighbouring elements rhodium and silver, with one less and one more electron per atom, important information connected with the electronic energy band forms can be obtained. The determination of the specific heats over a range of temperature of a series of binary alloys was, however, a very lengthy and laborious undertaking. In this connexion an important technical advance was recently made by Hoare and his associates. A method was developed for semi-automatic recording on punched tapes which can be fed into a computer; this in turn, suitably programmed, provides a print-out of the magnitude and standard errors of the various contributions to the specific heat. By this means, most of the routine drudgery has been eliminated, and the overall time requirements have been greatly reduced. A vast wealth of information has now been obtained particularly on elements of the nickel, palladium and platinum triads and their alloys. The results, while consistent with the general present theoretical outlook, provide many challenging problems to be faced in the future development of the theory of the metallic state.

Hoare's extensive contributions in research at Leeds were not made at the expense of other university activities. He played a full part in lecture and laboratory teaching, gave valuable service on many university committees and was actively interested in general educational matters outside as well as inside the university.

E. C. STONER

University News:

Liverpool

Following the success of an experimental Board of Industrial Studies, formed to encourage collaboration between the university and industry, the university is to appoint its first permanent director of industrial studies. He will take charge of an Industrial Studies Unit concerned with two main activities: improving the relation and mutual understanding between industry and universities; and developing courses of industrial studies for undergraduate and postgraduate students to help the transition from university study to industrial employment.

Announcements

THE members of the Advisory Committee for Scientific and Technical Information for the 1967-68 session are as follows: Dr F. S. Dainton (chairman); Dr J. W. Barrett; Mr H. P. F. Swinnerton-Dyer; Dr G. M. Dyson; Mr D. J. Foskett; Professor S. Gill; Professor S. P. Hutton; Dr N. Kurti; Mr J. Leicester; Dr R. M. Lodge; Professor W. J. M. Mackenzie; and Professor G. A. Smart.

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Mathematics*, Professor W. Olszak (Warsaw); *Chemistry*, Professor V. Bruckner (Budapest); *Physical Chemistry*, Professor A. Riad Tourky (Cairo); *Botany*, Professor I. Manton (Leeds) and Professor F. Skoog (Madison); *General Biology*, Professor E. Hadorn (Zurich); *History of Science and Medicine*, Professor J. Roger (Orleans-Tours).

DR ALAN T. WATERMAN, first director of the US National Science Foundation, has been awarded the Karl Taylor Compton Gold Medal by the American Institute of Physics.

APPLICATIONS for postdoctoral fellowships in reproductive physiology at the Harbor General Hospital (University of California, Los Angeles) are now being accepted for the year beginning July 1, 1968. Applicants should have an MD or PhD degree, and fellows supported by the US Public Health Service must be US citizens or have permanent resident status. US citizenship is not necessary for fellows supported by the Ford Foundation. Further information can be obtained from Dean L. Moyer, Department of Pathology, Harbor General Hospital, Torrance, California.

RESEARCH Fellowships in Forest Resources are awarded annually by Harvard University from the Charles Bullard Fund. These fellowships, which carry stipends of up to \$15,000, are open to men in public service, in academic careers and in private forestry. Further information can be obtained from the Committee on the Charles Bullard Fund for Forest Research, Littauer Center 119, Harvard University, Cambridge, Massachusetts.

A NEW permafrost map of Canada in colour has been published jointly by the Division of Building Research of the National Research Council of Canada, and the Geological Survey of Canada, Department of Energy, Mines and Resources. Copies of the map can be obtained at a cost of 50 c. either from the Division of Building Research National Research Council, Ottawa, or the Geological Survey of Canada, Department of Energy, Mines and Resources, 601 Booth Street, Ottawa.

THE manufacture of Dracone towed flexible barges, which are increasingly used for the transport of oil, drinking water and other cargoes, is to be transferred to the new Dunlop General Rubber Goods Division's factory at Skelmersdale, Lancashire, under exclusive licence from Dracone Development, Ltd., a subsidiary of the National Research Development Corporation. The Dunlop Dracone Consortium, until now manufacturer of the barges, has ceased to operate, but close association between Dunlop, Dracone Development, Ltd., and the National Research Development Corporation on the project will continue.

Meetings

PROCESS Instrumentation, October 9-20, Harwell (Post Graduate Education Centre, Building 455, AERE Harwell, Didcot, Berks.).

AUTUMN Metallurgical Days, October 16-19, Paris (Société Française de Métallurgie, Secrétariat, 47 rus Boissière, Paris XVI^o).

COMMISSIONING, Use and Maintenance of Reactor Instrumentation, October 23-November 3, Durley Hall, Bournemouth, and AEE, Winfrith (Harwell Post-Graduate Education Centre, Building 455, AERE, Harwell, Didcot Berks.).

DIET and the Central Nervous System, October 28, University of Aberdeen (J. L. Clapperton, Scottish Group, The Nutrition Society, Hannah Dairy Research Institute, Avr).

SIXTH International Congress of Allergology, November 5-11, Montreal (Dr S. O. Freedman, 1390 Sherbrooke Street West, Montreal 25).

MANUFACTURING and Processing in the Cosmetic Industry, November 13-15, Learnington Spa (Dr J. J. Mausner, Society of Cosmetic Chemists of Great Britain, 56 Kingsway, London, WC2).

THERMAL Conductivity, November 13-15, National Bureau of Standards, Gaithersburg, Maryland (D. R. Flynn, Building 226, National Bureau of Standards, Washington, DC).

DEVELOPMENT of Safer and More Effective Drugs, November 26-28, Washington, DC (Samuel W. Goldstein, Academy of Pharmaceutical Sciences, 2215 Constitution Avenue Northwest, Washington, DC).

BIOMATHEMATICS and Computer Science in the Life Sciences, March 14-16, 1968, Houston (Office of the Dean, The University of Texas Graduate School of Biomedical Sciences at Houston, Division of Continuing Education, P.O. Box 20367, Houston, Texas).

ERRATUM. In the communication "Effect of Previous Injection of Homologous Embryonic Tissue on the Growth of Certain Transplantable Mouse Tumours", by G. A. H. Buttle and Ann Frayn (Nature, 215, 1495; 1967), the third paragraph on p. 1496 should begin "The imferon (iron dextran) induced sarcoma² was found to be very sensitive to previous injection of embryonic tissue". The second line of the caption to Table 1 should refer to embryonic tissue suspension from B.S.V.S. mice. The data in Table 3 should refer to adult and foetal tissue, not maternal and foetal tissue. The twelfth line of the third paragraph on p. 1497 should begin a new paragraph: "If the Crocker tumour is used in place of the imferon induced sarcoma . . .". The first line of the last paragraph should read "The growth of tumours induced by 20-methyl cholanthrene".

Correspondence

Towards a Broader Curriculum

Sir.,—I read your report "Towards a Broader Curriculum" (Nature, 215, 1329; 1967) with the greatest interest. You should, however, realize that in one sizable part of Britain—Scotland—the sixth form curriculum is broader than in England and, at least in the four older Scottish universities, it is usual to spend four years over an honours degree. I write to suggest that it might be worth while for educationists concerned with the transition from school to university to take a good hard look at the system in Scotland which, after all, produces a significant number of graduates.

Yours faithfully,

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 9

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING ROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Disussion Meeting on "C.E.I. Examination Requirements—a Progress Report".

SOCIETY OF INSTRUMENT TECHNOLOGY (at the Royal Institution, 21 Albenarie Street, London, W1), at 6 p.m.—Sir Henry Jones, K.B.E.: "Sources and Uses of Energy" (Thomson Lecture).

Monday, October 9-Thursday, October 12

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2)—Conference on "The Economics of the Reliability of Supply—a Comparison of Standards Adopted in Various Countries".

Tuesday, October 10

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, SW1), at 10.30 a.m.—Meeting on "The Production of Fish".

ZOCLOGICAL SOCIETY OF LONDON (at the Zoclogical Gardens, Regent's Park, London, NW1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr E. Eastwood, C.B.E.: "Control Theory and the Engineer" (Chairman's Inaugural Address).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5.30 p.m.— Professor E. R. Laithwaite: "Making the Best of a Bad Job—What Engineering Is". (Lecture for Sixth Form Boys and Grits from Schools in London and the Home Counties. To be repeated on October 11, 17 and 18.)

UNIVERSITY OF LONDON (at Imperial College, Exhibition Road, London, SW7), at 5.30 p.m.—Professor L. A. E. Carleson (University of Uppsala): "Survey Type". (First of three Special University Lectures in Mathematics. Further lectures on October 11 and 12.)*

Institution of Mechanical Engineers, Automobile Division (at Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Annual Meeting. 30 p.m.—Mr C. H. Bradbury: "A Saga of Sound and Vibration" (Chair-6.30 p.m.—Mr C man's Address).

Wednesday, October 11

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./
I.E.E. MEDICAL AND BIOLOGICAL ELECTRONICS GROUP (at the Middlesex
Hospital Medical School, Cleveland Street, London, W1), at 2.30 p.m.—
Symposium on "Ultrasonics and Medicine".

PLASTICS INSTITUTE, POLYMER PROPERTIES DISCUSSION CIRCLE (at 11 Hobart Place, London, SW1), at 2.30 p.m.—Meeting on "Energy Requirement in Plastics Processing".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1), at 5 p.m.—Dr R. M. Loynes: "On the Concept of the Spectrum for Non-Stationary Processes".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, SW1), at 6.15 p.m.—Dr A. Dawson: "Disaccharide Intolerance".

SOCIETY OF ENVIRONMENTAL ENGINEERS (at the Euston Tavern, 73 Euston Road, London, NW1), at 6.30 p.m.—Mr G. Hooper: "Desiceants and Desiceators".

BRITISH SOCIETY FOR THE HISTORY OF PHARMACY (at the Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London, WC1), at 7 p.m.

—Dr Marie Boas Hall: "Apothecaries and Chemists in the Seventeenth —Dr Mari Century".

Thursday, October 12

CHEMICAL SOCIETY (at the Middlesex Hospital Medical School, Cleveland Street, London, W1), at 2 p.m.—Symposium on "Aspects of Organic Reactions Mechanisms".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, SW1), at 2,30 p.m.—Informal Discussion on "Estuarial Barrages in Relation to Water Resources Development". Mr S. E. H. Ford: Dee; Mr G. C. Marshall: Morecambe; Mr Ritchie M. Campbell: Solway.

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 New Cavendish Street, London, W1), at 5.30 p.m.—Mr K. Knowles: "Seasonal Variations in Demand".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion meeting on "The Influence of Changes in School Curricula on the Intake of Engineering Courses", opened by Mr G. B.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5.30 p.m.—Sir Lawrence Bragg, F.R.S.: "Electricity and Magnetism" (Civil Service Lecture).

Friday, October 13

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 2.30 p.m.—Colloquium on "Recent Advances in Measurement Circuits Using Semiconductor Devices".

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5.30 p.m.—Mr A. E. Brewster, Mr G. M. Gardiner and Mr R. J. Hodges: "Ultra-High-Speed Magnetic Printing and Display Systems".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the I.E.R.E., at the Middlesex Hospital Medical School, Cleveland Street, London, W1), at 5.30 p.m.—Symposium on "The Ariel III Satellite".

Saturday, October 14

JOINT BIOLOGY COMMITTEE (in the William Beveridge Hall, University of London, Senate House, Malet Street, London, WC1)—Conference on "Some Aspects of Plant Physiology".

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3.30 p.m.—Mr Michael R. K. Lambert: "Moroccan Summer—an Expedition to the High Atlas and Sahara".*

Monday, October 16

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 1.15 p.m.-Professor George Porter, F.R.S.: "The House of Michael Faraday".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, SW1), at 5 p.m.—Mr N. W. Hussey: "Prospects for Integrated Control in Protected Cultivation".

ROYAL INSTRUCTION (at 21 Albemarle Street, London, W1), at 5 p.m. – Professor G. Wilse Robinson: "Why is Oxygen Blue?" (Bourke Lecture).

BRITISH COAL UTILISATION RESEARCH ASSOCIATION (at the Institution of Civil Engineers, Great George Street, London, SW1), at 5.30 p.m.—Mr Harry Perry: "Current Coal Utilisation Research in the U.S.A." (Sixteenth Coal Science Lecture).

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "North Sea Gas—Internal Combustion Engines".

APPOINTMENTS VACANT

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER and an ASSISTANT LECTURER (with an interest in fluid merhanics) in the DEPARTMENT OF CIVIL ENGINEERING—The Staff Officer (586)(2). The University of Aston in Birmingham, Gosta Green, Birmingham, 4 (October 11).

LECTURER or ASSISTANT LECTURER, Grade B (preferably with an interest in the taxonomy and morphology of micro-organisms) in Microbiology, to the control of control of the control of con

RESEARCH ASSISTANT (with a good honours degree in mechanical engineering or physics) IN THE DEPARTMENT OF MECHANICAL ENGINEERING, to work on exciting new developments in the prediction of the fatigue life of metals—The Secretary, University College London, Gower Street, London, WC1

RESEARCH DEMONSTRATOR IN THE DEPARTMENT OF PHYSIOLOGY to work in the field of placental transfer and foetal nutrition of sheep—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire, quoting Ref. M.17.

RESEARCH STUDENT IN CHEMISTRY to work with the Head of the Department, Professor G. H. Williams, in the field of free radical chemistry—The Secretary, Bedford College (University of London), Regent's Park, London,

NWI.

SENIOR TECHNICIAN OF TECHNICIAN (preferably with experience with an electron microscope or in cell biology) to assist in research on fine structure and function of plant cells—Mr A. D. Greenwood, Botany Department, Imperial College of Science and Technology, London, SW7.

TECHNICIAN IN THE PHYSICAL METHODS SECTION OF THE DEPARTMENT OF CHEMISTRY to operate spectroscopic instruments—The Deputy Secretary, The University, Southampton.

TEMPORARY LECTURER/ASSISTANT LECTURER IN SOCIOLOGY—The Registrar, The University, Keele, Staffordshire.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Great Britain and Ireland

Ambassade de France. Service de Presse et d'Information. Culture in France. Pp. 24. (London: Ambassade de France, Service de Presse et d'Information, 1967.)

Proceedings of the Royal Irish Academy. Vol. 65, Section A, No. 5: Group-Similar Isometrics. By J. J. McMahon. Pp. 51–61. 2s. Vol. 66, Section A, No. 6: A Uniqueness Theorem for Fields of Elliptic Modular Functions. By D. L. McQuillan. Pp. 63–68. 1s. 6d. Vol. 65, Section A, No. 7: Spinors and Rotations. By D. J. Simms. Pp. 69–75. 2s. Vol. 65, Section A, No. 8: Thin Shells and Relative Probabilities in Hilbert Space. Pp. 77–91. 3s. 6d. Vol. 65, Section B, No. 11 and 12: New Ordovician Faunas from Grangegeeth, Co. Meath. By P. J. Brenchley, J. C. Harper, M. Romano and D. Skovington. Decordinaspis—a New Trinucleid Trilobite from the Ordovician of Ireland. By J. C. Harper and M. Romano. Pp. 297–308+plates 7–9. 4s. Vol. 65, Section B, No. 13: Anticancer Agents—III. Synthesis and Anticancer Activity of some Bisthosemicarbazones and Thiosemicarbazides. By V. C. Barry, M. L. Comalty, C. N. O'Callaghan and D. Twomey. Pp. 309–324. 4s. 6d. Vol. 65, Section B, No. 14: On the Origin of the Tertiary Granophyres of the Carlingford Complex, Ireland. By M. J.

Le Bas. Pp. 325-338+plates 10 and 11. 4s. 6d. Vol. 65, Section B, No. 1. Interglacial Deposits in Kildromin Townland, near Herbertstown, C Limerick. By W. A. Watts. Pp. 339-348+plate 13. 2s. 6d. (Dublin: Roy. Irish Academy, 1967.)

Other Countries

Thin Solid Films, Vol. 1, No. 1 (July 1967). Editors-in-Chief: L. Hollan and J. A. Dillon, Jr. Co-ordinating Editor: G. Siddall. (An Internation Journal on Their Science and Technology.) Pp. 1-83. Subscription rates Six issues per volume, 180s.; \$25; 90 D.fl. (Amsterdam: Elsevier Publishing Company 1867).

Six Issues per volume, 180s.; \$25; 90 D.fl. (Amsterdam: Elsevier Publishin Company, 1967.)

United States Department of the Interior: Geological Survey. Wates Supply Paper 1685: Magnitude and Frequency of Floods in the Unite States. Part II: Pacific Slope Basins in California. Vol. 1: Coastal Basin South of the Klamath River Basin and Central Valley Drainage from th West. By L. E. Young and R. W. Cruff. Pp. x1+272+plates 1 and 2 (Washington, D.C.: Government Printing Office, 1967.)

[11: Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Wildliffe Research 1965-66. Pp. 51 (Canberra: Commonwealth Scientific and Industrial Research Organization 1967.)

[11: Mauritius Sugar Industry Research Postitute 1. [11: Mauritius Sugar Industry Re

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Mauritus Sugar Industry Research Institute. Annual Report 1966pp. 149+12 plates + 24 tables. (Reduit: Mauritius Sugar Industry Research
Institute, 1967.)

Institute of Sera and Vaccines, Praha. Collection of Summaries, Vol. 6—
Papers Published by the Scientific Workers of the Institute in 1966. Pp. 41
(Praha: Institute of Sera and Vaccines, Research Institute of Immunology, 1967.)

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Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 123 (Juni 1967): Mikroorganismen in debWurzelregion von Weizen. Sammelreferat von Dr W. Gams. Pp. 77.
(Berlin-Dahlem: Biologischen Bundesanstalt für Land- und Forstwirtschaft, 1967.) 14 D.M.

[148]

Jamalea: Ministry of Agriculture and Lands. Bulletin No. 60 (New Series):

1967.) 14 D.M. Jamatea: Ministry of Agriculture and Lands. Bulletin No. 60 (New Series): Investigations, 1960–1961. Pp. iv+97. 5s. 6d. Annual Report of the Ministry of Agriculture and Lands for the year ending 31st December, 1962. Pp. 102. 6s. (Kingston: Ministry of Agriculture and Lands, 1967.) [148 World Health Organization, World Directory of Dental Schools 1963. Pp. 282. (Geneva: World Health Organization; London: H.M. Stationery Office, 1967.) 20 Sw. francs; 35s.; \$6.75. [148 Missao de Estudos Agronomicos do Ultramar (Junta de Investigacoes do Ultramar). Comunicacoes, Nr. 60: Bibliografia de Café/Coffee Bibliography, 1966. By Domingos B. Mariano. Pp. Ii+238. (Lisbon: Missao de Estudos Agronomicos do Ultramar). [148 Made of Iron. (Exhlbition assembled as a contribution to the Arts Festival of Houston, October 1968, held in honour of the inauguration of Jones Hall for the Performing Arts.) Pp. 288. (Houston, Texas: University of St. Thomas Art Department, 1967.) \$6.

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The Real Reason for the Brain Drain

WITH all the talk there has been in the past three years about the brain drain and the causes of it, it would have been unreasonable to expect that the Working Group on Migration of the Committee on Manpower Resources under Dr F. E. Jones would have been able to suggest a dramatic remedy for the continuing loss of scientists and engineers from Britain. It is, however, a considerable benefit that the committee has lent its authority to the abolition of several fairy stories about this social phenomenon. It is especially welcome that the committee has given as the principal reason for migration the likelihood that British engineers and scientists leave for the United States because American employers make them better offers. This simple truth has been apparent for several years, though it has often been concealed by the tendency of departing scientists to say that they have been won over not so much by money as by what are called "facilities"-gas chromatography equipment and the like. The report of the working group now published emphasizes that salaries are in practice important—young men starting their careers in the United States can hope to be paid three times as much as their counterparts in Britain if they choose to work in industry. Later on, American universities are able to win away the more distinguished academics by doubling British academic salaries in many cases. The working group would have been able to rub this point in more strongly if it had been able to devise some means of making an accurate comparison between the cost of living in Britain and in the United States—the chances are that those who stay at home are inclined to exaggerate the cost of living in the United States.

In the circumstances, it may well be that the most surprising feature of the brain drain is that substantial numbers of those qualifying as scientists and engineers still elect to remain in Britain. The figures which the Jones committee has been able to assemble are nevertheless enough to justify much of the alarm there has been about the brain drain. Since the beginning of the sixties, the number of qualified people leaving the United Kingdom for at least one year has more than doubled; in 1966, 6,200 people left the country. Engineers and technologists are now more than ever the dominant component of the brain drain and the 4,200 of them who left in 1966 accounted for no less than 42 per cent of the new supply of engineers and technologists three years before. By comparison with the rapid acceleration of the emigration of engineers and technologists in the past five years, the emigration of scientists remains constant at 22-23 per cent of the annual supply three years earlier. It is only fair to add that North America takes only about half of those who emigrate each year, although it does appear that the North American demand for scientists and engineers is increasing more rapidly than that of any other region. It is also fair to acknowledge that the emigration from the United Kingdom is offset to a large extent by the movement of people in the opposite direction. As recently as 1961, indeed, it seems that emigration and immigration were substantially in balance, but this has only heightened the extent to which the ebb and flow of qualified people has been thrown out of balance in the past few years by the rapid increase in emigration offset by a substantially stagnant rate of immigration. The working group has estimated that emigrants outnumbered immigrants by 2,700 in 1966. There is nothing in the committee's report to suggest that there is any sign of slackening in this loss of skilled people from the United Kingdom.

The same points are established by the working group in several different ways. For one thing the working group has convinced itself that industry in the United States offers posts of responsibility to young engineers and scientists at an earlier age than is customary in Britain. Then taxation seems to play a part—though not a direct part—in making life in Britain less attractive for young ambitious people. According to the calculations in the committee's report, only one engineer in 200 is able to earn a net salary of £5,000 a year in the United Kingdom, and he is likely to be in his mid-forties by the time he reaches that position. In the United States, by contrast, the chance that engineers will reach that happy condition is much more like one in four. Although Mr. Wedgwood Benn quite properly went out of his way to say, when the Jones report was published, that there is no case for altering British tax laws so as to decelerate the brain drain, there is probably a strong case for believing that the British system of taxation will be something to be reckoned with by those who make a practice of counting emigrants for as long as it persists. As the committee points out, for example, high marginal rates of tax make it expensive for employers to reward technical people as well as they would like to. The trouble here is that the pinprick of taxation is only one of several reasons why it is easier for prosperous countries like the United States to compete successfully with the less prosperous countries of western Europe for the services of the most talented people. Ironically, of course, the result is often to drain away the talent on which prosperity depends.

It is, of course, a great improvement that the reality of all this has at last been recognized and the Jones committee might have earned its keep if it had done no But it has also made a number of practical recommendations, some valuable and some which would increase the spate of public and somewhat idealistic speech-making which there has been about the brain drain. It is, however, entirely admirable that the committee has rejected proposals whose effect would be to restrict the right of technical people to emigrate if they choose. Even a curb on foreign advertising for technical people is turned down, no doubt to the chagrin of some of those in official circles who have from time to time advocated some course Instead, the committee has plumped for positive solutions and has in particular taken the view that the best way of preventing the brain drain is to arrange that Britain should become more prosperous than it is. It is not surprising, therefore, that a good deal of what it has to recommend consists of policy making in the broadest sense—it wants to be assured that expenditure on research and development will be used as efficiently as possible for increasing the national product and it wants better planning between Government and industry on the conduct of research, more mobility for people (which will be a familiar cry to the Fulton Commission on the Civil Service) and better management in industry. All this is sensible enough and possible as well.

The committee's recommendations on the way in which individual scientists and engineers are dealt with in industry are in many ways the most controversial. To many outside the technical professions, they will seem like a charter for the aggrandizement of scientists, but they are no less sensible on that account. The committee would like to see a "national policy" for paying good young engineers, technologists and scientists more money, and it has done a valuable service by pointing out that the way in which civil service scales of pay are at present linked to what is paid elsewhere by a doctrine of "fair comparisons" serves as a means of preventing industry from gaining a permanent advantage. But this, of course, is only half the tale. The committee would have done well

to preach a little more at industry, for it remains a puzzling phenomenon that even progressive industrial undertakings will often fail to recognize that good men are capital assets to a company and that the commercial price that should be put on them is often much higher than the rate at which it is possible to hire them on what is clearly a buyer's market. And it would have helped if the committee had done more to suggest how it would be possible to implement the recommendation that industry should give technical people greater "opportunities for promotion to the boards of companies". Would it not perhaps be a step in this direction if academic courses at the universities were more often accompanied by practical training in industrial techniques of various kinds?

The committee also indulges in the now conventional opinion that universities should "reverse the current tendency to train scientists towards academic achievement as an end in itself". This seems to be a continuing misconception about the character of scientific training and its relationship with the contributions which the people trained are likely to make to industry. It is odd that a committee which has correctly diagnosed the reasons why graduates in technical subjects emigrate has not of its own accord worked out that much of the unwillingness of young people to enter industry may be explained in the same way. Indeed, the chances are that the impoverishment of scientific and technical careers in industry contributes in an important way to the drift away from science in the schools. The danger is that by trying to impose an artificially practical pattern on scientific education in the universities, the committee and those in even more influential positions who adhere to the same view will make things worse and not better. To say this, of course, does not imply that there is no room for closer relations between the universities and industry-indeed the committee is right to urge that deliberate steps should be taken in that direction, and there is every likelihood that even modest numbers of research contracts placed in university laboratories would help to stimulate not merely an awareness of industry but a better pattern for university education as a whole. This, fortunately, is something that even universities now appreciate.

How Foundations Should Spend Money

THE Wolfson Foundation is planning to break new ground with its scheme to encourage projects in British universities directed at goals which are somehow linked with the prosperity of British industry. It is, of course, understandable that in the years after the war, British foundations should have been most of all concerned to help remedy the shortage of facilities and people for academic research. It is equally plain, now when the lesson is beginning to sink home that Britain is now most badly provided with facilities in science and technology, that the foundations should take a lead in the encouragement of more practical programmes of

research. In this, of course, there are some precedents to help. In the United States, for example, the Rockefeller Foundation has done wonders in the past decade by its encouragement of schemes for the improvement of agriculture. What the Wolfson Foundation is planning, however, is at once more imaginative and more difficult. In the past the foundations have been proud, and rightly proud, of their freedom to make grants for projects with much more freedom from practical considerations than other grant-giving organizations can aspire to. It will be interesting to see how successfully the Wolfson Foundation will be able to single out

deserving projects when the criteria of success are linked not with the promise of academic gain but, rather, with the promise of economic benefits of various kinds. One obvious difficulty is that the foundation will find itself having to encourage extremely speculative ventures. If the object, for example, is to improve the productivity of the electronics industry, there will be no point in looking for mechanical inventions which will actually improve the production process, for industry is

likely to have thought of projects like that off its own bat. By the same token, the foundation is unlikely to find much scope for its activities in projects of the kind which the Government establishments at present carry out. But this, however, seems to be fully appreciated. The object is somehow to devise new ways in which universities at present too detached from the problems of the economy and industry can make useful contributions.

Changes for Cambridge?

The University of Cambridge publishes this week its answer to the Franks Commission Report. In fact the comparison is unfair, because the committee under Dr W. W. Grave considered only the administrative functions of the university, and excluded consideration of admission procedures, the relationship between the university and the colleges, and the like, which took the Franks Commission so long to work out. Dr Grave, Master of Fitzwilliam, has taken only a year to produce the report, advised by a committee of three, Mr J. S. Boys-Smith, Sir Frank Lee and Lord Butler.

The report recommends a number of administrative adjustments. The Senate of the university, to which all holders of the MA belong, would give up some of its powers to the Regent House, the body representing the resident teaching and administrative members of the Senate. The Senate should retain only the power to elect the chancellor of the university and the high steward. The Regent House, which should consist of all teaching members of the university (and not only those already members of the Senate) should be responsible for electing the vicechancellor, conferring degrees, approving new courses leading to degrees, and deciding broad questions of building policy. At present, though, Regent House is often asked to determine detailed implementation of policy, and the report recommends that these detailed decisions be transferred to central bodies such as the Council of the Senate. The central bodies themselves should also be reorganized. At present there are three —the Council of the Senate, the General Board of the Faculties and the Financial Board; the report recommends that the Council of the Senate should become the supreme body, and that the other two should rank as committees of the Council. This change, the committee hopes, will reduce the continuous reference back that exists between the three administrative offices. The new council would consist of the chancellor, vicechancellor, two members from among the heads of colleges, two professors or readers and twelve members of the Regent House.

The report also seeks to make it harder for the Regent House to block new legislation. Requests for ballots should need at least twenty signatures, and proposals should not be rejected unless objectors are in a majority and number more than 100. In proposing changes in the tenure of the vice-chancellorship, the committee has reached a compromise between the two year period now in operation and the four year term which the University of Oxford has adopted. Rather half-heartedly, it recommends a term of three years. The election of proctors should not be changed, but women should be eligible. The report also recommends,

perhaps with the Prince of Wales in mind, that a press officer for the university should be appointed.

. . . and for Oxford

from our Oxford Correspondent

THE University of Oxford is ringing in the academic year with some long awaited changes. For the past three terms Congregation, the assembly of the senior members of the university, has been debating fourteen new statutes reframing the administration of the university according to the proposals of the Franks Commission. The statutes have now been published, and will become effective subject to the formal approval of the Queen in Council. From 1969 onwards, the vicechancellor, who will no longer have to be the head of a college but only a senior member of the university. will be elected by Congregation on the nomination of a committee. Since 1957 the term of office of the vicechancellor has been fixed at two years, a period too short for him to do much more than learn his duties, which include the chairmanship of all the more important committees of the university and the representation of the university to outside bodies. Under the new statutes vice-chancellors will remain in office for four years; the term of the present vice-chancellor will be extended until the election of his successor. The Hebdomadal Council, whose functions are comparable with those of a government cabinet, is to be made formally responsible for the administration of the university, and the administrative services which execute its decisions are to be unified under the registrar.

The new statutes differ from the proposals of the Franks Commission over the question of the composition of the General Board, the concern of which is with matters academic rather than administrative. At present the board consists of the vice-chancellor. certain other university officials, and one representative from each of the sixteen faculties. The Franks Commission had proposed that there be only five faculties, each with two members of the board, and that many of the present faculties be reduced to sub-faculties. A year ago, Congregation approved the scheme in principle, by 114 votes to 112. The Hebdomadal Council and the General Board itself, however, opposed the proposal "because it seemed to them that the new faculty boards would have no clear function to perform, and that the new sub-faculty boards (up to forty in number) would fragment academic administration too greatly". Congregation was therefore advised to reject the statute, and the constitution of the General Board will remain the same, with the important exception that no longer will all the faculties be represented. Instead, six board members will be chosen by Congregation from the nine arts faculties, and six from the seven scientific faculties.

No Swan Song Yet

To read the annual report of the Atomic Energy Authority is to forget that the organization is going through a crisis. There is, it is true, a little retrospective nostalgia about the early days at Calder Hall, now ten years away, but the report barely mentions the reduction in expenditure at Culham, or Lord Penney's imminent retirement and the urgent need for a successor. The report gives the impression that the authority will go on for ever, occasionally annexing new fields of research, but basically unchanged. Perhaps it will, but to outsiders this is beginning to look increasingly unlikely.

It has been another year of success for the authority, although expenditure on nuclear research and development was held at the 1965-6 figure of £38.5 million. Capital expenditure in this area did increase, from £8.5 to £11 million, and research expenditure in nonnuclear fields was up to £772,000, most of it spent on desalination. The trading fund, which covers sale of isotopes, electricity and nuclear fuel services, made a profit of £3.3 million, £0.8 million up on last year, despite a 25 per cent reduction in the sale of fuel because of the reduced requirements of the Central Electricity Generating Board for initial charging for new magnox stations. The resources of the trading fund are to be used to pay for the re-activation of the Capenhurst fuel enrichment factory. The changes at Capenhurst, the report reveals, involve the complete reconstruction of the larger process units of the plant, and the enlarged factory will supply enriched uranium for the first three advanced gas cooled reactors,

Dungeness B, Hinkley point B and Hunterston B.

The expenditure of the authority has declined this year, from £78.8 million to £71.5 million, and receipts, which include payments from the Ministry of Technology for military purposes, have increased by £4 million; as a result, the net expenditure is given as £17 million against £28 million last year. The development of reactor systems has of course continued, and the prototype fast reactor at Dounreay made progress. Contracts have now been placed for about half the work on the station, and the fuel specifications will be completed soon. An improved sub-assembly has been examined in some detail, and design detail of other materials has continued. The steam generating heavy water reactor at Winfrith was completed, and confirmed that this type of reactor can be built comparatively quickly, because all the principal components can be shopfabricated.

Getting round GATT

Mr Wilson's latest device for cutting the British import bill has been constructed with considerable tactical skill. GATT, the General Agreement on Tariffs and Trade, frowns on most easy ways of aiding the balance of payments—import surcharges, or direct subsidies to home producers—and import restrictions invite retaliation from other countries. The British Government is now proposing to establish a new indus-

try in Britain by allowing aluminium companies to negotiate special prices for the electricity which they use to smelt aluminium. The smelters will pay the generating boards a sum equal to the capital cost of the power needed, and will then buy the electricity at near cost price. Because of the advantages of building big, and the fact that no smelting plant so far contemplated could use the entire output of a large station, it is likely that the aluminium companies would take a proportion of the output of a 1,200 MW nuclear station, perhaps as much as 300 MW. Electricity could then be supplied to the smelters at prices as low as 0.5d. a unit if they were prepared to build plants next door to power stations. Transmission costs would increase this slightly if the power station and smelting plant were not next door to each other.

The financial arrangements for the deal are confused. In addition to the low cost power, investment grants at the rate of 45 per cent will be given for the smelter, but the aluminium companies say that they will also need investment grants for their share of the capital cost of the power station if they are to compete with smelters in the USA, Labrador and Newfoundland, where power costs are around 0.25d. per unit. The Ministry of Power has different ideas; it confirmed this week that investment grants would be available only for the smelting plant and not for the power station.

But if aluminium is to have these advantages, why should not other industries also jump on the bandwagon? This has been the perfectly understandable response from a number of industries. The Prime Minister made clear that the favourable terms would only be available in development areas and when the investment could be seen to aid the balance of payments. There is another important proviso, however; the arrangements will not be available to existing companies for existing operations. Only if they are prepared to sanction large expansion in their activities, or a completely new industry, and offer savings to the balance of payments (£30 million is the estimate for the aluminium companies), would the arrangements be negotiable. In other words, existing businesses using large quantities of electricity will have to put up very convincing cases—and a huge investment, of the order of £1 million for every 10 MW of powerbefore they are likely to get anywhere.

power for chlorine manufacture may be the best prospect outside the aluminium companies. Other possibilities include British Petroleum, with its new petrochemical interests, and possibly Courtaulds. The other processes which use electricity intensively—magnesium, sodium, potassium and calcium production, for instance, are probably not large enough to justify special arrangements. The British Steel Corporation produces more than 3 million metric tons of steel annually by the electric furnace method—almost 14 per cent of the total output—but since production is already in progress, and expanding, it is unlikely that the BSC could legitimately claim cheap power, even as a means of achieving its cherished aim of cutting steel prices. The first plan, put up by Rio Tinto Zine, is still the simplest;

The bid by Imperial Chemical Industries for cheap

RTZ would share a 1,200 MW station with the Atomic Energy Authority. The AEA share of the power would be used to run the uranium enrichment plant at Capenhurst, expanded—hopefully—to supply uranium to the Common Market countries, if Britain gets in.

New Look for Gas

It would have been difficult to predict ten years ago the speed with which the energy pattern of the United Kingdom has been transformed by the discovery of natural gas. As Sir Henry Jones, chairman of the Gas Council, explained in his Thomson Lecture to the Society of Instrument Technology on October 9, the picture changed on August 14, 1959, when the Slochteren field, containing at least 60×10^{12} cubic feet of gas, was discovered off the Dutch coast. It was not until 1964 that gas was found in the North Sea, and now Sir Henry puts the potential supplies at 3,000 million cubic feet a day for the next 20 to 30 years—as usual, he is more optimistic than the Ministry of Power, which believes that the fields can bring in 2,000 million c.f.p.d.

So far, all Sir Henry's optimism has been justified, but he is determined to preserve a sense of proportion. Over a full year, the ministry prediction is equivalent to 25 million tons of coal: perhaps, if this rate could be doubled within ten years, the coal equivalent would be 50 million tons a year. "Natural gas will provide a most valuable addition to our country's resources, but it will not replace all the coal used nor will it render imports of oil unnecessary." Sir Henry emphasized the Gas Council view that supplies must be built up quickly—"a high speed build-up seems to me to be absolutely essential." But negotiations on terms and conditions of gas supply had not been easy, he admitted, and expressed the hope that purchase terms could be settled fairly quickly.

Unlike his opposite number at the National Coal Board, Lord Robens, Sir Henry believes in nuclear power stations. Fission, or possible fusion reactions, will, he believes, soon be able to supply the cheapest heat available for very large installations working at high load factor, and the capital cost of nuclear stations will fall. But, as a reassurance to those in the gas, coal and petroleum industries, Sir Henry quoted the laws of thermodynamics. Fuel used in heat engines to generate electricity will, he thinks, always exceed the heat equivalent of the electricity produced, and the cost of electricity transmission, on a heat equivalent basis, will be greater than that for coal, oil or gas. For the provision of heat, he said, fuel burned at the point of use is likely to remain more economic for very many purposes. With that the coal, oil and gas industries will have to be satisfied.

Expanding Services

THE Post Office is undergoing a considerable reorganization in an endeavour to become a more forward-looking and efficient public service industry. The Government decided in August 1966 to convert the GPO from a civil service department to a public corporation, and the newly published report and accounts for 1966–67 (HMSO, 14s.) gives details of the form it will take, provided the requisite legislation is passed by Parliament. The new corporation, known as the Post Office, will take over in 1969, with responsibility for all activities of the present GPO except the savings department. Total assets, numbers of staff employed and plans for capital investment will put the Post Office among the ten largest industrial concerns in the world.

Activities will be divided into the two categories of Post and Telecommunications and, unless savings

can be made by particular co-ordination in some places, they will be run as separate services. Enquiries have been made into ways of raising profitability and productivity, by McKinseys Inc., in the Post section, and by the Organization and Efficiency Branch of the Inland Telecommunications Department in their own field. Recommendations are already being put into effect, and the saving of £500,000 a year in telecommunications is only a beginning.

Overall profit rose by £4 million to £44.3 million. of which £6.6 million came from the post services Higher charges accounted for most of the increased income of £21.8 million in the postal section, bringing the total income here to £340.6 million. Three-quarters of the increased expenditure went on pay awards, but manpower productivity rose by more than I per cent Mechanization is being introduced wherever possible. in the handling of post, for example, so that letters and parcels are arriving faster than before. Ninetythree per cent of letters now arrive within a day of Experiments are being made into parcel delivery services with cheaper local tariffs, and minibus post services. A new look is also creeping into postmen's uniforms. Large scale investigations were made into the possibilities of a two-tier letter system for maximizing labour efficiency, when charges would be related to speed of delivery. Work on the National Giro continues, and market research has given promising results.

In the telecommunications section income rose to £441.8 million, but profit fell by £1.6 million to £37.7 747,000 new telephone connexions were made, but there is still a waiting list of 115,000. because of shortages of equipment. The numbers of local, trunk and overseas calls rose during the year, and these were dealt with more efficiently, either by operators or by automatic exchanges. Improvement of service for subscribers has been given priority, with good effect. £211.6 million of the capital expenditure of £242 million was for expansion of the telecommunications system. Manpower productivity rose by more than 8 per cent, partly because it was realized that it takes one man, not two, to install a new phone. Both Telex and Datel services have continued to expand, but during the year the overall growth rate of the telecommunications section has slowed down. This is thought to be a temporary situation, due to the squeeze, and as rapid expansion of investment is expected to be required in the near future, minimization of costs is an important aim.

The Post Office seems to be preparing to launch into profitable corporation business with a splash, but despite this healthy state, increased post office charges are likely to be introduced. The Postmaster General. the Rt. Hon. Edward Short, has explained why these increases are thought to be necessary. The sheer size of the telecommunications expansion programme will be beyond the resources of the Post Office. More money will have to be borrowed from the Exchequer. for which interest will have to be paid. The interest bill stands at present at £60 million a year, and will rise further with the increases in borrowing. To ask why the telecommunications system should be selfsupporting is to come back to the question of how the resources of the country are best allocated. For this reason the proposed price increases are being put before the Prices and Incomes Board.

Trees and incomes Doar

Even More about Torrey Canyon

THE effect of the Torrey Canyon disaster of March 18 on marine life continues to be studied by biologists. Two teams—one from the Marine Biological Association at Plymouth, and one sponsored by the Ministry of Agriculture, Fisheries and Food—have, since April, been assessing the biological damage below low tide mark in the vicinity of Porthleven and Gunwalloe. The results of their observations are published in the Underwater Association Report for 1966–67.

It is now generally accepted that detergents, highly toxic emulsifying agents, do most of the damage to marine organisms. The data in the report indicate the symptoms of infected animals, and one noticeable feature is the differential vulnerability shown by some species. Among the Decapod Crustacea, the commercially important edible crab, Cancer paganis, and the lobster, Homarus vulgaris, are relatively resistant but become sluggish when seriously affected and crabs may lose their legs. Some molluscs, including the razor shell, Ensis siliqua, are extremely vulnerable and appear to enter a narcotized state before death. Black patches develop on the epidermis of certain echinoderms in weak concentrations of detergent, and in stronger concentrations the animals quickly die and fall into gulleys between rocks. Fish were noticeably absent in areas heavily treated with detergent and very few corpses were seen. also investigated two important ha The teams habitats—rock with Laminaria forest, and clean sand. Of the two, unhealthy and dead species were more common in the sand habitat: evidence of damage in the rock habitat was only seen for a limited distance offshore and, by the beginning of June, all corpses had disappeared and only healthy ones remained in rather depleted stores stocks.

The cause of the differential vulnerability is unknown. It may be caused by differences in habitat or differences in the feeding habits of the various organisms.

Hazards below Ground

THE Federal Radiation Council in the United States has now set out to provide guidance for the control of radiation hazards in uranium mining—chiefly an enhanced risk of lung cancer. The principal cause of the increased incidence of lung cancer among uranium miners is continued exposure to the radioactive decay products of radon-22. These are polonium-218, lead-214, bismuth-214 and polonium-214. It is known that some of the radon daughters contained in the air breathed by miners are retained in the respiratory system. The development of lung cancer may follow some ten to twenty years later.

The council measures the hazard in working levels, defined as any combination of radon daughters in 1 litre of air that will result in the ultimate emission of 1·3·10⁻⁵ MeV of potential α-energy. In unventilated underground mines, the concentration of radon daughters has been found to vary from a fraction of a working level to several hundred times the level. Although some degree of risk presumably exists at all levels of exposure, maximum incidence of lung cancer occurs when the cumulative exposure exceeds 1,000 working level months (WLM), when the miners are moderate to heavy smokers, and when they have worked in mines for ten or more years.

A significant reduction in the concentration of radon daughters in the air of underground mines has been achieved since 1960. The simplest control technique is by ventilation with fresh air, although studies have been made on possible procedures that might be used to prevent the diffusion of radon from rock into mine air. Another practice is to limit a miner's occupancy time in relatively high concentrations of radon or its daughter products. Despite the healthier working conditions introduced during the past decade, the council recommends that steps to make improvements should begin immediately and made operational as soon as possible. But the council recognizes that present regulations and technology are inadequate to ensure compliance with its recommendations. It puts its trust in research, development and education of the miner.

Wider Patent Laws

DELEGATES from twenty-four countries—Argentina, Australia, Austria, Belgium, Brazil, Canada, Czechoslovakia, Denmark, France, West Germany, India, Italy, Japan, Mexico, Netherlands, Norway, Poland, South Africa, Spain, Sweden, Switzerland, UK, USA, and USSR—were meeting this week in Geneva to discuss the plan for a Patent Co-operation Treaty which has been prepared by the United International Bureaux for the Protection of Intellectual Property (BIRPI). Agreement could imply a big step forward towards a more rational patent law.

Many attempts have been made to do this. Patent Group of the Union of the Industries of the European Community has already tried to unify the patent system of the Six, but that plan has, for the time being, been shelved. The Council of Europe has the Strasbourg Convention which standardized administration procedure in making a standard application form for all countries. The big problem is, however, to obtain wider coverage for patents; as things are, applications have to be made to each country separately and this takes much time and expense and even then may not be entirely satisfactory. The meeting in Geneva was to discuss plans for an international centre for patents where a patent application would be forwarded to by a national office. The centre would distribute the patent application to any country nominated by the inventor. How much work this centre would do is one of the topics debated this week. It has been suggested that the centre should issue a report of novelty; an immediate anticipation search should be carried out by the centre or the national patent office but using the same standards, and a certificate of patentability should be issued by the centre after a patentability examination has been carried out by the centre or the national patent office. The certificate would have indicative value only and national offices would be under no obligation to recognize it. The British attitude to these and other proposals should be clarified when the report of the Banks Committee on Patent Reform is published in the middle of next year.

Facing the Food Shortage

An international seminar on Change in Agriculture is to be held in September 1968 and is being organized by the University of Reading with the co-operation

of the Rockefeller Foundation. The steering committee under Professor Bunting of Reading consists of eminent agriculturists from both sides of the Atlantic. It is drawing up a timetable for ten days of intensive and, it hopes, constructive work on improvements in agriculture. Case studies of various agricultural projects in areas of both high and low productivity will be presented, in the hope that some general principles will emerge as to how best to improve farming methods in unproductive areas. Technical knowledge is available to improve crops, in some cases to three times the present level, but the best means of applying new methods are as yet undefined. It is hoped that constructive suggestions will emerge during discussion at the seminar, which will be attended by about 150 people, including conomists, educationists, sociologists and agriculturists. The steering committee hopes that by restricting the attendance at the seminar, results will be produced. Detailed reports of the proceedings will be published.

Airlines Grounded

The latest victims of economic hardship in the United Kingdom are the airlines, whose operating figures for the first half of 1967 do not show the usual healthy upward trend. Compared with the same period in 1966, the number of passengers carried has risen by only 2·2 per cent to 5·46 million—in recent years, increases have been nearer 10 to 15 per cent a year. Although the airlines have increased their capacity by 8·6 per cent to 7,051 million seat miles, passenger miles have only increased by 5·3 per cent to 3,915 million. Freight carried has dropped by 13·5 per cent to 149,309 short tons, although total load has risen by 5·2 per cent to 523·4 million short ton miles.

The worst hit seem to have been the shorthaul services within Europe. Comparing the June 1966 and June 1967 figures, British European Airways have carried 6.6 per cent fewer passengers, and the load factor is down from 67.4 per cent to 60.1 per cent. In the same period, freight fell by 26.8 per cent and mail by 29.1 per cent. The private companies fared even worse; freight fell by 36 per cent, and mail by 60 per Although the seamen's strike in June 1966 gave the airlines extra traffic which they could not expect to maintain, the figures show that the weight of cargo carried has increased very little over the past five years. Longhaul services, fortunately, do not seem to have been as badly affected. BOAC carried 3.3 per cent more passengers, and 6.1 per cent more mail, but 10.7 per cent less freight.

The real blow to the airlines seems to have been in the holiday business. The inclusive tours to Europe expanded only modestly this year, although the annual increase has usually been between 40 and 80 per cent per year.

Since inclusive tours can be paid for in sterling, it is unlikely that the £50 currency limit can be blamed. In any case, it is the less expensive tours which have been hardest hit, which suggests that general economic conditions have dictated a holiday at home for many who might otherwise have gone abroad. Within Britain, improved rail services have affected airline traffic. With these latest statistics, it is easy to see why the Air Transport Licensing Board recently sanctioned increases in fares.

Social Science at Boston Spa

ELEVEN miles of library shelving were added to the existing twenty-five when Mr Patrick Gordon-Walker, Secretary of State for Education and Science, opened a new extension of the National Lending Library for Science and Technology at Boston Spa, Lincolnshire, on October 5. At the same time, a new service for social scientists was inaugurated. From now on the library will hold journals and periodicals devoted exclusively to the social sciences as well as those previously held in which the subject was mentioned. There are at present no plans to build up a stock of social science books but, following the report on libraries by the University Grants Committee, the future policy of the library is under discussion.

More than 500,000 requests have been dealt with in the past year, for photocopying papers, as well as borrowing books, and the stock of 650,000 volumes makes the library the largest scientific lending library in Europe. A new list has just been published of the 28,000 current periodicals that are held by the library.

Efficient use of scientific information becomes more important each year as the volume of literature increases, and the staff of the library have for several years been organizing courses of instruction in this field. The first courses, five years ago, were held for research students, but became impossibly oversubscribed. The present plan is to instruct academic staff and university librarians in the hope that they can run courses for their own students. In the meantime, the library is using modern handling techniques such as conveyor belts, and is streamlining cataloguing methods so that requests for information can be dealt with in the fastest possible way.

Foundation for Prosperity

THE Wolfson Foundation has decided in principle to set aside up to £500,000 over the next five years for agreed projects in applied science education which in the judgment of the trustees are most likely to improve the economic position of the United Kingdom and help the modernization of British industry. It is intended that universities should apply for quite substantial sums of money to carry out specific projects of this kind and it is apparently understood that the total sum now allocated is unlikely to be able to support more than half a dozen projects or so. At the same time, the trustees of the foundation are breaking new ground by their determination to seek out, by discussion with universities, proposals for projects that will qualify for In deciding where to place money, the support. foundation has apparently decided that it will give particularly favourable consideration to universities which have established in the natural course of events close relationships with local industries.

The motive behind this new development is the argument that the Government and the foundations have in the past twenty years done much to stimulate academic research and have, unwittingly, contributed in the process to the strong emphasis on academic problems now apparent in university research of various kinds. In its new work, which will consume somewhat less than half of what the foundation is likely to spend in the next five years on higher education as a whole, the Wolfson Foundation is looking for kinds of research

and development projects which are quite new, at least in British universities. It has in mind, for example, the way in which a number of universities in the United States have been able to surround themselves with research institutes with specialized interests in various kinds of industries. Among the kinds of projects it would like to encourage in Britain are, for example, schemes which might yield industrial and economic benefits by making it possible to manufacture electronic components more reliably and therefore more cheaply. No doubt it would look favourably on projects whose results might be a shortening of the time spent on developments of all kinds. But it seems also aware of the way in which the effectiveness of British industry could be enhanced by the development and introduction of schemes for training specialists of various kinds with great despatch.

More Facts about Schools

The latest Statistics of Education (HMSO, £1 7s. 6d.) are a rich lode for sociologists. As well as providing further evidence of the distaste for science in the schools, the tables provide fascinating information on the sociology of immigration. In the country as a whole, immigrant children (defined as those who were born outside the UK, or whose parents came to the UK after January 1, 1956) make up only 1.8 per cent of the school population. But there are wide variations —in the Inner London Education Authority 12·1 per cent of the pupils are immigrants, while the Welsh authorities have only 0.1 per cent, despite Tiger Bay. In the northern region, children from India and Pakistan make up the vast majority of immigrant children, 87.8 per cent between them, but in London they make up only 15.1 per cent, and West Indian children take the largest share, 46.6 per cent.

Perhaps these differences go some way towards explaining the differences in the knowledge of English among immigrant children. In London 55 per cent of the children have no problem with their English, while only 3.5 per cent have no English at all; in the north 7.1 per cent have no English, and only 44.7 per cent can claim that their English is entirely adequate.

The figures disclose a further decline in the numbers of children studying the mathematics/science subjects in the sixth form. This group accounted for 53 per cent of the maintained school sixth forms in 1963, but it was down to 47.8 per cent in 1966. The actual numbers in this option had declined from 47,140 in 1965 to 46,599 in 1966. Some educationists may see it as a comfort that the number of children in the sixth form who mix science subjects with the humanities continues to increase. This group now accounts for 12.9 per cent of the sixth form population, against 8.7 per cent four years ago; the actual numbers in this option have almost doubled since 1963.

Galapagos Preserved

WHILE the Ministry of Defence contemplates the destruction of Aldabra by an air staging post, the Galapagos Islands at least are in good hands. The Charles Darwin Foundation for the Galapagos Islands, founded in 1959, has had a research station on Santa

Cruz Island since 1961. Scientists have been carrying out ecological survey work with a view to deciding how best to preserve these islands, whose animals (most notably the finches) resembling but differing slightly from those of mainland South America made Darwin realize the full importance of geographical isolation in evolution. Many of the animals belong to a subspecies peculiar to these islands, and the marine iguana, unique in the world, is also the only sea-going lizard. The scientists are producing a plan for the conservation of this wildlife. The government of the Republic of Ecuador, which owns the islands, already gives financial support to the research station, and will shortly pass a law declaring parts of the archipelago a national park. Within these areas the wildlife will be completely preserved. There may be some careful direction of the forces of selection to protect any animals in danger from others. The seals may be culled to prevent them from harming the fish population. The flamingo, which was thought to be in danger of extinction, has been found an area where it can live, and it is now out of danger.



Marine iguanas in the Galapagos.

The national park of the Galapagos Islands could become an attraction for luxury tourism. Visitors will come on cruising ships and by air, to tour the islands by boat, under strict supervision. They will be expected to pay heavily, and help to subsidize the conservation work. Private enterprise, which is involved in the air traffic to the islands—there is already an air strip contributes to the research station. But the Darwin Foundation is still forced to appeal for money to replace the forty-year-old ex-Cornish fishing vessel, Beagle II, which has been essential to the research staff in their work on the islands. Anglia TV has made a colour film, "The Enchanted Isles", which is to have a special royal showing at the National Film Theatre on November 27, when the foundation hopes to raise £20,000 to build a ship that will stand up to the rough South American seas.

NEWS AND VIEWS

Don't Bring Back the Ether

It is probably too much to hope that the exchange of views between Professor Herbert Dingle and Professor W. H. McCrea which appears in this issue will put an end to a long-standing argument in special relativity. By now there is all too much evidence to show that issues like these have a habit of springing to life long after the stuffing seems to have been knocked out of them by the force of pure reason. It is Dingle who mentions Zeno's paradox, which shows that he too is fully aware of the pitfalls which can exist in what seem to be the most elementary kinds of arguments. But defining limits for the length of the diagonal of a square is child's play compared with the process of synchronizing clocks in special relativity, which means that nobody should be surprised or even alarmed that distinguished people occasionally pay close attention to these matters.

Dingle's criticism of special relativity is, of course, pretty radical as these thing go. He seems quite genuinely convinced that the theory of special relativity is seriously in error, and that there is a kind of conspiracy to hide from this supposedly self-evident and presumably unpalatable truth. He also considers that there has been something of a breach of professional propriety in the way in which his detailed assertion of his case against special relativity (Nature, 195, 985; 1962) provoked only a reply from Professor Max Born (Nature, 197, 1287; 1963). To many people, of course, that will seem an over-generous tribute to Dingle's case against special relativity, yet it is also true that Dingle's persistence with his argument is deserving of respect. He has been concerned not so much to twist the tail of orthodoxy as to draw attention to what he has for many years considered to be a serious defect in the accepted doctrine. McCrea's commentary on this argument will bring the controversy to an end for most people. It is earnestly to be hoped that it will also satisfy Dingle.

A part of the confusion which this controversy has occasioned arises because what may be called the Dingle contradiction is in many ways suggestive of other paradoxes in relativity—the clock paradox, for example. It is therefore important to be clear about the nature of Dingle's protest. He says, quite accurately, that Einstein's postulates in special relativity lead directly to the familiar Lorentz transformation for the co-ordinates of space and time and, in particular, to the notion that the time recorded by moving systems-clocks, for example, or radioactive atoms-appears to be dilated when matched against a stationary system for recording time. For example, short-lived mesons in the cosmic rays appear to observers on the surface of the Earth to last long enough to reach the ground. But time dilation works in both directions. From the point of view of quickly moving mesons in the cosmic rays, the lifetime of radioactive

atoms on the surface of the Earth will seem to be unnaturally prolonged.

All this is entirely consistent with that one of the twin postulates of Einstein's special theory which has it that there is no objective difference between inertial frames of reference moving relatively to each other with constant velocity. Dingle constructs an argument by which he claims to show that this reciprocity of time dilation implies a contradiction. If it implies that some clocks in motion must run slow, it also implies that the same clocks run fast compared with some stationary system. What McCrea has done is to point out that Dingle's construction is an attempt somehow to evade the awkward truth that in relativistic mechanics there are serious physical limitations of the freedom with which separate events can be synchronized in time. In other words, McCrea argues that Dingle has thrown to the winds some of the quite elementary precautions which should be taken by those who choose to venture into this important field. In the circumstances, it is no great surprise that he is able to establish a reductio ad absurdum.

McCrea has done a public service by the trouble he has taken in his demonstration of these points. The chances are that most people will be persuaded by what he has to say. It does not follow, of course, that those who, for one reason or another, find Einstein's version of the theory of special relativity unpalatable will promptly be forced to toe the conventional line. After all, there is nothing to prevent those who want to bring back the ether, or who would like the velocity of light to be otherwise than constant, from seeking other ways of saving the appearances. As the saying goes, it is a free country, and there is nothing to prevent people from tilting at windmills if they choose. It is also worth recalling that McCrea's demonstration will not put an end to discussion of that paradox which, in its most popular form, alleges that a relativistic space traveller will be found to have aged less quickly than those who have stayed at home. The point here is that the symmetry of the problem in special relativity is upset by the mere fact that the traveller is set in motion by the deliberate application of external forces.

There remain two quite serious aspects of the charges which Dingle has levelled against orthodoxy, and they also deserve an answer. The least contentious of them concerns the way in which theories of any kind can be challenged and destroyed. There is, of course, no question that a flagrant internal inconsistency is intolerable, and must be got rid of if the theory is somehow to survive. In practice, however, the special theory of relativity has been enormously successful in the past half-century, and in spirit as well as in detail has come to pervade the whole of modern

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physics. Even though Dingle may say that an abandonment of strict relativity in the sense of Einstein's essential postulate would not necessarily imply the end of Lorentz transformations, it is exceedingly hard to believe that a postulate which seems entirely helpful in most of its applications should nevertheless lead to a real inconsistency, and it is no surprise that the resolution of the paradox which McCrea has provided is in a sense semantic. But in circumstances like these, where a theory is lent conviction by the sheer breadth of its agreement with experiment, it would seem incumbent on those who would overthrow it to produce not merely a contradiction but a constructive alternative.

There is also the problem of whether Dingle is right in saying that there has been a breach of professional concern for professional integrity in the way in which his original assertion has not been answered in the detail which he himself considers to be necessary. This raises interesting questions about the function

of the scientific literature as a whole. It is, in particular, important to know just to what extent the profession of science should come smartly to a halt when somebody cries scandal and says that there is something wrong with the essential character of an important theory. Dingle is right to seize on the importance of propaganda. The man who first spots an inconsistency has a duty to bring it to the attention of others, if necessary with vigour. But, especially now that most people are disciples of Popper, an inconsistency is more likely to be welcomed than ignored. Science may not be conspicuously more free from selfdeception than other kinds of intellectual activity, but everybody knows that it flourishes by upsetting applecarts. If, in these circumstances, an allegation of scandal should be ignored, that in itself is an entirely proper reason for asking whether the allegation can be well founded. It is immodest of Dingle to have plumped for the alternative supposition that the profession of science is at fault.

Can Learning be Transferred?

THE function of learning and memory would be much more easily understood if there were some chemical structure in the brain in which information could be reckoned to be stored, and this no doubt is why there has been such interest in the past two years in experiments with the object of determining whether learned behaviour can be transferred by injecting brain extracts from trained donor rats into untrained recipients. As yet, however, there is no positive evidence to suggest that transfer really occurs and there is nothing to suggest what the responsible substance might be—protein, RNA or some other. One obvious difficulty in experiments like these is that of measuring with some semblance of precision the degree to which learned behaviour can be supposed to be transferred from one animal to another by the injection of brain extract or of RNA. Another is, of course, the problem of knowing what materials are being transferred in any series of experiments.

Materials in Archaeology

from G. F. Freeguard

PRIMITIVE man was essentially unable to alter the properties of those materials which were naturally available to him, and therefore his development rested on his ability to learn by practical experience to adapt them to his requirements. Subsequently, in establishing further his mastery over his environment, man began to make new materials on an empirical basis, a process which eventually led to the manufacture of materials on a scientific basis. The annual meeting of the Materials Science Club at Banbury (September 22–23) was entitled "Materials in Archaeology" and presented an opportunity for a distinguished group of archaeologists to discuss with materials scientists the specialist knowledge which can help to unravel some of the secrets still held by the past.

The understanding of natural material may be

In the circumstances, it is no surprise that experiments so far have been in many ways conflicting. Some people claim that various learning schedules can be transferred by means of brain extracts. Others have failed to replicate these experiments. Adám and Faiszt have now (p. 198) carried out a series of experiments designed to define conditions under which training and habituation should be carried out if transfer effects are to be recognized most easily. Although the new experiments do nothing to define the chemical nature of transfer factors if these exist, there is at least the possibility that they will help to reconcile some of the conflicting reports that now appear in the literature. There is no doubt that, in this field, more accurate attention to the objectives and the significance of the experiments which are carried out cannot fail to be a boon. Whether in the long run the reality of transfer factors is established is, of course, another matter and a more problematical one.

illustrated by the building of the brochs—stone, round, tower-like structures which only occur on the northern and western coasts and islands of Scotland and which were probably built from 100 bc to ad 100. They represent the highest development ever reached in building with dry, undressed stone. The hollow wall construction which is tied together by lintels—also used extensively over the entrance of mural cells and internal wall openings—illustrates well the adaptation of a structure to the availability of materials, sandstone and metamorphic rock slabs. Observation confirms that the lintels have been able to withstand severe stresses imposed either by differential settling of the massive stone walls into which they are locked at both ends, or by imperfect horizontal alignment of the beds on which the lintels were being placed. The

strength of sandstone lies partly in the ability of the material to deform elastically: a spectacular demonstration of this can be seen in the Hunterian Museum, Glasgow, where a sandstone slab a few centimetres thick and 2.5 metres long supported at its centre sags by about 20 centimetres at its ends. Plastic deformation of poorly cemented sandstone is also possible by intergranular movement, but such deformation can only occur very slowly and must have been useful in coping with subsidence. In either case many lintels are markedly curved.

An insight into the evolution of particular technologies can be obtained from the analytical study of the composition of the artefacts. This is well illustrated in both metallurgy and ceramics. In the latter case recent X-ray diffraction studies of glasses through the ages have been applied to characterize the opacifying agents used from 1450 BC.

To quote as an example a particularly well known object, the white opaque decorations on the Portland Vase are due to Ca₂Sb₂O₇—since antimony oxide was chiefly used up to the third century AD. Tin oxide was not used until well into the Christian era and arsenic not until the eighteenth century. As an illustration of how it is sometimes necessary to modify ideas in the light of extensive analytical information, it has recently been shown that calcium fluoride is present as the agent in Chinese glass of the seventh to tenth centuries AD, whereas it had been thought for many years that such glasses were not developed until the nineteenth century.

The renovation and preservation of antique objects are of great importance if posterity is to be able to inherit the wealth that has so far been discovered. A report on the work of the British Museum in this field concluded the meeting, describing some of the solutions that have been found in the treatment of both metallic and non-metallic objects. Modern materials have a significant part to play in this work, since the aim is always to enable future workers to be able to improve on any restoration, perhaps by use of new methods and materials that may then be available.

A full account of the meeting will be published in the November edition of the *Materials Science Club Bulletin*, together with the text of the address by Professor F. C. Frank, this year's recipient of the A. A. Griffith medal. The interest generated by the meeting has resulted in the formation of a group to interchange information and give scientific assistance to field workers. Enquiries from scientists interested in participating will be forwarded to the organizers.

Improving Wheat

from a Correspondent

In recent years it has been possible to develop chromosomally deficient or aneuploid lines among the varieties of the commercial wheat of agriculture (Triticum aestivum). These lines are important, because each chromosome can be marked cytologically and followed in the generations after hybridization. It is this ability which enables the chromosomes of one variety to be replaced, one at a time, by chromosomes from another variety. In this way varieties of wheat which differ by only a single chromosome can be produced; each chromosome substitution can then be assessed for its economic benefits, and varieties better than those in current use may be produced. For this approach to

be successful, however, substitution lines must be developed on a large scale, a task beyond the scope of any one research institute, let alone any one worker.

Recently Riley and Law suggested that this difficulty could be overcome by co-ordinating European work with wheat aneuploids, and as a result a conference, supported by the Wates Foundation of the Royal Society, was held earlier this year at the Plant Breeding Institute at Cambridge to investigate the possibilities of co-ordination. During discussion it emerged that most European aneuploid material was still at an early stage, and that the first function of a co-operative would be to establish an information service by way of a newsletter through which contact could be maintained. The ultimate aim, however, should be collaboration at the work level, and a start was made on a project in which four key varieties from different parts of Europe will be involved in a reciprocal substitution The European Wheat Aneuploid Coprogramme. operative, as it will be called, includes representatives from twenty-one countries.

As well as discussing collaboration in Europe, the conference heard descriptions of work elsewhere. The history of the development of wheat cytogenetics is to a great degree the history of the development of one variety, "Chinese Spring". E. R. Sears, from the USA, who began the work with "Chinese Spring", gave a description of the development of an euploid research in his laboratory and of the range of an uploid material available in this important variety. G. Robbelen, from West Germany, gave an account of the development of a monosomic series—that is, aneuploids deficient for a single chromosome-in the variety "Wachtel" by backcrossing to "Chinese Spring"; this illuminated the great importance of maintaining precise cytological control throughout the backcross programme to avoid the possibility of "univalent shift", a process in which particular monosomics are lost. This need for precise control has also been realized in the substitution programme at Cambridge. Although monosomics can be used, the best technique employs a cytologically recognizable chromosome such as a telocentric. The requisite mono-telocentric lines could be produced relatively quickly if telecentric chromosomes from "Chinese Spring" were substituted concurrently with the development of monosomics.

Other contributions from Europe showed that a total of twenty-six varieties, representing many of the best agricultural varieties, are about to be or are being developed into monosomics. In most cases the monosomics are being developed by backcrossing the variety on to an established set of monosomics. Because this requires at least seven or eight generations of backcrossing, many of the monosomic sets are still at an early stage in their development. But the wide range of varieties being exploited in this way offers an opportunity, albeit at a later date, for an effective study of chromosomal differences at the population level.

Studying the Sea

from a Correspondent

OCEANOGRAPHERS are a versatile group. At a recent meeting at the National Institute of Oceanography, representatives of the Challenger Society and the marine laboratories discussed physical oceanography, biology, chemistry, and ship design.

J. Crease and J. C. Swallow dealt with deep currents and methods for their measurement. The use of arrays of recording instruments moored with subsurface buoyancy independent of the research vessel is now beginning to increase the amount of data which can be collected and to make possible synchronous observations of temperature and other variables. Improvements in the design of the mooring gear and in the manufacture of current meters were actively discussed. While most deep currents so far recorded are of low velocity, considerably higher velocities not caused by turbidity currents at the bottom have been seen to occur from time to time. Attempts to predict the worst possible conditions, for the information of engineers and others, must try to take into account these exceptional events.

Prediction of changes in flow and water level is a goal for oceanographers, and S. Ishiguro showed that within the relatively restricted area of the North Sea it could be done very quickly with an analogue model. This approach has already provided a fuller understanding of the combination of physical and meteorological factors which occasionally causes damaging increases in water level at vulnerable points on the English and continental coasts.

By the use of models of another sort, S. Thorpe presented the phenomena which occur between stratified fluids of different densities; of particular interest was the demonstration of internal waves breaking not at the crest but on the slope half way between trough and crest, a phenomenon which has been filmed occurring in a thermocline at sea.

For those whose fate it is to spend some part of their lives at sea it is most gratifying to find out how much research is now put into ship design. A. Silverleaf emphasized that it is only in the past twenty years that methods of measuring the waves and other factors which determine sea state have been available. As a consequence, laboratory basin experiments are now more realistic and measurements of ship behaviour at sea can be made in relation to sea conditions at the time.

It is generally assumed that fluctuations of the non-conservative constituents of sea water are due to biological activity, but C. P. Spencer showed that suspended inorganic particles are also capable of markedly affecting the quantities of dissolved nutrients. The effects of variations of temperature and salinity on the growth of crab larvae were described by J. D. Costlow. One of the more striking results was the appearance of extra larval stages not observed in natural populations when larvae were subjected to a particular temperature and salinity regime; these phenomena were shown to be under endocrine control.

The convenience of working on a large and thus relatively stable ship was stressed by J. Gilpin-Brown when he described the physiological work recently carried out by Professor Denton and himself on board RRS Discovery. Much of the work was on buoyancy mechanisms in various invertebrates and particularly the cephalopod *Spirula*. The trick of substituting ammonium for sodium ions in the body fluids to increase buoyancy, so far described only in Cranchiid squid, has now been observed in several quite different squid and in a species of crustacean. The well known subjective impression that the colour of oceanic invertebrates is related to depth was put on a more

objective basis by P. J. Herring's work on carotenoproteins and this is clearly a large field for further investigation.

The application of statistical analysis to plankton samples should not be undertaken by those who become easily depressed. M. V. Angel managed by persistence to prove that single samples of ostracods were significant in certain respects, particularly in that the order of abundance of species was consistent in ten repeated horizontal hauls made at the same depth. But the degree to which numbers can vary even from closely spaced samples was apparent from P. Foxton's series of observations on a swarm of salps. The variations were, however, caused by meaningful changes in animal density, not by sampling errors, and were correlated both with hydrological parameters and with time. To mark a particular spot in a moving water mass so that the same patch of water can be revisited over a period of time would appear to require something more effective than a parachute drogue supported by a surface buoy. The later observations were therefore treated with some reservation.

Problems of Classification

from J. G. Hawkes

To what extent and in what manner can the results of comparative studies in biochemistry, microbiology and immunology be used in classifying plants, animals and micro-organisms? To what degree should one draw conclusions on evolutionary relationships from the data obtained from these studies? These were the basic questions discussed at a symposium on chemotaxonomy and serotaxonomy, arranged by the Systematics Association at the Department of Botany, University of Birmingham, on September 15 and 16. Can one, for instance, classify a group of organisms by reference to one series of biochemical characters, such as amino-acid sequences in peptide chains, bile salt differences, anthocyanins or immunoelectrophoretic spectra? And is it permissible to infer evolutionary sequences from the degree of complexity of a series of related substances, following what appears to be a logical sequence, stage by stage, from species to species? It was felt that the answer to such questions should be a qualified negative; biochemical data can indeed be of value when placed in perspective, together with a range of other characters from the organisms studied, such as morphological and cytological ones; such data should not be used alone, nor should they be given weighting as of greater importance because they are in some way more "fundamental". Nor should surprise and disillusionment be engendered if no pattern of correlations between biochemical and other characters emerges, as, for instance, with essential oils in eucalyptus species. Such completely uncorrelated characters can be of little value in classification and, equally, one must guard against ascribing a special significance to a series of biochemical characters just because the techniques by which they are elucidated are highly difficult, tedious or ultra-sophisticated.

It is also dangerous, as emerged in the discussions, to confuse presumed evolutionary sequences with systems of classification. At times, perhaps, it is possible to infer the former from the latter, but it is always essential to understand clearly whether one

is trying to form a classification based on character similarities and correlations or trying to understand the phylogeny of a group of organisms by referring to characteristics observed only in the living representatives.

Another point discussed with some animation was whether all characters of living organisms are adaptive, even though many of them may appear not to be. Possibly the obvious phenotypic effect of a pleiotropic gene whose other, less obvious, feature is influenced by selection pressures may lead us astray at times. It was generally accepted that all variants might be adaptive, but perhaps what did not emerge too clearly was how much of this adaptive variation was based on present selection pressures, and how much was caused by pressures acting in the remote past, so setting a pattern for a particular group from which it cannot now escape.

Although one must admit that the Systematics Association had taxonomists primarily in mind when it arranged this symposium, there were few "pure" taxonomists present; and indeed most of the audience were either biochemists, microbiologists or taxonomists who had already used biochemical techniques in some way or other. No doubt, however, taxonomists and others will read the papers when they are published by the Systematics Association. The combination of papers and discussions from workers who are actively using a wide range of biochemical and serological techniques led to lively discussions and a feeling that the symposium had served a useful purpose. To me it seems that the strong links being forged between descriptive and experimental disciplines, and the wide and excitingly different biochemical and biophysical techniques now being introduced into taxonomic studies, are some of the most exciting developments in contemporary biology.

Symmetry Conserved

from our Molecular Biology Correspondent

Two articles about the sub-units of aldolase illustrate the scope for error in the evaluation of quaternary structure in enzymes. Aldolase is by no means the only case which has been the subject of this kind of ambiguity, and there are others which are still unresolved.

In this column last June two papers were briefly discussed which gave apparently conclusive evidence that mammalian aldolase consists not of four sub-units, as had initially been suggested, but of three, two of them identical. This was based on the one hand on molecular weight determinations on the intact and dissociated enzyme, and on the other on measurements of the number of catalytic sites and on hybridization studies. It now turns out that the conclusion is wrong, and that the enzyme has after all four sub-units—a result altogether more compatible with current concepts of enzyme structure.

Morse, Chan and Horecker (*Proc. US Nat. Acad. Sci.*, 58, 628; 1967) have renounced their earlier conclusion; they find four C-terminal tyrosyl groups per molecule, which indicates the presence of four chains (whereas three C-terminal and N-terminal groups have been reported at various times by several workers in the past). But there still appear to be only three active sites in the intact enzyme. Whether one site is blocked in the enzyme as isolated, or whether the attachment

of substrates at three sites inhibits the fourth, is not yet known.

The most comprehensive new evidence on the quaternary structure of aldolase comes, however, from Penhoet, Kochman, Valentine and Rutter (Biochemistry, 6, 2940; 1967). In the first place they have prepared hybrids of aldolases from different tissues by acid-dissociation and reaggregation. A mixture of the two native aldolases, A and C, yields a set of five components on hybridization, which all possess the molecular weight of intact aldolases. Whereas the parent molecules alone produce no new species on dissociation, each hybrid gives rise to a complete set of five components, made up of combinations of the two types of When these are made from equimolar sub-unit mixtures of radioactively labelled A and unlabelled C, their specific activities are in the ratio 1:0.75:0.5:0.25:0, the first and last corresponding to reconstituted A and C. It follows that the combining unit is the quarter-molecule— α in aldolase A, and γ in C—and the species formed on hybridization are α_4 (intact A), $\alpha_3\gamma$, $\alpha_2\gamma_2$, $\alpha\gamma_3$ and γ_4 (intact C). These elegant experiments are supported by electron microscopy of intact aldolases, which shows clearly the presence of four globular sub-units in intact molecules, arranged tetrahedrally.

It is interesting to examine with hindsight the published molecular weight determinations on aldolase and its sub-units. For intact aldolase, two leading laboratories produced values of 142,000 and 160,000. Whereas this discrepancy might still be regarded as marginally tolerable, the corresponding values for dissociated sub-units were 51,000 and 37,000, and this is obviously sufficient to cause an irreconcilable difference in numerology. This demonstrates that alarming differences in molecular weights are still possible, even in the most expert hands. The conditions of dissociation were different in the two studies, however, and it must be supposed that the trouble arose from incomplete dissociation, perhaps obscured by non-ideality. (Penhoet et al. have also redetermined the molecular weight of the intact enzyme by high-speed sedimentation equilibrium, and obtain values in the range 150,000-157,000.)

The results may be seen as a vindication of Kawahara and Tanford, who had the correct answer for the number of sub-units. It suggests also the advantages of dissociation by 6M guanidine hydrochloride, which Tanford and his associates have advocated, and have shown in a series of painstaking investigations (the most recent of which have just appeared in J. Amer. Chem. Soc., 89, 5023, 5030; 1967) to eliminate all measurable non-covalent interactions in all of the large number of protein chains which have been examined. Further reflexions on the experimental discrepancies concerning aldolase are to be found in the paper by Penhoet et al., and make salutary reading.

T4 Messenger RNA

from our Cell Biology Correspondent

It is well known that when T even phage infect $E.\ coli,$ some phage specific proteins are made early in the infective cycle, and as the cycle progresses the synthesis of these 'early' proteins ceases and other 'late' proteins are made. Is the mechanism regulating the cessation of 'early' protein synthesis and initiation of 'late' protein

synthesis at the level of mRNA transcription or translation? Although the answer to this question still remains obscure, some recent experiments have eliminated some of the possible mechanisms that have been

proposed in the past.

Hall et al. (1964) found that most T_2 mRNA species made early in infection (before the phage DNA replicates) are still present at late times when 'early' proteins are no longer being synthesized; this suggests regulation at the translational level. One, perhaps unlikely, possibility considered was that ribosomes by some modification are rendered incapable of binding 'early' mRNA late in infection. But as Friesen, Dale and Bode (J. Mol. Biol., 28, 413; 1967) and Baldi and Haselkorn (J. Mol. Biol., 27, 193; 1967) have shown, this is not the case. Baldi and Haselkorn report briefly that about half the radioactivity given in a pulse at late times during T_4 infection is incorporated into 'early' mRNA and this binds to ribosomes equally as well as 'early' mRNA made at early times. Friesen et al. confirm this; they found 'early' mRNA present at late times and associated with ribosomes just as it is early in infection. (The relative amounts of 'early' and 'late' mRNA at late times remain debatable; Geiduschek et al. (1966) and Kasai and Bautz (1967) claim the concentration of 'late' mRNA is 30 times that of 'early' mRNA.)

One explanation of these results is that although 'early' mRNA at late times is associated with polysomes it is not translated. The 'early' messenger mRNA itself may be altered, or part of the translating machinery other than the ribosomes may be modified; for example a new class of tRNA made at late times may read codons restricted to early mRNA as missense or nonsense. Alternatively, since Greene and Korn (J. Mol. Biol., 28, 435; 1967) have now shown early that mRNA is unstable, and if the instability of mRNA results from its participation in translation, then 'early' mRNA may be translated, albeit imperfectly, at late times and yield non-functional protein.

Greene and Korn measured the half-life of $T_A mRNA$ at all stages during infection, using C14 uracil pulse labelling and actinomycin D to stop transcription, and found both 'early' and 'late' mRNA has a half-life of 3.5 min at 37° C. Furthermore, in the presence of actinomycin the rate of protein synthesis decays with the same half-life as the mRNA. There was no evidence for stable mRNA of any sort. Earlier this year Bose and Warren (Biochem. Biophys. Res. Commun., 26, 285; 1967) had reported that $m\hat{R}\hat{N}A$ for the early enzyme deoxythymidylate synthetase is unstable. These results eliminate Edlin's (1966) suggestion that the cessation of early protein synthesis occurs by competition, for the protein synthesizing machinery, between unstable 'early' mRNA and accumulating stable 'late' mRNA.

In the light of these experiments and given that at late times new species of mRNA appear (Geiduschek et al., 1966), it seems likely that the regulatory process involves interplay between translational and transcriptional controls and there is ample evidence that in other systems the processes are interrelated.

Induction of Meiosis

from a Correspondent

New arrangements of genetic material arise from recombination and segregation of genes at meiosis.

Mitosis, however, serves to pass genetic material through many cell generations without any gene rearrangement. But what are the mechanisms which underlie the entry of a cell to the meiotic process from a previous mitotic way of life? An answer would help to explain how pairing of homologous chromosomes is brought about (for a cytogenetic approach see Riley and Chapman, *Nature*, **216**, 60; 1967), and would ultimately enable genetic analysis of higher organisms to be carried out at will in cell culture.

Yeast cells can be induced to enter meiosis and sporogenesis by transferring them from a nutritionally rich growth medium to a poor, acetate-containing, sporulation medium. Some of the biochemical and cytological changes that occur during this switch from mitosis to meiosis have been published in two papers by Croes (Planta, 76, 209 and 227; 1967). visible indications of the onset of meiosis and spore development are an increase in nuclear and cell volume and cytoplasmic vacuolation, and are apparent two hours after transfer to the sporulation medium. Simultaneously there is a sharp increase in oxygen consumption and a small increase in protein content; but these, along with the RNA content, decline as spore formation continues. This lowering of metabolic activity is surprising since the gross morphological changes of sporogenesis, such as spore wall formation, occur 14 to 20 hours after transfer. Experiments with ethionine, which blocks both meiosis and spore maturation, showed that these processes are most readily blocked if the inhibitor is added during the first two hours after transfer, while less severe inhibition results if ethionine is given 4 to 6 hours after transfer. Further experiments showed that even before transfer to the sporulation medium the cells are prepared for meiosis. Ethionine given for two hours immediately before transfer caused a 99 per cent inhibition of subsequent meiosis and sporogenesis, while mitosis in that two hours was inhibited by only 26 per cent. experiments show that the potentiality for meiosis is latent in the cell at the time of transfer and it is contact with the acetate in the sporulation medium that causes the immediate induction of meiosis.

In the second paper Croes defines some of the metabolic patterns in the cell which correlate with the ability to enter meiosis. The number of meiotic cells induced is greatest if the transfer to sporulation medium is made when the culture is passing from the log to the stationary phase of growth. This transition occurs when glucose in the growth medium is exhausted by fermentation and the accumulated ethanol is then consumed in respiration through the glyoxylate cycle. This change from fermentation to respiration accounts for the stimulation of oxygen consumption by the sporulation medium as the acetate serves as the carbon source for the glyoxylate cycle. Although these metabolic changes appear significant in triggering meiosis, they seem far removed from the meiotic process itself. There are still many unanswered questions-does this radical change in cellular metabolism affect the types of RNAs and proteins synthesized? Are there special "meiotic" proteins? How does the intracellular environment affect the behaviour of the nucleus? Finally, are there parallel changes in cells of higher organisms as they enter meiosis? A fusion of the biochemical and cytogenetic approach to meiosis should be very rewarding.

The Case Against Special Relativity

H. DINGLE

In 1962 Professor H. Dingle published an argument that the theory of special relativity is invalid. In what follows he restates his case, which is then answered by Professor W. H. McCrea.

FIVE years ago' I gave, as the culmination of several similar efforts, a simple proof that the special relativity theory was untenable. This received only one reply from an acknowledged authority, namely, Professor Max Born, who unfortunately, as he himself said2, assumed that I had expressed myself badly, and replied to what he thought I had meant to say. My assurance that what I had meant was what I had said has remained unnoticed. Nevertheless, the theory has continued to be accepted and used as though it were unquestioned.

This does not accord with the general view of the ethics of scientific practice, and, in a matter so fundamental as this, it is not only abnormal but dangerously so. It is understandable that there should be hesitation in believing that a theory so firmly established, and apparently supported by a great weight of evidence, should be disproved as simply as my letter suggested, but it is equally hard to believe that, if such a simple disproof contained a fallacy, no exposure of that fallacy (which, it may be added, there have been numerous private but unsuccessful attempts to extract from recognized authorities), should have been forthcoming. This criticism of the theory, in various forms, has been published repeatedly, during a period of almost nine years, in physical, astronomical and philosophical journals and in four books, in Britain and in America, without eliciting a single published comment. Reluctance to correct errors in such matters is not a customary feature of scientific discussion, so the natural inference is that there is here no error to correct. The balance of probabilities is therefore fairly even, but in fact that is all irrelevant because the matter must be settled not in that way but by reasoned argument. What my argument showed was that the theory was untenable because it required each of two clocks to work steadily and continuously both faster and slower than the other. I do not think it can be maintained that this is physically possible, and therefore the decision rests on the validity or otherwise of the proof that the theory does in fact require that. That is a matter of pure reason, not of opinion or probability, and therefore it admits of a conclusive solution here and now. Furthermore, the point in question is quite specific and must be dealt with specifically, not submerged in more general considerations concerning the abstract functions of scientific theories. It is of course quite permissible, and indeed inevitable if progress is to be made at all, to use theories that are unproved: it is another, and quite impermissible matter, to base experiments on a theory known to be false. To facilitate assessment of the argument I give it here in an extended form, including explicitly details which were only implicit in the former statement in Nature.

Consider the following situation.

$$egin{array}{cccc} N^ullet & & B^ullet & \longrightarrow & v \ & A^ullet & & H^ullet \end{array}$$

A and H are two relatively stationary, regularly running, clocks. B and N also are two similar relatively stationary, regularly running, clocks, moving with uniform velocity v with respect to A and H. (The distances AH and BNare independent and arbitrary.) A and H are set so that a pulse of light which leaves A when A reads T_1 , and is instantaneously reflected back from H when H reads T_2 , returns to A when A reads $T_3 = 2T_2 - T_1$. N is similarly set in relation to B. The readings of A and H are denoted by t, and those of B and N by t'.

The following are three successive events during the process.

 N^{\bullet} $B^{\bullet} t' = 0$ $A^{\bullet} t = 0$ H^{ullet}

Here B is adjacent to A and both are observed to read 0. $B^{\bullet} t' = t'_1$ (event E_1) $H^{\bullet} t = t_1$

Here H is adjacent to B, H is observed to read t_1 and B to read t'_1 .

Here A is adjacent to N, A is observed to read t_2 and N

All this is quite independent of theory: it is a simple description of a possible physical process. A theory is required when we wish to determine two independent things: (i) the values of t'_1 , t'_2 for given values of t_1 , t_2 , or vice versa; and (ii) the relative rates of A and B which these values imply.

Now apply Einstein's theory⁴, supposing A fixed at the origin of the K co-ordinate system and B fixed at the origin of the k system.

(i) t and t' are related by the Lorentz transformation, so that

$$t_1' = at_1 \tag{1}$$

(ii) This is determined by choosing a pair of events and comparing the intervals between the readings of A and B at those events.

Einstein chose events E_0 and E_1 . At these events A reads 0 and t_1 , respectively, and B reads 0 and t'_1 , respectively. The reason why A must be held to read t_1 at E_1 is that H reads t_1 at this event, and on this theory the process by which A is set in relation to H synchronizes it with H.

Thus, between events E_0 and E_1 , A advances by t_1 and B by $t'_1 = at_1$ by (1). Therefore

$$\frac{\text{rate of } A}{\text{rate of } B} = \frac{t_1}{at_1} = 1/a > 1 \tag{3}$$

 $\frac{\text{rate of } A}{\text{rate of } B} = \frac{t_1}{at_1} = 1/a > 1$ But now choose events E_0 and E_2 . At these events A reads 0 and t_2 , respectively, and B reads 0 and t'_2 . respectively. The reason why B must be held to read t_2' at E_2 is that N reads t_2' at this event, and on this theory the process by which B is set in relation to N synchronizes it with N.

Thus, between events E_0 and E_2 , B advances by t_2' and A by $t_2 = at_2'$ by (2). Therefore

$$\frac{\text{rate of } A}{\text{rate of } B} = \frac{at_2'}{t_2'} = \alpha < 1 \tag{4}$$

Equations (3) and (4) are contradictory: nence the theory requiring them must be false. Einstein4, in his paper, gave only (3), and accepted it as giving the unique value of the rate-ratio: he did not check the result by considering the interval between E_0 and E_2 . Had he done so he would undoubtedly have seen that his conclusion was erroneous.

I regard this as a conclusive proof that the special relativity theory is untenable, and the consequences of this fact, however improbable they may seem now (they would certainly not have seemed so in 1905), must therefore be accepted. The resistance to acceptance arises not from reason, as my long experience shows, but from incredulity, and this, in its turn, from some very deep-seated misapprehensions which it is impossible here to explore fully, but which can be indicated sufficiently, I hope, to remove something of the almost compulsive predisposition to regard criticism of special relativity as necessarily misconceived.

(I) It is often held that the logical structure of the theory is unassailable, and therefore the theory can be disproved, if at all, only by experiment: hence, any such paper disproof as the foregoing must necessarily be fallacious and there is no need to waste time in discovering where the fallacy lies. This was expressed by Professor Max Born, in the letter previously referred to, in the following terms²: "The simple fact that all relations between space co-ordinates and time expressed by the Lorentz transformation can be represented geometrically by Minkowski diagrams should suffice to show that there can be no logical contradiction in the theory."

The error here lies in oversight of the fact that a physical theory must contain not only a mathematical structure but also a correlation between the mathematical symbols and observable quantities: a perfectly logical theory may therefore fail physically in the second of these requirements. This oversight calls for much more general consideration, because it characterizes almost the whole of modern physical theory, in which so often a mathematical possibility is assumed automatically to be a physical possibility also, whereas mathematical symbols have a far wider range of significance than is possible to the physical objects whose properties they are taken to represent. This is a matter for later discussion: here I must restrict myself to a single example showing the inapplicability of Professor Born's statement.

The equations, 8-6=2 and 6-8=-2, are mathematically valid and equivalent examples of the general equation, a-b=c. They are both geometrically applicable to a physical situation: thus, if we walk 8 miles north (+) and then 6 miles south (-) we end 2 miles north of our starting point; and if we walk 6 miles north and then 8 miles south we end 2 miles south of our starting point. But they are not both applicable to physical objects: you can get 6 apples from 8 by leaving 2 behind, but you cannot get 8 apples from 6 by leaving -2 behind, but you cannot get 8 apples from 6 by leaving -2 behind. If Professor Born's argument were sound we should be able to say: the simple fact that all numerical values of a, b and c expressed by the equation a-b=c can be represented geometrically by lines drawn to north and south should suffice to show that there can be no logical contradiction (and, by implication, nothing wrong) in the theory that you can get 8 apples from 6.

(II) The resistance most commonly felt by practical physicists to the disproof of the theory arises from a conviction that the experimental evidence for it is too strong to be overcome by a mere piece of logical jugglery which, in face of it, has no more weight than Zeno's proof that Achilles could not overtake the tortoise. This again reveals a misconception needing far more extended treatment than is possible here, where all that can be said is that it is due to an oversight or misreading of the facts of history. There is no existing experimental evidence for Einstein's theory that does not give exactly the same support (whatever that may be) to a quite different theory advanced earlier by Lorentz⁵. Both theories have the same mathematical structure (the Maxwell-Lorentz electromagnetic equations plus the equations of the Lorentz transformation) but give it All that the quite different physical interpretations. experiments so far performed (for example, those showing increase of mass with velocity, extended lifetimes of cosmic ray particles, etc.) show is that if we assume the electromagnetic equations we must correct them by the Lorentz transformation; they throw no light at all on the physical interpretation of the equations.

The physical differences between the theories are profound: here are a few. Lorentz ascribes the contraction of rods and slowing down of clocks to an *ad hoc* physical effect of the ether on moving bodies; Einstein

ascribes them to an ad hoc modification of kinematics at high velocities. Lorentz's theory is impossible without an ether; Einstein's (because of its relativity postulate) is impossible with one. Einstein's theory makes a velocity greater than c logically impossible; Lorentz specifically restricted his theory to "a system moving with any velocity less than that of light", and, from the nature of its effects, it must break down well short of that velocity, just as Boyle's law breaks down well before the volume of a gas shrinks to nothing; it makes the "light barrier" no more necessarily impassable than the "sound barrier". Einstein's theory merges space and time into an unimaginable "space-time"; Lorentz's leaves them independent, as in ordinary understanding. The physical consequences of these differences when very high macroscopic velocities are attained are enormous and ominously incalculable.

Until the First World War, Lorentz's and Einstein's theories were regarded as different forms of the same idea, but Lorentz, having priority and being a more established figure speaking a more familiar language, was credited with it: thus Poincaré, as late as 1912, spoke of "le principe de relativité de Lorentz", even in a paper in which he was discussing Einstein's view of the action of light on molecules. It was not until 1919, when the eclipse observations compelled acceptance of Einstein's general theory, that "the special theory of relativity" became uniquely ascribed to Einstein, and the ideas associated with the name in the minds of physicists became an incompatible mixture of Lorentz's and Einstein's—a fact that preserved the theory from disproof, since any attack on the relativity aspect could be met by an appeal to Lorentz's non-relativistic ideas, and criticisms of those could be disposed of by a reversion to relativity. Thus, for example, the "FitzGerald contraction" was variously regarded as an actual physical effect and as a mere appearance, according to the needs of the occasion.

Whittaker' partly exposed the confusion, but, as a pure mathematician characterizing a theory by its mathematics alone, he saw it as merely a wrong assignment of priorities, and entitled his chapter on the supposedly single theory, "The Relativity Theory of Poincaré and Lorentz". The fact that there were two distinct theories, physically poles apart, was thus obscured. If Einstein's paper, however, had never been written, all the experiments now held to "prove" Einstein's theory would still have been performed and held with the same conviction to prove Lorentz's. Is it conceivable, it would have been asked, that a moving body can experience a resistance to acceleration (increase of mass) unless there is an ether to provide the resistance? Indeed, this very phenomenon was cited by Lorentz in support of his theory before Einstein's paper appeared. The very experiments now held to prove a theory dismissing the ether would have been held to prove its indispensability.

An important point in the present discussion, however, is that the disproof of Einstein's theory given above leaves Lorentz's intact. Both agree down to equations (1) and (2), but the process by which, according to Einstein, A and H, and B and N, respectively, are synchronized does not synchronize them on Lorentz's theory, because one pair, at least, must be moving in the ether. If we suppose the other pair at rest, then they are truly synchronized, but the moving pair are not, any more than clocks on relatively stationary aeroplanes, moving rapidly through the air along the line joining them, would be synchronized by a similar process with sound waves. If, then, we attach conclusive weight to already performed experiments, we must consider Lorentz's theory proved and seek a rational basis for his ad hoc postulates.

But those experiments are not conclusive, for they do not dispose of the alternative possibility, advanced by Ritz, that the velocity of light is c with respect to its source alone. Einstein's theory is a logical deduction

from two postulates: (a) the postulate of relativity the absence of an absolute standard of rest, that is, of an ether, and (b) the postulate that the velocity of light in space is c, whatever the motion of its source. Lorentz's theory denies (a) and accepts (b); Ritz's theory accepts (a) and denies (b). Contrary to general belief, Ritz's theory (that is, the simple hypothesis just stated, not necessarily his tentative development of it, which he later described as a "Scheusal-Theorie"—horror theory) has never been tested. Deductions from double star observations are inconclusive10, and the various laboratory experiments with hypothetical particles as sources and the assumption of the wave equation, $c=n\lambda$, with its usual interpretation, all involve a circular argument. If Ritz's hypothesis is correct, the electromagnetic theory of light, in its present form at least, is not, for that requires the velocity of light to be independent of that of its source. Thus we must not presuppose any part of the electromagnetic theory in testing Ritz's hypothesis. But all tests involving hypothetical particles, or interference as it is usually understood, do just that. To take but one example, in the experiment of Alväger, Nilsson and Kjellman¹¹, beams of γ-radiation from a vacuum tube, showing spectrum shifts suggesting sources moving with high velocity, travelled through space with the same velocity as beams from particles in the tube showing no spectrum shift, and it was concluded that Ritz's hypothesis was disproved. But suppose the beams had travelled with different velocities. Then the electromagnetic theory would have been disproved, and so the evidence that the sources were particles moving with the supposed velocities would have disappeared. Such an experiment therefore could not possibly have tested Ritz's hypothesis. For a true test the source must be a body observed to move with a known velocity and not one inferred from a theory that rules the hypothesis out of court before the test has begun.

The following aspect of the situation may clarify it for some readers. The Maxwell-Lorentz electromagnetic equations and the Newtonian mechanical equations had in common the co-ordinates (x, y, z, t) which were related to space and time measurements in an understood way, and their values when the physical system under consideration was referred to a relatively moving system of co-ordinates were taken, in 1905, to be given by the Galilean transformation. This left the mechanical equations unchanged in form (that is, they were relativistic), but not the electromagnetic equations. ("The [electromagnetic] theory appeared to be unsatisfactory only in one point of fundamental importance. It appeared to give preference to one system of co-ordinates of a particular state of motion. . . . In this point the theory seemed to stand in direct opposition to classical mechanics, in which all inertial systems which are in uniform motion with respect to each other are equally justifiable as systems of co-ordinates"12.) Electromagnetic theory was accordingly taken to require certain observable events to occur (for example, a fringe-shift in the Michelson-Morley experiment) when a piece of apparatus was moved. In fact these events did not occur, that is, electromagnetic phenomena were relativistic (invariant to motion), but the equations were not. The latter, however, would be relativistic if the transformation equations were not those of Galileo but those of Lorentz. Einstein's theory was that they were so, and the effect of this on mechanics was then far beyond the possibility of experimental test because the necessary velocities in a mechanical experiment were unattainable.

But if the Galilean transformation is the correct one, the assumption of the Lorentz transformation must give discrepancies with observation in mechanics corresponding to those found in electromagnetism under the Galilean transformation. This is what is now shown to be the case; the assumption of the Lorentz transformation in mechanics requires one clock to work both faster and slower than another. The fact that this can be seen to be contradictory in advance of observation, whereas the result of the Michelson-Morley experiment could not be foreseen, is due simply to the fact that we already know far more about clocks than about light. Whether or not a particular mathematical possibility can be realized physically can be known prior to experiment only when we have sufficient knowledge of the physical situation concerned, and we know enough about clocks to know that one cannot, at the same time and in the same sense, be working both faster and slower than another. If we had as much knowledge of the structure and behaviour of light sources and light beams as we have of clocks (or apples), a fringe shift in the Michelson-Morley experiment would be as obviously impossible as the contradictory behaviour of clocks (or the obtaining of apples by the compensating creation of negative ones). And, just as the Michelson-Morley experiment is only one of a number showing the breakdown of electromagnetic theory under the Galilean transformation, so the experiment with moving clocks is only one of a number showing the breakdown of mechanical theory under the Lorentz transformation. Another, for example, is revealed in the possibilities of mutual observation by widely separated observers¹³. It is clear that a change of transformation equations, as proposed by Einstein, merely transfers the discrepancy with observation from one set of phenomena to the other: a change in the theory of one set (almost certainly electromagnetism, as quantum phenomena more than suggest), by giving the ether additional properties (Lorentz) or discarding it (Ritz) or by some other means not yet conceived, would now seem to be the only possibilities open of reconciling mechanical and electromagnetic phenomena in a single theory (which may or may not be a unified field theory).

The net result, then, of these considerations concerning experiments is that none yet performed disproves either Lorentz's or Ritz's theory, and because neither theory is disproved by the earlier rational argument which was fatal to Einstein's (on Ritz's hypothesis, equations (1) and (2) become simply $t_1' = t_1$ and $t_2 = t_2'$, leading to a rateratio of unity for all event-intervals), these theories remain in the field. A valid experiment to test Ritz's hypothesis (such, for example, as that suggested earlier to which observable sources are used) is clearly called for.

(III) Another apparent possibility of saving Einstein's theory lies in the supposition that equations (3) and (4) are not really contradictory because they refer not to objective phenomena but merely to appearances: A appears to go slow when observed from B, and B appears to go slow when observed from A. Again it would take too much space to show—although I do not think anyone familiar with the subject will have much difficulty in perceiving it—that if this were so the whole theory would be concerned merely with appearances and could not possibly lead to an explanation of any of the objective phenomena for which the theory was designed. All that is practicable here is to point out that this was not Einstein's interpretation of the result, nor has it been that of any of his followers when dealing with this point alone and not seeking an interpretation that will dispose of some other difficulty. Here are the deductions which Einstein makes from equation (3)4: "If one of two synchronous clocks at A is moved in a closed curve with constant velocity until it returns to A, the journey lasting t seconds, then by the clock which has remained at rest the travelled clock on its arrival at A will be $\frac{1}{2}tv^2/c^2$ second slow. Thence we conclude that a balance-clock at the equator must go more slowly, by a very small amount, than a precisely similar clock situated at one of the poles under otherwise identical conditions". We need not ask if these deductions are valid; all we need to notice is that precisely the opposite deductions, valid or invalid, can be made from equation (4). It is inconceivable that if Einstein had noticed this he would have selected only the equatorial clock as the one which was going slower than the other. Moreover, he

added a footnote to say that the result did not apply to a pendulum clock. This would have been meaningless if it were not the actual physical working of the clocks that was in question but merely an accident of the observer's standpoint. As evidence that the general interpretation of the result agrees with Einstein's, it is sufficient to cite the universal belief that asymmetrical ageing of separated and reunited clocks or persons is required by Einstein's

Allied to this is the misconception that equations (3) and (4) refer to different physical situations. That is not so. The events E_0 , E_1 , E_2 , are successive events in a single process; there is no change in the physical conditions during that process. Also, there is no "change of co-ordinate system". Such systems appear in the argument only implicitly in the use of the Lorentz transformation to derive equations (1) and (2), and there is no change of system anywhere in the derivation. Whether you regard A as stationary and B as moving, or vice versa, makes no difference whatever: throughout, the primed symbols refer to the clocks B and N and the unprimed symbols to the clocks A and H, no matter how you describe them.

The situation is quite clear: the only difference between the arguments leading to (3) and (4) is in the events chosen for comparing the clock rates. If Eintsein's theory is valid the following questions arise. How is it possible for the ratio of the intervals recorded by two identically constructed, regularly running, clocks, between the same pair of events, to vary with the events chosen (in other words, how can the ratio of two constant quantities be variable)? Second, if it is possible, why must the events that alone give the "correct" ratio be chosen from the set occurring on one and not the other of the clocks? Third, if they must be so chosen, how does one (consistently with a theory in which the only feature in which the clocks differ-motion-can be ascribed indifferently to one or the other) discover on which clock the valid set of events occurs? I think it is self-evident that these questions are unanswerable. There can be no doubt that, if this criticism of the theory had been made in 1906, it would at once have been seen to be fatal and Einstein would have been the first to acknowledge it, for then reason was the de facto as well as the de jure arbiter in such a matter. In 1967, however, the obvious has become the inconceivable, and it has to meet the prejudice, independent of reason, that every apparent objection to special relativity is merely evidence of incomprehension and can accordingly be ignored. Unless faith in reason is restored, and prejudice determinedly uprooted, the outlook in the present age is black indeed.

I have introduced a discussion of the implications of the matter, not at present for their own sake but in order to remove obstacles, which experience has shown to be formidable, to concentration of attention on the simple alleged disproof of the theory. I hope it will not have the opposite effect of diverting attention to itself. As I have said, most of the points raised demand fuller treatment

later. But the disproof is complete in itself. Unless some specific error is found, and clearly exposed, in the passage of the foregoing argument extending from the words, "Now apply Einstein's thoery ..." to "... hence the theory requiring them must be false"—an error of such a character that it invalidates equation (4) without, at the same time, invalidating equation (3)—it must be accepted that the special relativity theory is untenable, no matter how unexpected or unwelcome or perplexing or fraught with difficulties the implications and consequences may be.

I would point out also that what I have advanced is not a theory which, in the traditional scientific manner, can be left to be justified or condemned by experiment. No experiment can do either, for the conclusion follows rationally from the premises. If there is no error in the reasoning, the only relevant experiments—and they are urgently demanded-are those designed to show where, and not if, the theory is wrong. Furthermore, it does not seem yet to be sufficiently realized that the nature of modern experiments makes imperative a change of attitude to the relegation of fundamental problems to decision by experiment. It was safe enough to await a measurement of the velocity of light through air and water before deciding for the wave or particle theory of light, and the convincing nature of the result justified suspension of judgment. But, so deeply involved are the special relativity theory and the electromagnetic theory of light in the whole of modern physics, that if experiments of the modern type continue on the assumption that special relativity is tenable when it is not, the results, sooner or later, are as likely as not to lay waste a county. Truth is immortal but human lives are not, and they have claims to protection, even at the cost of admitting an error in physical theory that should never have been made. The recent tragedy at Aberfan shows how bitterly regrettable the consequences may be when hindsight is not anticipated by foresight, and the consequences there were slight compared with those conceivable here. I hope, therefore, that this matter will no longer be allowed, by neglect, to take its own natural and possibly disastrous course, but will be faced squarely and promptly, with no aim but that of arriving at the truth, whatever it may be.

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Why the Special Theory of Relativity is Correct

by W. H. McCREA

I give first a brief presentation designed to facilitate a reply to Professor Dingle's present statement and to the one1 he gave in 1962. So far as applicable, I use Dingle's present notation (which is not identical with what he used in 1962).

Let Ox be a rigid rod graduated in the usual way; let similar clocks be fixed to the rod at points along the rod, and let them be synchronized by a standard procedure (that described by Dingle). If anything happens at the position x of any one of the clocks, let t be the reading of that clock at that event E, say. We speak of the event E

as the event (x, t). Let O'x' be a second rigid rod in motion along Ox with uniform velocity $v \neq 0$. Let Ox' be graduated in the same way as Ox; let clocks similar to those attached to Ox be fixed to O'x' at points along O'x', and let them be synchronized amongst themselves by the standard procedure. If anything happens at the position x' of any one of these clocks, let t' be the reading of that clock at that event E', say. We speak of the event E'as the event (x', t').

According to the theory of special relativity, this system is possible, supposing Ox, O'x' to belong to inertial frames K, k, say. The theory then asserts that E, E' are one and the same event if and only if the parameters satisfy the relations

$$at' = t - vx/c^2 \tag{I}$$

$$at = t' + vx'/c^2 \tag{II}$$

E

where $a = (1 - v^2/c^2)^{\frac{1}{2}}$, supposing 0 < a < 1 and supposing the zero points of the various quantities are suitably chosen. This is one way of writing the Lorentz transformation (being the one used by Dingle in his earlier paper¹).

Consider in k the particular clock B permanently fixed at O', so that every event at B has x'=0. Then from (II) for every such event

$$at = t'$$
 (III)

[Take, for example, the case $a=\frac{1}{2}$. Equation (III) means that if clock B reads t' then that K-clock past which B is moving reads 2 t'; at 1 o'clock by B it passes a K-clock reading 2 o'clock, at 2 o'clock by B it passes a K-clock (a different one, naturally) reading 4 o'clock, and so on.]

In the immediate operational interpretation of (III), as just illustrated, t' is the reading of one and only one clock and t is the reading of a different clock for each different value of t. I repeat that, so far as our discussion is concerned, every event to which (III) applies happens to clock B.

If we next consider in K the particular clock A permanently fixed at O, then every event at A has x=0 and from (I) we have for every such event

$$at' = t$$
 (IV)

This is obviously what we expect from (III) because now K, k have exchanged roles. In (IV), t is now the reading of one and only one clock, and t' is now the reading of a different clock for each different value of t'. Manifestly the parameters t, t' do not have the same meanings in (III), (IV). Every event to which (III) applies happens to the clock B; every event to which (IV) applies happens to the clock A.

[If we do require both (III) and (IV) to hold good we get simply t=0=t', since $a^2\neq 1$. That is, (III), (IV) are both satisfied for the unique event that happens to both clock A and clock B, namely their single mutual encounter. This is obviously entirely consistent with what has just been said.]

No particular or preferred observer is concerned in these results. If a cine-camera anywhere in any state of motion takes a sequence of pictures of clock B, each picture will show clock B with some reading t' and, adjacent to B, a K-clock reading t'/a, the K-clock being a different one in each picture. If the same or any other camera takes pictures of A, each picture will show A with some reading t and, adjacent to A, a k-clock reading t/a, the k-clock being a different one in each picture.

I turn now to Dingle's allegation that the theory used above "must be false". In his present paper, this is based simply on his claim to have inferred the contradictory statements (3) and (4) of his paper from the theory. So we have to do only with the logical consistency of the theory. It may help if I enumerate a sequence of arguments; the first alone is sufficient to refute Dingle's contention, but I hope the rest throw further light on the subject as a whole.

(i) Dingle's assertion is obviously and demonstrably wrong. Using no more than the Lorentz transformation in his algebra, he claims to derive two different values for the same quantity. But the transformation is linear and any result it gives can only be unique. It is trivially impossible for it to give two different answers to the same question. If Dingle obtains two different answers it must be because (a) he has made a slip in the algebra, or (b) his quantities are not well defined, or (c) what he treats as the same quantity are two different quantities.

(ii) Dingle has not made any mistake in the algebra, but in his present paper he deals with objects to which the theory explicitly denies a meaning. We consider events E_0 , E_1 , E_2 defined and described in frames K, k as follows (these being apparently the events similarly denoted by Dingle):

Event	K-description	k-description
E_0 A, B encounter each other	x = 0, t = 0	x' = 0, $t' = 0$
E_1 , H , B encounter each other	$x=x_1, t=t_1$	$x'=0$, $t'=at_1$
E_2 A, N encounter each other	$x=0$, $t=at'_{B}$	$x' = x_2' l' = l_2'$

Here, and in physics generally, event means something happening at a particular position at a particular instant. The crucial feature is that an observer experiences an event if, and only if, the event is part of his own history, that is the event is in his own world-line.

In Dingle's system in his present article A and B are the only observers who experience the event E_0 , or are "at" event E_0 ; H and B are the only observers at E_1 ; A and N are the only observers at E_2 . Dingle arrives at his conclusions because in practice he does not adhere to the standard concept of an event. He asserts, "The reason why A must be held to read t_1 at E_1 is that H reads t_1 at this event, and on this theory the process by which A is set in relation to H synchronizes it with H... The reason why B must be held to read t_2 at E_2 is ...". A is not "at" E_1 in any sense admitted by the theory and it simply has no meaning whatever within the theory to speak of what A must be held to do at E_1 . B is not at E_2 and it has no meaning to speak of what B must be held to do at E_2 .

Just before his formula (3), Dingle proceeds to state "between events E_0 and E_1 , A advances by t_1 . . .". Because A is never at E_1 , this phrase is meaningless and so Dingle's (3) is meaningless. Correspondingly his (4) is meaningless.

(iii) Naturally there is an event E_{1A} , say, at which A reads t_1 . This event has x=0, $t=t_1$ and so clearly $E_{1A} \neq E_1$, thus corroborating what has just been said.

(iv) Dingle's language requires a meaning for what the clock A reads "at" some event involving B even though A is not at the place of that event. In other words, he wants to say what A does "when" B does something, although A and B are not adjacent. Indeed, Dingle expressly uses this phraseology in his 1962 paper. But this restores the notion of distant simultaneity.

About the first thing that relativity theory does is to deny any operational meaning to the notion of simultaneity at two different places. Naturally, this fundamental feature in the theory is not affected in the slightest by any arbitrary conventions we may adopt for the synchronization of clocks. The latter is merely a particular way of putting the readings of two relatively stationary clocks into 1–1 correspondence with each other.

(v) While Dingle's (3) and (4) are meaningless as they stand, the quantities involved can of course be assigned operational meanings in terms of readings of the relatively moving clocks A, B. The formulae do not then tell us about the "rates" of the clocks. They become simply two different ways of putting the readings of A, B into 1-1 correspondence with each other. There are infinitely many different ways of doing this! Being no more than ways of attaching labels, there can be no question of any two of these ways being "contradictory".

these ways being "contradictory".

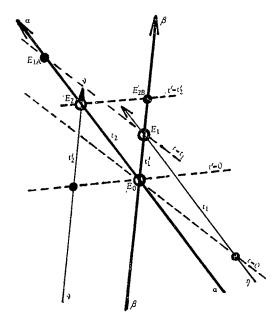
(vi) In his 1962 paper, Dingle started from equations (I), (II) as we have written them (but in his earlier notation) and then derived precisely our equations (III), (IV). He then asserted, "every symbol has exactly the same meaning in both cases", and he claimed to infer a contradiction. His assertion is false, because here he is not talking about the same thing, but two different things. As we have seen, equations (III) and (IV) concern two distinct sets of events, and so they cannot contradict each other. More exactly, the equations concern distinct sets apart from the unique common event for which t=0 and t'=0, and for this event (III) and (IV) are clearly both satisfied.

(vii) While in Dingle's system A and B are the only observers who experience event E_0 , it is of course meaningful to say that other observers can observe event E_0 . Indeed, if any observer Ω anywhere in the universe takes a motion picture of A or B and if one exposure shows the encounter of A with B, then we say that Ω has observed the event E_0 . This exposure would show A and B in juxtaposition with both clocks in this case reading zero. Every observer who observes E_0 will get precisely this same picture.

Suppose now that the motion picture taken by Ω shows also the clocks H, N of Dingle's system. Then in the exposure showing the event E_0 , clocks H, N will appear showing some particular readings. If another observer Ω^* at some different place in the universe makes a corresponding motion picture, then in Ω^* 's exposure showing the event E_0 clocks H, N will appear showing some other particular readings different (in general) from those in Ω 's exposure. This is because the various light-travel times from the clocks to Ω and to Ω^* are all different. Thus there is no unique reading, and no preferred reading, of H or of N to be associated with the event E_0 . This inference does not depend on any arbitrarily selected graduation of the clocks. Thus we have another, possibly more "operational", refutation of Dingle's criticism.

(viii) We may draw a simple space-time diagram in which α is the world-line of A, and so on. Then the events E_0 , E_1 , E_2 are as shown. This makes it perfectly clear that α does not go through E_1 and so there cannot possibly be a reading of A "at" E_1 , this having nothing to do with the manner in which the clocks happen to be graduated. This was the essential point of Born's comment².

In this diagram we may treat t, t' as oblique cartesian co-ordinates. Then, using these co-ordinates, equation (III) is the equation of the world-line β and equation (IV) is the equation of the world-line α and E_0 is their unique point (0,0) of intersection. This shows more clearly than any-



thing else the difference between the two sets of events for which (III) and (IV) hold good.

The diagram shows the line $t=t_1$ through E_1 meeting α in the event E_{1A} . Thus E_{1A} is the event at which A reads t_1 . As we have said, Dingle's formula (3) has to do with the correspondence between events E_1 , E_{1A} ; but we learn nothing by setting up this correspondence and so there is nothing in it to be contradicted, or to contradict anything else.

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Fluidization

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Industry is making increasing use of gas and liquid fluidization, but the theory behind the process is imperfectly understood. Improved application of some earlier techniques, however, is beginning to reveal something of the behaviour of bubbles and solids during gas fluidization.

"FLUIDIZATION" is a good example of an industrial process which is widely used although the underlying science is imperfectly understood. In a "fluidized bed" a mass of solid particles is made mobile by means of an upward flow of gas or liquid, and the pressure drop through the bed balances its weight. The bed behaves like a liquid, there is good mixing of solids, a uniform temperature distribution and excellent heat transfer between the walls and the gas or liquid. These properties make the use of fluidized beds attractive for many operations where solids are contacted with gases or liquids especially if close temperature control is important. Gas fluidized beds are much more commonly used than liquid fluidized beds although the latter are employed in some water treatment and ore leaching processes. The first uses of gas fluidized beds were concerned with ore roasting and coal combustion, but during the past 25 years they have become very widely used in a variety of catalytic processes; the well known "cat cracker" was the first of these.

The nature of the solids movement obtained when the fluidizing medium is a gas is different from that produced by a liquid. In the latter case, as the liquid velocity is

increased beyond the value necessary to support the bed this flow rate is known as the minimum fluidization velocity or U_{mf} —the particles separate from each other and begin to move about the bed in a complex manner in response to eddies in the liquid flow. The bed expands and continues to do so progressively, if the liquid velocity is further increased, until ultimately the individual particles are carried out of the bed. In a gas fluidized bed, on the other hand, as the gas velocity is increased beyond U_{mf} , the bed expands only to a very limited extent before it becomes unstable and voids or bubbles of gas are formed. These bubbles pass upwards through the bed in a manner very similar to bubbles rising in a liquid. Any further increase in the gas flow rate leads to the formation of more bubbles. The bed thus consists of two regions, or phases, the bubble phase and the dense phase, which has a density very close to that of the bed at U_{mf} and through which gas passes interstitially at a flow rate close to U_{mf} . Although the movement of particles is rapid and appears to be quite random, it is only a complex repetition of a motion resulting from the rise of bubbles through the bed. Each bubble draws up a "spout" of particles behind it, and this upward flow is compensated by a steady downward drift elsewhere in the bed.

There is a certain amount of overlap between the behaviour of the liquid fluidized and gas fluidized type of bed. Light particles of less than about 50µ diameter exhibit a limited degree of liquid bed-type expansion while, on the other hand, liquid voids are obtained when very dense media such as lead or tungsten powder are

fluidized by liquids.

Because of the greater use of gas fluidized beds, their ever increasing size and cost, and the more complicated flow pattern obtained in them, they have been much more widely investigated than liquid fluidized beds. Nevertheless, much empiricism is still necessary in their design. Empirical measurements are expensive and time consuming to carry out on the scale necessary and there is thus a considerable financial incentive to obtain a better understanding of the factors controlling the performance of gas fluidized beds.

The first step in designing a gas fluidized bed is to know the minimum fluidizing velocity of the bed material. Typical values for spherical particles are 0.3 cm/sec for a silica-alumina catalyst of less than 0.1 mm diameter and 40 cm/sec for copper shot of 0.4 mm diameter Although various semi-empirical correlations have been developed from which U_{mf} can be calculated, these can often be unreliable when applied to industrial materials,

and the value is best measured experimentally.

In most cases it is necessary to operate gas fluidized beds at gas velocities in excess of U_{mf} in order to obtain sufficiently rapid solids mixing and good rates of heat transfer from wall to bed. Because a large fraction of the gas is then passing through as bubbles it is important to know how effectively this gas contacts the solid. Until recently there was no direct evidence at all on this point and such information as existed came from a study of the "overall" behaviour of the bed using a variety of "models" to explain the results. In these models it was generally assumed that the solids were perfectly mixed with the gas passing through in plug flow, but in two One stream comprised the gas flowing interstitially through the dense phase at approximately U_{mf} , the other being the gas passing through as bubbles and which was thought to have little contact with the solids. Some interchange of gas between these two phases was assumed to take place and the extent of this interchange was determined experimentally in some cases by injecting a pulse of a suitable tracer gas into the fluidizing gas and examining the attenuation of the emergent pulse. The results obtained, however, were often difficult to interpret unambiguously and extrapolation of the results obtained in one bed to another was very speculative.

Much of the basic investigation into the behaviour of gas fluidized beds has been carried out in small laboratory equipment a few inches in diameter. Because, however, bubbles I ft. or more in diameter are frequently encountered in industrial practice the interpretation of the laboratory experiments is sometimes suspect. Various types of probes have been inserted into experimental, and operating, gas fluidized beds to identify the bubble pattern within. These have depended on the change produced in the optical density, local heat transfer and, especially, dielectric constant, of the bed when a gas bubble surrounds the probe. The picture is usually complicated and often confused. Probes have also been used to investigate particle movement and changes in

the density of the particulate phase.

More recently, direct unambiguous information about the behaviour of bubbles and of the dense phase has been obtained by the use of two complementary techniques. Neither technique is new but previously they had not been used to the best advantage. The first is the use of "two dimensional" fluidized beds. Such beds, which may be up to 10 ft. high and 2 ft. wide, are only about 0.5 in. thick, so that the bubbles span the gap between the trans-

parent walls and are visible as shown in Fig. 1. Although at first sight one might expect a large "wall effect", which would affect the flow pattern, in fact, whenever a direct comparison has been possible between a two dimensional bed and a conventional bed the flow patterns have been found to be essentially similar. The use of a coloured gas, such as nitrogen dioxide, enables the gas flow pattern to be seen directly and the corresponding use of coloured particles demonstrates the solids movement. In addition, changes in the density of the dense phase can be measured using X-ray or γ-ray attenuation. The second technique is the use of X-ray cinephotography to observe the shape and velocity of bubbles in a cylindrical bed, typically of 6 in. diameter. By using solids of different X-ray opacity the particle movement can also be seen. With each of these techniques considerable advantage has been obtained by studying the bed with a single bubble rising through it. In this way the basic patterns of gas flow and particle movement associated with a bubble have been discovered and can be applied to the more complex situation of a bed in which many bubbles are present.

These techniques, which were developed principally at AERE, Harwell, have shown that the solids are not in random movement but are displaced in an ordered manner by the bubbles as they rise through the bed. The bubbles, which are empty of particles, are essentially spherical (or cylindrical in a two dimensional bed) except for an indented base. As a bubble rises the particles flow around

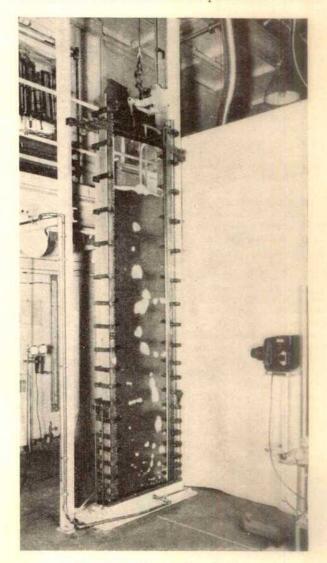


Fig. 1. View of large two dimensional bed.

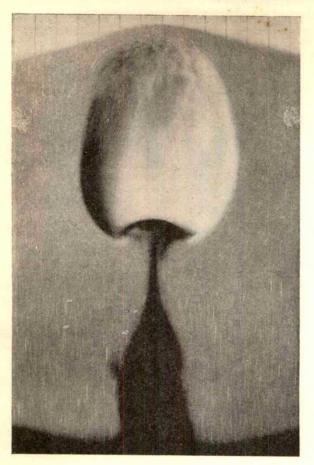


Fig. 2. Wake and drift profile associated with a rising bubble,

it in streamline motion and as a direct consequence each horizontal layer of particles is displaced into the classical 'drift profile" that is characteristic of this type of streamline motion. In addition to this, the indented base of the bubble, constituting about the lower quarter of the sphere volume, contains a "wake" of circulating particles that are travelling with the bubble. A photograph of a bubble in a two dimensional bed is shown in Fig. 2. The drift profile and wake are visible because the bubble has passed from the lower region of the bed, which contains dark coloured particles, up into the lighter coloured upper region. Experiments have shown that the particles in the wake are more widely spaced than in the rest of the dense phase. The wake is unstable and periodically sheds material and re-forms as the bubble rises. Eventually it is deposited on the surface of the bed. Thus particles are progressively carried up from the bottom of the bed to the top. If the bubble pattern is ordered, then so is the solids flow pattern. Because of the rapid bubble flow in most industrial beds the overall result is very similar to perfect mixing, but the difference between truly random mixing and bubble mixing can be significant where the local flow of solids is important.

The two dimensional gas flow studies have shown that the gas flow pattern associated with a bubble is critically dependent on the ratio, α , of the bubble velocity to U_{mf} . The latter generally varies as the square of the particle diameter, whereas the bubble rising velocity is relatively independent of the bed material and, like a gas bubble in an inviscid liquid, depends on the square root of its diameter. Thus, in a bed of large particles (usually this means particles greater than about 350μ in diameter) U_{mf} will be greater than the bubble velocity and $\alpha < 1$, whereas in finer materials U_{mf} will be much less and $\alpha > 1$. The former is the normal state of affairs in beds

containing large lumps of, for example, coal, while the latter state exists in beds of catalyst particles. When a is less than unity interstitial gas passes into the base of the bubble and out through the roof. Thus the gas comprising the bubble is continually changing and it is incorrect to consider the bubbles as forming a separate stream of gas. When a is greater than unity a region of gas recirculation, termed the gas cloud, is formed around the bubble. The gas in the bubble recirculates continually through the bubble as it rises penetrating only a limited distance into the dense phase. The extent of this penetration diminishes as a increases until at a values greater than about 10 the cloud boundary virtually coincides with the bubble boundary. The bubble gas only mixes with the solids in this penetration region, and so the smaller the cloud the poorer the contacting efficiency. Some exchange does take place, however, between the cloud and the interstitial gas because of molecular diffusion and also because of an imperfectly understood "cloud shedding" phenomenon. In Fig. 3 a gas cloud, of nitrogen dioxide, is shown around a bubble travelling in an air fluidized bed at an α value of 2.5. Some gas which has been shed from the cloud can be seen below the bubble.

The existence of this cloud was predicted by Davidson' as a result of calculations based on a simple model representing the hydrodynamics of gas flow in the dense phase adjacent to a rising bubble. More sophisticated models have since been proposed by Jackson² and Murray³. These enable the size of the cloud to be calculated more accurately and it is now possible to predict with a fair degree of confidence the efficiency of gas—solids contacting in a bed if the bubble velocity and U_{mf} are known. The former can be calculated from the bubble size and the latter from the particle size. Unfortunately, no satisfactory theory exists which enables the bubble size distribution in a bed to be predicted. Thus, either the bubble size must be guessed or else empirical measurements made.

Observations of the behaviour of rising bubbles, both in the two dimensional bed and also by the X-ray technique, show that they are continually splitting up and coalescing. A protuberance forms on the roof of a bubble and cuts the bubble into two as it rises. Bubble splitting in this way sometimes results in the formation of two separate bubbles, but very often a rapid recoalescence of the "daughter" bubbles occurs. In addition, the coales-

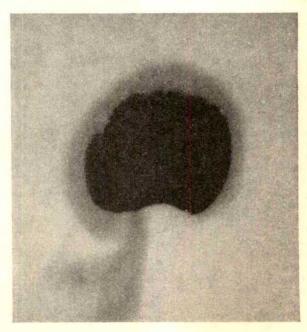
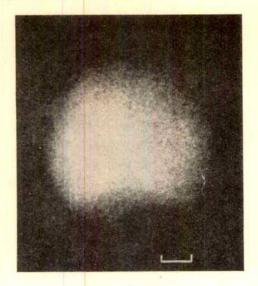
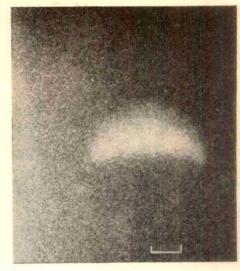


Fig. 3. Gas cloud around bubble, a = 2.5.





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Fig. 4. X-ray photographs of bubbles in (a) crushed coal and (b) 'Synclyst' (a silica-alumina cracking catalyst). Scale, 1 cm.

cence of separate bubbles occurs very frequently so that the average bubble size increases with height in the bed. Bubble size can play a vital part in determining the efficiency of gas-solids contact in many circumstances and it is therefore important to be able to predict the size and pattern of bubbles that will be formed in a given situation. This is an important, and difficult, aspect of fluidization on which further research is badly needed.

When a is greater than unity the extent of gas interchange between the cloud and the interstitial gas will markedly affect the efficiency of gas-solids contacting. At present the magnitude of this exchange can only be estimated and here again there is a need for further

research to explain this phenomenon.

Apart from these two important areas of ignorance there remain many other interesting features of the gas fluidized bed that would repay further theoretical and experimental investigation. In particular, the mechanics of the behaviour of the dense phase in a bubbling bed are not understood in detail. It is known that the shape and velocity of rise of bubbles are influenced by the physical nature of the fluidized particles. For example, X-ray experiments have shown that bubbles in a bed of irregularly shaped particles, such as sand, have smaller wakes and rise at greater velocities than bubbles in similar, but spherical, material. Also, the size of the bubble wake region increases as the particle size of the fluidized material is decreased. Bubbles in low density spherical material of very small particle size, for example, 50µ diameter, have large wakes so that their shape approaches the characteristic spherical cap form observed for large air bubbles in water. In Fig. 4 the difference in shape between bubbles produced in coarse coal and in a fine catalyst can be seen. In addition, the movement of a bubble through a bed of fine material can generate eddies within the dense phase and this enhances the mixing of particles. This very liquid-like behaviour is entirely absent from beds of coarser particles.

Such observations as these immediately suggest that there is interference, or contact, between neighbouring particles. Generally the voidage of the dense phase is about 0.4: this means that, even if the particles are ideally arranged in the most open form of packing, rhombohedral, the separation between particles is only about one-sixteenth of a particle diameter. As a bubble rises the dense phase flows around it and there is a relative motion between adjacent particle layers around the bubble. Because of the close packing of the particles it is reasonable to expect that there is interference between neighbouring particles close to the bubble during this shearing movement. The formation of the particle wake, and the solids circulation therein, may even be consequences of this particle interference.

Striking evidence in support of the existence of interparticle contacts, but not necessarily in support of the explanation advanced above, is the well established fact that a fluidized bed of conducting particles exhibits a significant overall electrical conductivity. Both liquid and gas fluidized beds behave in this manner. property has been exploited to heat gas fluidized beds of carbon particles by resistance heating in such processes as the manufacture of acetylene. Liquid fluidized beds of conducting particles have also been suggested for use where high surface area electrodes are needed. Until the mechanism of the conductivity is known, optimization of the design and operation of such beds will be entirely a matter of trial and error.

The evidence in support of the existence of interparticle contacts, and particularly this last observation of the electrical conductivity of a bed, is sometimes cited in order to attempt to invalidate the gas flow theories of Davidson, Murray and Jackson because these neglect the effects of any such contacts. The attempted argument does not necessarily follow. Interparticle contacts could occur without significantly affecting the gas flow pattern, and the striking agreement between observation and the predictions of Jackson and of Murray shows that it is valid to neglect interparticle forces when dealing with this aspect of the behaviour of bubbles. But they must be taken into account when considering the detailed

mechanics of the dense phase.

To sum up, experiments have now given a good understanding of the type of flow patterns obtained inside fluidized beds. Mathematical models have enabled some of the behaviour of gas bubbles in gas fluidized beds to be predicted, but much knowledge important to the better design and optimization of such beds is still required. One disturbing feature is that attempts to produce better models have produced mathematical equations that are insoluble and it seems that more experimental information on the fundamental properties of the dense phase is needed. The latest models were discussed at a recent conference at Eindhoven, but there were no signs of an imminent "break through" in this field.

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Some New Behaviour-disrupting Amphetamines and their Significance

by

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Department of Psychiatry and Surgery, Medical College of Alabama, Birmingham, Alabama The effect of a series of amphetamines has been investigated, and the results suggest that substitution in the para-position is important for hallucinogenic properties.

AMPHETAMINE is the paradigm of a central stimulant drug, and it is also known to cause a paranoid psychosis closely resembling a schizophrenic illness in certain addicts. Peretz et al.1 first reported that a derivative of amphetamine-3,4,5-trimethoxy amphetamine—was hallucinogenic in man. Its effects were very similar to those of its close chemical relative mescaline. Smythies et al.2 have developed a behavioural test for animals, based on the Sidman Avoidance Schedule and the Bovet-Gatti profiles, which has proved to be reliably predictive of the hallucinogenic effect of a drug on man. We used this test to show that of the nineteen possible methoxylated phenylethylamines, only three were active in disrupting behaviour—the 3,4,5 compound (mescaline itself), the compounds 2,3,4,5 and 2,3,4,5,6, in order of increased activity. All the other 16 were inactive. Thus it appears that for hallucinogenic activity, at least 3 methoxy groups are required, and that these had to be in the 3,4,5 configuration. The two other active compounds were more lipid-soluble than mescaline, and are not metabolized by amine oxidase; mescaline being metabolized by diamine oxidase but not by monoamine oxidase.

In this present investigation, we have examined the following series of methoxylated phenylisopropylamines (amphetamines): the 3,4,5, 2,4,5. 2,4,6 and 2,3,4 trimethoxy compounds; the 2,3, 2,5, 3,5 and 3,4 dimethoxy compounds; and the 3 monomethoxy derivatives 2, 3, 4. Each compound was tested on two animals exactly as described in our previous communication². The results in this series were quite different from those in the ethylamine series.

In the trimethoxy amphetamines activity was in decreasing order: $2,4,5 > 3,4,5 > 2,4,6 \gg 2,3,4$. A typical Bovet-Gatti hallucinogenic profile was obtained for the first three, whereas the 2,3,4 compound was almost inactive. A very similar result was obtained in human studies by Shulgin³, who found the 2,4,5 compound to be seventeen times as active a hallucinogen as mescaline, the 3,4,5 compound to be approximately twice as active, and the 2,3,4 compound to be inactive.

In the dimethoxy series, we found the 2,3, 2,5 and 3,5 compounds to be inactive, but the 3,4 compound was highly active, 12.5 mg/kg being roughly equivalent to 25 mg/kg mescaline.

The monomethoxy series was the most interesting. The ortho-compound showed moderate amphetamine-like activity over a wide dose range.

Para-methoxy amphetamine (PMA), however, proved the most potent hallucinogen we have so far tested (with the exception of LSD). In a dose of 3·1 mg/kg, it produced a typical "low dose hallucinogenic" Bovet-Gatti profile, quite distinct from the Bovet-Gatti profile for amphetamine. In doses of 6·2 mg/kg, it disrupted bar-pressing behaviour completely, and induced bizarre behaviour in both the rats tested. Although the rat could walk about normally, and appeared to be able to eat and drink normally.

mally, it frequently walked backwards—a typical mescaline effect. It would show exaggerated startled responses in the absence of external stimuli and would frequently engage in strange behaviour reminiscent of shadow boxing—rearing and pawing in the air. If placed on a table, it would walk apparently normally towards the edge and fall off, and would do this repeatedly if replaced on the table. This period of abnormal behaviour lasted one day in one rat, when the animal died, and one week in the second rat, when this animal also died.

Thus the present data would suggest that the essential feature of the "hallucinogenic" molecule in this series is the para-methoxy group. All compounds lacking this were inactive and compounds with additional groups were variously weaker. The different results in the phenylethylamine series may be due to the fact that the apparently inactive compounds may be too easily oxidized by amine oxidase to produce behavioural effects following parenteral injection. It is known that the 3,4-dimethoxyphenylethylamine is a much better substrate for amine oxidase than is mescaline^{4,9}. Once the molecule is protected from amine oxidase by α-methylation, then the behavioural reaction to the drug, injected parenterally, may more truly reflect activity at the site of action. To test this hypothesis, we pretreated an animal with 50 mg/kg iproniazid 3 h before injecting 6.2 mg/kg of para-methoxyphenylethylamine (PMPEA)—which is also o-methyl paratyramine. Neither of these given alone produced the slightest effect on behaviour, but when they were given together. behaviour was completely disrupted and toxic effects rapidly appeared: the rat lay on its side twitching slightly, and died in a few hours. The same dose of iproniazid given before injecting 12.5 mg/kg mescaline increased the effect of mescaline only two-fold and no toxic effects appeared. This experiment is being repeated with lower dosages of PMPEA. This increase in the toxicity of 4-methoxyphenylethylamine following MAO has also been reported to produce an intense rage reaction and hyperthermia in cats10. It has also been found that paramethoxy amphetamine at 25 mg/kg was lethal in cats after producing a similar rage reaction.

The severe and prolonged disruption of behaviour produced by PMA suggests an irreversible inhibition of an enzyme—possibly catechol o-methyl transferase. Because 3-methoxylation is the normal method of inactivating catechol amines, it seems not impossible that catechol o-methyl transferase might be blocked, or its action distorted in some way, by 4-methoxylated phenylethylamines or isopropylamines. A second hypothesis is that because amphetamine is usually para-hydroxylated in the rat, the interference with this reaction by the para-methoxy group might have behavioural results.

Thus it would be of interest to analyse by biochemical and pharmacological techniques the mechanisms of action of PMA. The action of PMA on species that do and do not parahydroxylate amphetamine would also be of interest. Human testing with PMA would clearly require great care. The potent and long lasting hallucinogen STP has been identified as a derivative of dimethoxy amphetamine.

Finally, these findings suggest the hypothesis that schizophrenia may be associated with 4-methoxylation of catecholamines or of tyramine, or with some disturbance in whatever biochemical mechanism is affected by these paramethoxylated compounds. In this respect, the recent reports by Boulton et al.7, that one "pink spot" excreted by schizophrenics is paratyramine, and by Jenner et al.*, that a manic depressive patient was excreting very large amounts of paratyramine metabolites, are of interest. Our data suggest: (1) that o-methyl paratyramine produced in the synapse (separated from MAO) would have profound and long lasting effects on behaviour, and that the fact that 3,4-dimethoxyphenylethylamine (DMPEA) is inactive in humans on parenteral injection may be due to its rapid breakdown by MAO, and that it also might disrupt behaviour if locally produced in the synapse; and (2) that the psychosis found in amphetamine addiction may be associated with the production of 4-methoxy amphetamine

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Inertial Confinement of Extended Radio Sources

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The confinement of extended radio sources may be a dynamic rather than a static process where the internal pressure of the source is balanced by the ram pressure of the intergalactic gas.

In this article, we discuss the nature of those strong radio sources in which the radio emitting regions are separated from the parent object by distances comparable with or greater than the dimensions of the sources. These radio sources are often double, with the parent object (commonly a giant elliptical galaxy) situated between the sources and roughly at their centroid. Descriptions of such sources, of which Cygnus A is a well known example, have been given by Maltby, Matthews and Moffet1 and by Burbidge, Burbidge and Sandage².

Apart from information concerning the radio spectra, present observations of strong radio sources provide little more than the luminosities and approximate dimensions of the components, although with higher resolution studies now becoming possible it seems that a certain amount of fine structure can be observed. In the case of Cygnus Athe emitted power in the radio frequency range is estimated to be 4.4 × 1044 ergs/sec, with the two (approximately equal) components separated by about 79 kparsec and having diameters of about 35 kparsec. From such information the usual procedure is to estimate the minimum total energy, E_{min} , in particles and the magnetic field required to produce the observed luminosity by the synchrotron mechanism. Thus, for Cygnus A, if the energy densities of relativistic electrons and nuclei are equal, and if the magnetic field fills the emitting volume uniformly, the minimum energy is of the order of 3×10^{59} ergs, and the corresponding magnetic field strength, H_0 , is about 7×10^{-5} oersted^{1,2}. If the magnetic field fills only a fraction, f, of the volume of the source, then the energy required decreases by a factor $f^{3/7}$ and the magnetic field increases by a factor $f^{-2/7}$; for example, if $f = 10^{-2}$, then $E_{min} \approx 2 \times 10^{66}$ ergs and $H_0 \approx 3 \times 10^{-4}$ oersted. For sources other than Cygnus A, similar calculations imply

 $E_{min} \approx 10^{67} - 10^{61}$ ergs and $H_0 \approx 10^{-6} - 10^{-4}$ oersted. The significance of these calculations is limited by their over-simplification of the problem. Nevertheless, an important result is implied, namely, that the internal pres-

sure of the emitting region is at least $H_0^2/8\pi$. The actual pressure might exceed this value considerably, for a comparable pressure must be exerted by the energetic particles involved, and the configuration need not correspond to the minimum energy configuration for the observed luminosity. One would also expect the emitting regions to be roughly in hydrostatic equilibrium in a co-moving frame of reference so that regions of high magnetic pressure tend to have low particle pressure and vice versa. To maintain the pressure within such an object (hereafter called a "plasmon", a term introduced in a slightly different sense by Shklovskii⁸) which is well separated from its source and presumably in near equilibrium with its surroundings, an external confining pressure of at least $H_0^2/8\pi$ is necessary.

It has been suggested that the confinement could be provided by an intergalactic magnetic field, and that such a field would also produce the observed alignment of the plasmons with the object from which they have been ejected4. This would require an intergalactic field strength at least equal to the minimum value of H_0 required for any observed source of the type under discussion. That is, the intergalactic field would have to have a strength $H_m \ge 7 \times 10^{-5}$ oersted, as deduced for Cygnus A. pressure exerted by the intergalactic magnetic field, however, should not exceed the total pressure of the interstellar medium in our own galaxy, which implies that H_m is less than about 1×10^{-5} oersted. This is probably a gross overestimate of H_m (ref. 5), but it is none the less far too small to confine and collimate the Cygnus A plasmons. Moreover, if the intergalactic field were the confining agency, the pressure inside all similar objects would be the same (that is, $H_{m^2}/8\pi$), and there is no reason to believe that this is the case.

With essentially the same arguments, one is led to reject the possibility that the confinement is due to the static pressure of the intergalactic gas in a "hot" universe. a conclusion which is also implied by observations of the

X-ray background⁵. The only alternative seems to be that the confinement is essentially dynamic rather than static, with the internal pressure of the plasmons being balanced by the ram pressure of the intergalactic gas. $\rho_0 v^2 \approx H_0^2/8\pi$, where ρ_0 is the density of the intergalactic gas and v is the current speed of the plasmon. Note that this requires only that the intergalactic gas should be sufficiently dense and makes no demands on the pressure of this gas or the intergalactic magnetic field strength, both of which could be quite low. In addition, the internal pressure of the plasmons can vary, for the ram pressure depends on the speed of the plasmon, a quantity which does not have to be the same in all cases and which may vary in time for any one case. The collimation of plasmons with the parent object cannot be due to the intergalactic magnetic field in these circumstances, but must result from a directed ejection, presumably controlled by the distribution of matter in the parent object.

Perhaps the most interesting aspect of this suggestion is that it permits us to deduce a rough lower limit for the density of the intergalactic medium. Taking $v \approx 3 \times 10^{10}$ cm/sec and $H_0 \approx 7 \times 10^{-5}$ oersted (as required for Cygnus A), we then find $\rho_0 \geq 2 \times 10^{-31}$ g/cm³. There is a slight uncertainty caused by the possibility that relativistic effects might be important; however, we do not consider this likely. It seems more probable that the internal pressure of the Cygnus A plasmons is much larger than the minimum value we have assumed, and that v is somewhat smaller than the speed of light. Thus the actual value of ρ_0 is probably substantially larger than 2×10^{-31} g/cm³, at least in clusters of galaxies. This argument is not without flaws, but we believe that it is useful, because this is probably the only case in which we can be reasonably sure that we are observing a direct interaction involving the intergalactic medium. That is, the strong radio sources provide the best available intergalactic probes.

If these arguments are inverted so that a lower limit is obtained for v, then they provide a counter to the suggestion that the plasmons which constitute the radio sources such as Cygnus A are gravitationally bound to the parent object³. If the plasmon is confined by ram pressure alone, then taking an upper limit of ρ_0 to be 10^{-27} g/cm³, we find that $v \ge 4 \times 10^8$ cm/sec with $H_0 = 7 \times 10^{-5}$ oersted, which is well above the escape speed for even a very large galaxy. If ρ_0 is taken equal to the "cosmological" value of 2×10^{-29} g/cm³, then $v \ge 3 \times 10^9$ cm/sec. This suggests that the Cygnus A plasmons must be moving at speeds as nearly as great as the speed of light and hence that their age is only 10^5 – 10^6 years.

These arguments refer only to the maximum ram pressure of the intergalactic gas. This is attained in the vicinity of stagnation points on the interface between the plasmon and the impinging intergalactic gas. Because the normal pressure can drop to quite low values elsewhere on the interface, it is not immediately clear that confinement of the plasmon can be produced by ram pressure effects alone. It is not possible to give a completely general treatment of this problem, allowing for arbitrary distributions of magnetic field and plasma within

the moving cloud, but we have been able to construct a simple model which illustrates how the confinement occurs. First, note that as a result of the drag exerted by the intergalactic gas the plasmon must be decelerating, so that in a co-moving frame there is an effective gravitational field directed parallel to the direction of motion of the plasmon. Consider a quasi-equilibrium situation in which this gravitational field is essentially uniform throughout the plasmon and produces an acceleration $(-a\hat{z})$ cm/sec², where the z axis is directed back along the trajectory of the plasmon and z=0 at the stagnation point. Let the combined particle and magnetic energy in the plasmon be & per unit mass at each point, and further assume that ε has the same value throughout the cloud. The total pressure distribution within the plasmon consequently obeys the simple hydrostatic law $p = p_0$ exp (-z/h), where $h = \varepsilon/a$ is an effective scale height. The Newtonian approximation for hypersonic flow is appropriate in this situation, and hence the external pressure acting normally on the surface of the plasmon is $\rho_0 v^2$ $\cos^2 \xi$, where ξ is the angle between the incident flow direction and the outward normal to the surface (Fig. 1). The shape of the plasmon (which is axisymmetric) is obtained by equating the external and internal pressures

$$p_0 e^{-z/h} = \rho_0 v^2 \cos^2 \xi \tag{1}$$

Thus

$$r = 2h \tan^{-1} \left[e^{z/h} - 1 \right]^{1/2} \tag{2}$$

with the maximum radius of the plasmon being πh . The

calculated shape is shown in Fig. 1.

We emphasize that this model calculation is designed only to illustrate the general principles of inertial confinement of a plasmon, and not necessarily to give an accurate representation of any particular strong radio source. Nevertheless, it does provide good qualitative agreement with observations for the general shape of the radio sources. It should be noted that any intergalactic magnetic field which exists tends to be compressed and hence amplified at the surface of the moving plasmon, similar to the case of the interplanetary magnetic field at the surface of the magnetosphere. Instabilities of this surface would tend to mix filaments of strong field with energetic particles from the interior, thus giving rise to synchrotron radiation in addition to that resulting from the internal magnetic field of the source.

If we accept this estimate for the maximum crosssection of the plasmon, then the drag due to the intergalactic gas is roughly $\pi^3 h^2 \rho_0 v^2 = Ma$, where M is the total mass of the plasmon. Because $a = \mathrm{d}v/\mathrm{d}t$ and $h = \varepsilon/a$, this can be regarded as a differential equation for the motion of the plasmon which integrates to give

$$v = \left[v_0^{1/3} - \frac{1}{3} K^{1/3} t\right]^3 \tag{3}$$

where v_0 is the initial speed and $K=(\pi^3\epsilon^2\,\rho_0/M)$. The time required to stop the cloud is $t_s=3(v_0/K)^{1/3}$. For the case of Cygnus A, if we take $Mv^2/2=\epsilon M=E_{min},\,f=10^{-2},$ and $v_0=3\times 10^{10}$ cm/sec, then $M=2\times 10^6 M_{\odot},\ \rho_0\geqslant 4\times 10^{-30}$ g/cm³, and $t_s\leq 2\times 10^5$ years. This is comparable with the

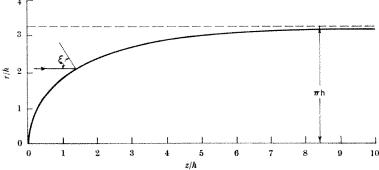


Fig. 1. Calculated shape of plasmon.

present age of the object, suggesting that the deceleration might be quite severe. In its present state Cygnus Acould continue to radiate for 3×10^7 years or more, which is much longer than ts. The plasmon does not radiate significantly, however, after it halts, because as it decelerates it expands, and the resulting adiabatic cooling of the plasma together with the reduction of H_0 quenches the effectiveness of the synchrotron mechanism. This could explain why the components of double radio sources are not seen to be separated by more than about 200

kparsec from the parent object.

We are severely hampered in pursuing theories of this type by a lack of knowledge of physical conditions in the radio sources. In particular we would like to know the quantities f and H_0 in a source such as Cygnus A. These could in principle be found by radio observations with sufficiently high resolution (which would yield an estimate of f), and from X-ray observations. If the X-rays emitted by Cygnus A result from inverse Compton scattering of the cosmic black body radiation by the energetic electrons in the source, then the ratio of the X-ray and radio luminosities is roughly $8\pi W f H_0^2$, where $W \approx 1.6 \times 10^{-12}$ is the energy density of the black body radiation. According to our previous estimates for fHo2 in Cygnus A, the X-ray luminosity should be approximately 4×10^{42} ergs/ sec (if f=1) or 2×10^{43} ergs/sec ($f=10^{-2}$); both figures are below the present observational limits?

Our attention has been directed to a recent paper by Ryle and Longair⁸ which is relevant to the present work. On the basis of a simple kinematical model, Ryle and Longair have shown that one can deduce the age and current speed of the plasmons as well as some information about their deceleration and rate of change of luminosity. By combining these results with our arguments on the dynamics of plasmons one should be able to reduce the number of free parameters quite substantially, bearing in mind, however, the limitations imposed by the assumptions involved.

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Infrasonic Pressure Waves from the Aurora: a Shock Wave Model

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The anisotropic radiation of infrasonic waves from moving aurorae is explained by a shock wave model. The acoustic waves, as observed on the ground at a site ahead of the moving aurora, are amplified in the direction parallel to the auroral motion by superposition of wave fronts if the auroral form is moving at supersonic speed.

DURING periods of great geomagnetic activity, infrasonic waves with an amplitude of a few dynes/cm2 and with periods from 40 to 80 sec have been observed at low latitude stations to come from a source in the polar regions. The times of occurrence and azimuths of arrival of the travelling pressure waves indicated a source near the local time region of maximum activity in the auroral Recent observations of infrasonic associated with auroral activity made in the auroral zone at the Geophysical Institute at College, Alaska, show that travelling pressure waves are radiated when large scale auroral forms move with supersonic speed2.

The association of auroral infrasonic waves with high speed auroral motions resulted from direct observations of infrasonic signals from visible aurorae as well as from statistical studies. The local midnight reversal in the direction of auroral motions during a typical auroral sub-storm³ corresponds to the statistical reversal at local midnight in the azimuths of arrival of auroral infrasonic waves from east of the magnetic meridian to west of this direction. This observed simultaneous reversal of auroral motions and azimuths of incoming infrasonic waves implies that the auroral infrasonic waves are radiated principally in a direction parallel to the motion of the auroral forms.

An example is given in Fig. 1 to show the relation between the direction of motion of a supersonic auroral form and the direction of the observed infrasonic wave which was generated by the moving aurora. The successive positions, at intervals of 1 min, of the auroral arc are projected from the 100 km level onto a map of Alaska in Fig. 1 showing that the infrasonic wave, indicated by the

long arrows, was radiated in a direction parallel to the motion of the arc.

The infrasonic wave from this moving are is shown in Fig. 2 with several other examples, as the superposed pressure versus time traces from four microbarographs with the time scales shifted to give waveform alignment for these coherent waves. At 0613 h local time, just 7 min after the arc passed over the observing station at College, Alaska, the infrasonic signal arrived from an azimuth of 304° from an elevation angle of about 60°. All the

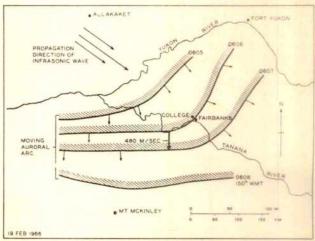


Fig. 1. Projection of a fast moving auroral arc, at one minute intervals on a map of Alaska with the direction of the associated infrasonic wave.

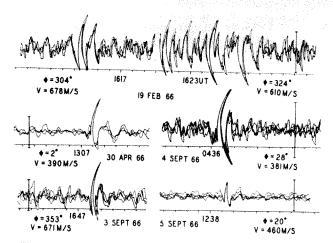


Fig. 2. Superposed pressure versus time traces of four microbarographs for infrasonic waves observed in the auroral zone. Time scale is in minutes and vertical bars represent 5 dynes/cm² pressure change.

examples found by Wilson and Nichparenko² of infrasonic waves generated by aurorae showed that only aurorae moving with supersonic speed produce infrasonic waves. This fact strongly suggests a shock wave mechanism for generating auroral infrasonic waves.

A typical shock wave pressure versus time profile does occur for many of the observed auroral infrasonic waves. Several examples of these are shown in Fig. 2 on April 30 and September 3, 4 and 5, 1966, in which a positive pressure spike is followed by a longer negative phase. The more complex waveforms, such as that shown in Fig. 2 at 1625 UT February 19, are thought to be due to multiple path propagation effects, the complex geometry of the emitting auroral forms, or superposition of signals from several sources. Continuous sinusoidal waveforms such as those resulting from a harmonic source are never observed in auroral infrasonic waves.

Any model for the generation of infrasonic waves by moving aurorae must explain the following three principal characteristics: the pressure wave is radiated highly anisotropically in the direction of the auroral motion; detectable acoustic energy is radiated only if the motion of the form is supersonic; and the waveform is like that of a shock wave and not sinusoidal.

A model that will produce the observed characteristics of auroral infrasonic waves is described here. In Fig. 3 an auroral form is shown moving from right to left parallel to the surface of the Earth, with a speed v. If the auroral form produces an acoustic pulse of constant phase as it passes through each interval of path Δx_{η} , then the moving auroral form will produce an acoustic shock if v is greater than c, the speed of sound. The shock front will propagate to the ground with a speed c as shown in Fig. 3 where $\theta = \arcsin c/v$. In this model, c is taken to be constant at

all heights and the radiation of the acoustic pulse from each interval Δx_{η} is assumed to be isotropic. The first acoustic energy in the shock arrives at P from the interval shown in Fig. 3 as Δx_0 along the ray path R_0 , where R_0 is normal to the shock front. The delay time for the arrival of this initial acoustic energy from the interval Δx_0 is given by $t_0 = R_0/c = h/c \ (1-(c/v)^2)^{-\frac{1}{2}}$. Although the emission of acoustic energy by the aurora is continuous as it moves, it is useful to describe the process by a numerical approach. The path along x is divided up into finite intervals Δx_{η} and an amount of energy $U \Delta x_{\eta}$ is emitted within each interval where U is the acoustic energy emitted per unit path length by the moving aurora.

The amplitude of the acoustic shock received at the ground point P at any instant of time is the scalar sum of the acoustic pulses radiated isotropically from each interval Δx_{η} . The pressure versus time profile of the shock received at a point P will depend on the shape of the acoustic pulse emitted by the aurora in each interval Δx_{η} , the delay time of each pulse, and on the degree of attenuation of each pulse.

In order to make the numerical integration to find the profile of the shock at P, Fig. 4 was constructed to show the calculated delay time of each acoustic pulse from each interval Δx_n at the instant of time when the shock front has just reached the point P. The x co-ordinate x_n of each interval of path length Δx_{η} is plotted along the ordinate in km. The origin of x is at the zenith over P and x increases toward the right in Fig. 3. The delay time for the pulse from each interval Δx_{η} , measured in seconds along the abscissa in Fig. 4, is the time for propagation of each acoustic pulse from its position in space at the time t_0 to the point P. Thus the delay time for the pulse from the interval Δx_0 is zero; the delay time for the pulse from the interval at x=0 is 45 sec and for the pulse from $x_{\eta} = 100$ km is 18 sec as shown in Fig. 4. If it is assumed that the acoustic pulse from each interval Δx_{η} has a 20 sec period with symmetrical positive and negative phases, as shown in Fig. 4, and that geometrical attenuation occurs, then a numerical sum of the pulses emitted along the path of the aurora from x = 180 km to -40 kmgives the shock pulse shown in Fig. 4. From this figure it can be seen that there is no significant contribution of acoustic energy to the shock pulse from acoustic pulses in intervals for which the delay times are greater than about 40 sec. The x co-ordinates for the Δx_n intervals for which the acoustic pulses have a delay time of 40 see are 130 km and about 2 km. Thus, for this example in which v=2c, the shock energy received at P due to the motion of the auroral acoustic source comes from the region of path from 130 km away to about overhead. Thus the supersonic motion of the source of the auroral acoustic pulse toward the zenith of the observing site at P causes the generation of a shock pulse radiated in the forward direction by the superposition of the infrasound emitted from each interval Δx_{η} of the auroral path. The

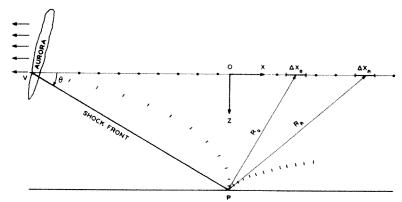


Fig. 3. Shock wave model for supersonic auroral form. Short bars converging on observation point P show positions of acoustic pulses from intervals $\varDelta x_{\eta}$ at time t_{q} .

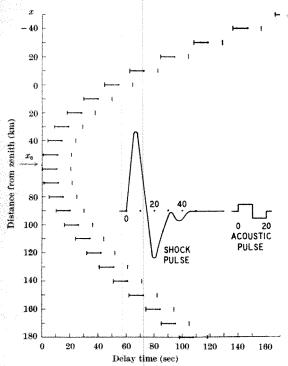


Fig. 4. Delay time diagram for acoustic pulses from auroral path intervals Δx_{η} at time t_0 . The acoustic pulse from any interval Δx_{η} and the integrated shock pulse observed at P are shown for case where v=2c.

the highly directional properties of the radiation of infrasound in the forward direction with respect to the supersonic aurora.

To determine how much the actual sound propagation would deviate from that assumed for c constant at all heights a ray tracing calculation was carried out using a standard sound velocity profile for the atmosphere with an isotropic source of sound radiating at the 100 km level. The ray paths for each 10° of initial propagation direction are shown in Fig. 5. The deviation of the actual wavefront from a spherical wavefront which would result if c were constant at all heights is shown in Fig. 5 by the relative position of the short bars on the rays for 10°-60° with respect to the solid circle which represents a section of the spherical wavefront. Only for nadir angles greater than 50° is there a significant deviation due to the actual sound profile in the lower atmosphere. Now from the model we find that there was significant contribution to the shock pulse only for intervals from overhead out to about 130 km. Thus for the range of x_n which is important the nadir angles are less than 50°. The approximation of constant sound velocity used in the model is therefore sufficiently good.

Sound that is radiated at the 100 km level in directions that are within 30° of the horizontal, as indicated by the 60° cone in Fig. 5, will propagate within the mesospheric sound channel. That radiated within 60° of the zenith will either be absorbed at higher altitudes or refracted back to the Earth to produce weak secondary signals.

The model predicts that the delay time for propagation along the path R_0 will be shorter for higher velocity auroral forms and that the horizontal trace velocity of the shock

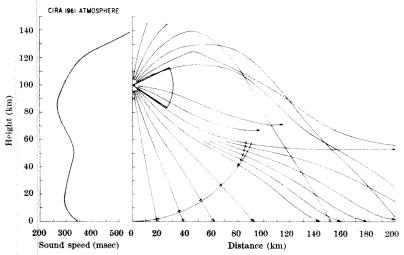


Fig. 5. Sound velocity profile and ray tracing diagram for an isotropic source at 100 km level.

amplification of the shock pulse can be seen in Fig. 4 to be a factor of about 10 greater than that which would be received from a single acoustic pulse from the radiation in one interval. The piling up of the wave packets from "upstream", x > 0, relative to the spreading out of those from "downstream", x < 0, can be seen in Fig. 3, in which the positions in space of the spherical wavefronts from each interval Δx_{η} are plotted as short bars for the instant of time t_0 . Thus the acoustic pulses from intervals for which $x_n > 0$ are all close together so that their superposition at P causes the shock pulse to build up in the forward direction. Now from Fig. 4 it can also be seen that if an aurora started at x=0 and moved away from P in the negative x direction, and it the difference in the delay time between successive pulses was greater than the pulse width, then no constructive superposition would occur.

This model is able to explain the shock wave like character of individual auroral infrasonic waves: the amplification of the basic auroral acoustic emission and

wave will be equal to the velocity of the auroral form. The model requires that, in the frame of reference moving with the aurora, the acoustic pulse generated in the aurora has a constant shape and phase. This would be true for either the mechanism of heating by auroral electrons suggested by Maeda and Watanabe or for that of electrodynamic drift suggested by Piddington⁵ for the generation of the initial acoustic energy in the aurora itself for conditions of constant auroral electron flux. The observations of auroral infrasonic waves made in the auroral zone are consistent with this shock wave model3.

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DNA Synthesis and DNA Content of Leucocytes in Acute Leukaemia

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Inhibition of division of blast cells is common in untreated acute leukaemia of childhood. During relapse, while a patient is on maintenance therapy, the proportion of blast cells in cyclic division

THE labelling of blast cells in acute leukaemia with tritiated thymidine (3H-TdR) in vitro1,2 and in vivo2,3 has shown that only a small proportion of these cells is engaged in DNA synthesis at any given time. There are strong indications that only part of the leukaemic cell population is engaged in cyclic division, and it has been suggested that the cells eventually mature into sterile blasts which do not divide2, although some of these cells could be a mitotically dormant reserve. The work of Mauer and Fisher³ has indicated that the generation time of the blast cells in untreated acute leukaemia of children may be as short at 15-20 h.

A short pulse labelling with 3H-TdR can provide information about the percentage of cells in DNA synthesis, and quantitative cytochemical determination of the DNA content combined with the labelling can give the relative distribution of the cells in the various compartments of interphase activity^{4,5}. This approach has been applied to the analysis of populations of blast cells in leukaemic children in two phases of the disease: in the untreated acute state and at the onset of relapse after a period of

Sixteen children suffering from acute leukaemia were first studied before treatment was started. Fourteen examinations were performed on eleven patients in an early phase of relapse; in eleven instances the patient was on chemotherapy at the time of investigation. The bone marrow was examined every 8 weeks and occasionally at other times. When blast cells formed more than 10 per cent of the total nucleated cells in the marrow or were present in the peripheral blood, the patient was considered to be in relapse.

The methods used for the identification of the cells, analysis of DNA content, *H-TdR labelling and measurement of cell size have been described in detail elsewhere⁵⁻². Some of the marrows were incubated with Some of the marrows were incubated with 5 $\mu c.$ of tritiated uridine (*H-UR, specific activity = 5 c./mmole) for 1 h; incorporation of the 3H-UR was

studied by autoradiography.

The percentage of blasts synthesizing DNA (labelling index) in the bone marrows of fourteen cases of acute lymphoblastic leukaemia before treatment varied from $0 \cdot 1 - 10 \cdot 2$; in the peripheral blood taken at the same time the labelling index was 0·1-7·2. The ratio of the labelling index in the bone marrow to that in the blood was 0.8:1 to 7.0:1. A similar relationship has been reported previously^{1,2}. A very low peripheral blood labelling index was seen in one patient with a high blast cell count (900,000/mm³) and in another with only 135 blasts/mm³. A case of acute myelomonoblastic leukaemia had a labelling index of 10.2 in the bone marrow and 7.7 in the peripheral blood, and one of acute myeloblastic leukaemia had a labelling index of 7.5 in the bone marrow and 5.5 in the blood. .

The distribution patterns for the DNA content of the cell populations were similar; most of the cells had DNA contents close to the normal diploid amount. Post-DNAsynthetic, premitotic (G_2) cells with 4n DNA contents formed less than I per cent of the population. A few unlabelled cells had DNA contents between the 2n and 4n modes; these may have represented an uploid cells of atypical DNA content. Estimation of the DNA content by measurement of Feulgen dye absorbance cannot detect the small differences associated with increase or decrease of one or two chromosomes. Chromosome analyses were made on seven of these cases, six of which were normal. In this series no marked aneuploidy was encountered; others have reported that normal chromosome numbers are present in about 50 per cent of cases of acute leukaemia*. In the case of acute myeloblastic leukaemia it was possible to identify myeloblasts and myelocytes, although the latter were morphologically abnormal. The DNA distribution pattern of these two cell types indicated that a hold-up at the 2n mode occurred at both stages of differentiation.

Figs. 1a and b show the relation of DNA content, 3H -TdR labelling and cell size in two cases of acute lymphoblastic leukaemia. In one of them (Fig. 1a), there was evidence of a bimodal population in the size distribution of the 2n cells; in other cases, however, this bimodality was absent (Fig. 1b). It would seem therefore that size alone is unlikely to be a sound criterion for distinguishing cells in G_1 which are actively proliferating, as opposed to those which are no longer participating in regular cyclic division. In some of the patients there was evidence of considerable cell growth as the blast cells moved through the cell cycle, but in other cases this was not particularly marked. Autoradiographs of cells incubated with 3H-UR were exposed for 4 weeks, at which time 78-98 per cent of the blasts were labelled, the intensity of labelling tending to be greater in the large cells. Some of these large cells were only weakly labelled, however, and some of the smallest cells were highly labelled, which points to a non-uniformity of metabolic activity independent of the cell size. Furthermore, aneuploid cells out of cycle may be large and confuse the picture.

The overall rates of DNA synthesis, as indicated by ³H-TdR grain counts, were measured in those cases in which there were sufficient normal cells in the preparations. No significant difference was detected between the acute

lymphoblastic leukaemic and normal cells.

During the response of the cases of acute lymphoblastic leukaemia to chemotherapy with 6-mercaptopurine and prednisone, or vincristine and prednisone, the total numbers of blasts and of labelled cells in the peripheral blood fell rapidly, and there was a progressive decline in the percentage of blast cells synthesizing DNA. There was no apparent relation between the initial labelling

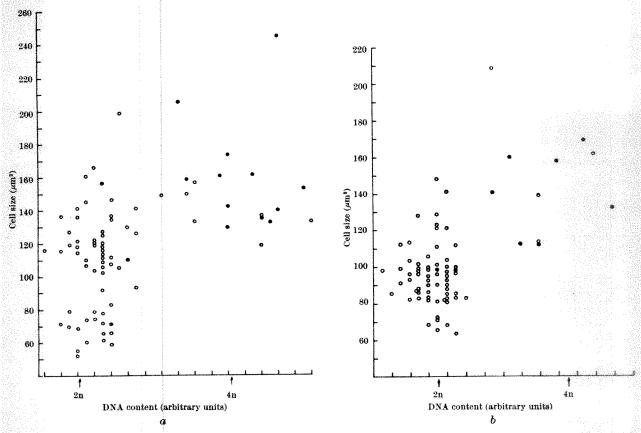


Fig. 1. Relation between cell size, DNA content and DNA synthetic activity in lymphoblasts in the bone marrow of two patients (a) and (b) with untreated acute leukaemia. •, Lymphoblasts synthesizing *H-TdR; O, lymphoblasts not synthesizing *H-TdR.

index in bone marrow or blood and the clinical course of the leukaemia.

It is clear that the labelling index was frequently higher in relapse than on first presentation (Table 1). The chromosome analyses and the estimations of the DNA content of the interphase cells indicated that there was a higher incidence of aneuploid cells in relapse than in the untreated cases. Fig. 2 shows the distribution patterns of the DNA content of the cells from two patients in relapse; there was a reduction in the percentage of cells at the 2n mode and the appearance of more unlabelled cells between the 2n and 4n modes, indicating an increase in the frequency of aneuploid cells. The distribution was similar to that seen in a blast cell crisis in chronic myeloid leukaemia4. The distribution of DNA contents of the DNA-synthesizing cells of the patients treated with methotrexate indicated that the high labelling index could not be caused by the release of the methotrexate block by the 3 H-TdR; such a release is associated with the formation of a synchronous wave of DNA synthesis, so that after 30 min most of the cells synthesizing DNA would be expected to be near the 2n mode. Evidence of a hold-up at G_{2} was observed in some patients treated with cyclophosphamide. This type of block has been reported in several types of cell systems following treatment with alkylating agents 9,10 .

At the first examination of a patient with a tetraploid acute lymphoblastic leukaemia, who had relapsed for the first time 7.5 years after the original diagnosis, following a long unmaintained remission, 17 per cent of the blasts in the peripheral blood were synthesizing DNA: bone marrow was not obtainable at this time. The leukaemic cells remained tetraploid until the patient's death 6 months later and no alteration of the modal DNA content was observed during the response and subsequent relapse on chemotherapy. The 3H-TdR grain counts were

Table 1. COMPOSITION OF LEUKAEMIC CELL POPULATIONS IN ACUTE LEUKAEMIA IN RELAPSE

Case No.	Age (yr)	Time since diagnosis of leukaemia (months)	Therapy at time of examination*	Bone i Percentage blasts	narrow Labelling Index†	Total blasts/mm ³ of peripheral blood	Chromosome No.
1	6	17	Cyclo	34	13-4	0	Hyperdiploid (47-48)
2	4	7	6-MP	80	8.2	2,900	Hyperdiploid (53-54)
3	3	(i) 20	MTX	80	70.0	0	Hyperdiploid (47-60)
		(ii) 24	Cyclo	18	17.5	Ó	
4	3	15	Cyclo	36	32.0	28	taran da araba da ar
5	6	35	Rubido	75	7.1	260	Mainly normal, few cells hypod/ploid (43-45) and polyploid (92+)
- 6	. 9	15	Cyclo	78	17.1	180	Hyperdiploid
7	. 3	5	Comp (8)	95	24.0	2,000	
8	17	(i) 92	Pred	9	18-0	95	Tetraploid (92)
		(ii) 96	Cyclo	98	47.0	*****	Tetraploid (by DNA estimation)
9	4	(i) 2	6-MP	80	17.6	1.500	- Alleria
		(ii) 3	Comp (3)	78	16-2	9,500	Hypodiploid (43-45)
10	5	2	6-MP	78	14.0	0	Hypodiploid (41-45)
10 11	6	6	Comp (6)	8	20.0	150	Hypodiploid (40–45)

^{*}Therapy on which relapse occurred. Cyclo, cyclophosphamide; Pred, prednisone; 6-MP, 6-mercaptopurine; Rubido, rubidomycin; Comp. previously treated by short-term combined chemotherapy with cyclophosphamide, vincristine, methotrexate and prednisone, and subsequently unmaintained for the number of weeks shown.

† 1,000 blast cells counted.

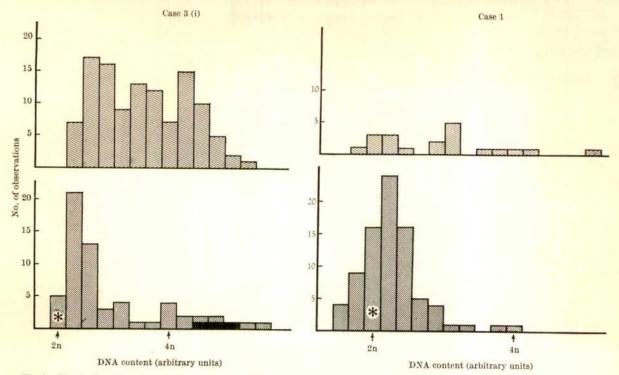


Fig. 2. Distribution of nuclear DNA content of lymphoblasts in the bone marrow of two patients in relapse. Stippled columns, cells not labelled with *H-TdR; black columns, mitotic figures. * = 2n modal value for lymphocytes.

approximately twice that of the normal cells (acute lymphoblastic leukaemia blasts, mean grain count = 73, myelocytes, mean grain count = 31); this is comparable with the relative 3H-TdR grain counts over the diploid and tetraploid cell lines in regenerating liver 11. Fig. 3 shows the DNA distribution in this patient.

This investigation confirms that the cell population in untreated acute leukaemia has a low labelling index and a large proportion of the cells with DNA contents close to the 2n mode. The pattern of cell behaviour does not remain constant, however, when the evolution of leukaemia is modified by chemotherapy; a far higher proportion of the population may be committed to division during a relapse while on maintenance therapy. This indicates that the "maturation" of the leukaemic blast cell to form a non-dividing blast is not a constant feature of the disease and may be affected by the overall rate of expansion of the cell population. It is unlikely that the change in the population can be explained on the grounds that the non-dividing blasts are more susceptible to chemotherapy than the leukaemic

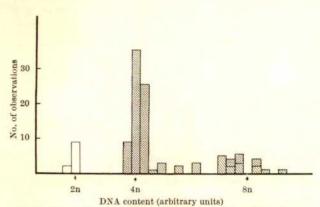


Fig. 3. Distribution of nuclear DNA content of tetraploid leukaemic cells in peripheral blood. White columns, normal lymphocytes; stippled columns, blast cells not labelled with "H-TdR: hatched columns, blast cells labelled with "H-TdR.

cells committed for division, because the labelling indices fell during the initial response to therapy. The higher incidence of aneuploid cells in the marrows of the patients in relapse may have been due to the effects of chemotherapy. It is improbable that the rate of de novo formation of aneuploid cells would have undergone any significant change during the few months between presentation and relapse, but it is probable, in the presence of the chemotherapy, that the aneuploid cells had a biological advantage so that their contribution to the population became noticeable. This change in the population of leukaemic cells during the course of the disease is in marked contrast to some of the model leukaemias in mice, the individual cells of which maintain fairly constant growth characteristics at all times12. This study suggests that generalizations about the growth characteristics of leukaemic blast cells without particular attention to the phase of the disease at which they are investigated should be made with caution.

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Radiosensitization of Bacterial and Mammalian Cells by Substituted Glyoxals

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Radiobiological Research Unit, Harwell, Didcot, Berkshire Methylglyoxal preferentially sensitizes anoxic bacterial and mammalian cells to the lethal effects of ionizing radiation. The radio-protective effect of anoxia on mammalian cells is effectively abolished in the presence of methylglyoxal, whereas sensitization of oxygenated mammalian cells is slight.

SEVERAL different classes of compound enhance the lethal effects of X-rays on various bacterial and mammalian cell systems. In view of the suspicion that anoxic foci are largely responsible for the radiation resistance of some tumours, the potential therapeutic usefulness of agents which could act preferentially in sensitizing anoxic cancer cells to X-ray induced cell killing is obvious. Some, but not all, sulphydryl reagents exert such an effect on bacteria¹, but they are in general too toxic for use with mammalian cells.

The effectiveness and the mechanisms of action of those chemical sensitizers which react with SH groups, such as N-ethylmaleimide, p-mercuribenzoate, iodoacetic acid and iodoacetamide have recently been reviewed by Bridges². Adams and Dewey³ have demonstrated, with bacterial

systems, the sensitizing properties of diacetyl, CH₃C.C.CH₃, and various other carbonyl compounds. These authors have produced experimental evidence to support a most interesting theory which accounts for the radiosensitizing properties of compounds in which carbonyl groups constitute part of a conjugated system of double bonds. According to theory compounds of this type can capture and stabilize, through resonance, hydrated electrons produced by the ionizing radiation. The radical anion produced is longer lived than the free hydrated electron and thus possesses an extended sphere of activity compared with the hydrated electron. Many such compounds which can form long lived radicals during irradiation, however, can also react with SH groups and it is still not clear what contribution is made by this latter property to their radiosensitizing activity.

Methylglyoxal, CH₃C.C, has been shown to inhibit

protein synthesis in bacterial cells and to prevent cell division⁴. This compound is widespread in nature and reacts reversibly with many naturally occurring thiols. In view of the interesting reactions of methylglyoxal with thiols, and especially with glutathione, we decided to investigate the possible radiosensitizing action of this substance and of certain other substituted glyoxals. The experiments which are reported in this article demonstrate unequivocally the radiosensitizing properties of methylglyoxal for bacteria and for mammalian cells in culture.

Methylglyoxal was obtained as a 45 per cent aqueous solution (Aldrich Chemical Co., Milwaukee). Before use, this was redistilled at the water-pump. An aqueous fraction was obtained first, followed by a yellow green oil distilling over the approximate range $50^{\circ}-70^{\circ}$ C and dissolving in the earlier fraction to give a colourless, homogeneous distillate. The methylglyoxal contents of such distillates when estimated gravimetrically as the bis (3-nitrobenzoylhydrazone)⁵ ranged from 26 per cent to 42 per cent (w/v); the distillates had an acidic reaction

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and they were stored in the dark at 4° C. In addition. methylglyoxal was prepared by oxidation of acetone with selenium dioxide. The crude product, boiling point 32°-60° C at 13 mm, was dissolved in dry ether and dried over anhydrous sodium sulphate overnight. The ether was removed and the residue distilled at 12 mm when a fraction, boiling point $45^{\circ}-50^{\circ}$ C, was collected, the receiver being cooled in a mixture of acetone and solid carbon dioxide. The product contained $42 \cdot 0$ per cent carbon and 6.37 per cent hydrogen; calculated for C₃H₄O₂, 3/4 H₂O: 42·1 per cent carbon and 6·48 per cent hydrogen. Riley, Morley and Friend⁶ give the boiling point as 54° C at 10 mm, and Moulds and Riley⁷ give 57°-60° C at 20 mm. They report products containing a small proportion of water. When the commercial or freshly prepared samples were analysed by gel filtration on a column of 'Sephadex G-10' they were shown to contain at least four components differing in molecular weight, which absorbed ultra violet light at 2800 Å (Fig. 1). It is probable that these components are various polymeric forms of methylglyoxal. Gas chromatographic analysis also revealed the presence of at least four components. Samples of methylglyoxal from both sources proved to have similar biological properties.

Glyoxal was obtained from Hopkin and Williams, London, as a 30 per cent aqueous solution. Propylglyoxal was supplied by Professor A. Szent-Györgyi of Woods Hole, Massachusetts. Phenylglyoxal was obtained from Koch-Light Laboratories, Colnwood, England, in the form of a polymeric glass. This was dissolved in chloroform and the chloroform was removed by distillation, leaving a syrup which was distilled *in vacuo*. The pure monomer was collected in the boiling point range 87.5°-89° C at 10 mm (lit. boiling point 95°-97° C at 25 mm), then dissolved in ten volumes of hot water and allowed to crystallize, when white needles of the monohydrate separated and were dried in air, melting point 81°-83° C.

When methylglyoxal, as a neutral solution (0.2 molar), was given intraperitoneally to female mice of the Harwell R stock, weighing between 20 and 25 g, the LD_{50} (30 days)

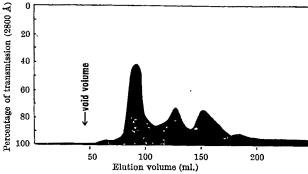


Fig. 1. Fractionation of methylglyoxal (2 molar) on a column (1.5 \times 90 cm) of 'Sephadex G–10'. The column was developed with distilled water at a flow rate of 26 ml/min and the absorption of the cluate at 2800 Å was measured and plotted against the clution volume.

was approximately 7 mmoles/kg (500 mg/kg). Doses of less than 6 mmoles/kg had no obvious toxic effects. LD_{50} values (30 days) for glyoxal and phenylglyoxal were 7 mmoles/kg and 1.8 mmoles/kg, respectively.

Sensitization by methylglyoxal was first demonstrated with the bacterium Serratia marcescens. Preliminary experiments established that the bacteria were not killed in the presence of methylglyoxal until concentrations greater than 5×10^{-2} molar were reached. Cells of Serratia marcescens grown in nutrient broth at 30° C were collected during the logarithmic growth phase, suspended in phosphate buffer (M/15, pH 6.8) and irradiated at 4° C with gamma rays from sources of cobalt-60 at a dose rate of approximately 4.8 krads/min. Nitrogen was bubbled continuously during and for 6 min before irradiation. Surviving bacterial cells were estimated by colony counts after plating on nutrient agar and incubating at 30° C for 24 h. Irradiations were made in the presence and absence of methylglyoxal, both in oxygen and in nitrogen. In most of the radiation experiments, methylglyoxal was

used at a concentration of 2×10^{-3} molar.

The cell line known as "clone A" originally derived from the ovary of the Chinese hamster by T. T. Puck (and subsequently cloned several times) was also sensitized by methylglyoxal. At the time of the experiments reported here, about 80 per cent of the population comprised cells containing the normal diploid number of twenty-two chromosomes. Cells were grown and manipulated according to the methods previously described. ments involved the application of methylglyoxal to cells, the growth medium was replaced by a pre-warmed, balanced salt solution to which was added the required amount of the drug from a cold, 0.4 per cent solution. Cells remained in contact with the drug for 20 min, whereupon the bathing fluid was removed and the cells irradiated. For irradiation in anoxic conditions, the cells, at four petri dishes for each dose, were sealed in a 'Perspex' container which was gassed with nitrogen for 10 min before irradiation (no oxygen could be detected in the effluent gas by a Beckman oxygen analyser). Cells were irradiated at room temperature in batches of four replicate plates for each dose. In all cases, the irradiations were performed with the bathing fluid removed in order to obtain full anoxia under nitrogen and to avoid unilateral absorption of energy, because the dishes were interposed between two Siemens therapeutic machines arranged to deliver 250 kVcp X-rays (8 m.amp, half value layer 1.2 mm of copper) at 620 rads/min. Immediately after irradiation the cells were washed with 199 medium, then given complete growth medium before incubation for 10 days in a gas (5 per cent carbon dioxide and 95 per cent oxygen) incubator. Control batches received all the appropriate treatments including sham irradiation in situ. inary toxicity experiments, all these manipulations were performed, with two exceptions: no tests were performed under anoxia and there were no irradiations. Surviving cells in all experiments are defined as those which produced colonies of fifty or more normal descendants after 10 days of incubation at 37° C; the only effect of irradiation studied is therefore cell killing. Two identical toxicity experiments were performed. In each case, four concentrations of methylglyoxal in a balanced salt solution were tested, and results of exposure of hamster cells to the drug for 20 min are shown in Table 1.

In the case of the irradiation experiments, the Harwell Atlas computer was programmed to analyse the results by an iterative least squares technique. The following relationship was assumed between surviving fraction (S) and dose (D)

where n is the extrapolation number and $D_{\rm 0}$ is the dose increment in rads to reduce any surviving fraction F on the exponential region of the survival curve to 0.37F. Survival values were determined at least twice for all treatments and, where no significant differences between

 $S = 1 - (1 - e^{-D}/D_0)^n$

Table 1. TOXICITY OF METHYLGLYOXAL TO CHINESE HAMSTER CELLS

Concentration of methylglyoxal in balanced salt solution

10-4 molar

Nil

10 ⁻² molar	Nil
10-3 molar	85.3 ± 4.89
10 ⁻⁴ molar	102.9 ± 4.41
10−5 molar	101.9 ± 3.67
Control (balanced salt solution)	100 ±1.47

Table 2. RADIOSENSITIZING ACTION OF METHYLGLYOXAL ON THE BACTERIUM, Servatia marcescens

SOLVER HER COURTS											
Survival curve	Extrapola- tion No.	$D_{\mathfrak{o}}$ value (rads)	Ratio of D_0 values								
Curve 1: methylgiyoxal in oxygen	3·03 ± 1·35	1,104 ± 38·4	$\frac{1.608}{1.104} = 1.45$								
Curve 2: control in oxygen	3.96 ± 2.08	$1,608 \pm 110.4$									
Curve 3: methylglyoxalin nitrogen	n 1·24 ± 0·54	5,083 ± 796-8	8.554 = 1.7								
Curve 4: control in nitrogen	1.40 + 0.52	8.554 + 864-0	5.083								

experiments were detected by a computer test of consistency, the data were pooled for final analysis.

For Serratia marcescens, each experiment produced the same qualitative effect of methylglyoxal, but significant differences in extrapolation numbers between experiments made it impossible to pool the data. Accordingly, the results of a typical experiment are shown in Fig. 2 and the values of the parameters n and D_0 derived from these curves are listed in Table 2. In all the experiments the effect of methylglyoxal was found to be independent of dose for there was no significant difference in extrapolation number between curves 1 and 2 or between curves 3 and 4 (compare ref. 11). After this independence had been established, the ratios of the D_0 values with and without methylglyoxal were used as a measure of the relative effect of the drug. In all cases these were greater than unity, and a combined test of all values showed that the effect of methylglyoxal was significant (P < 0.1 per cent) both in oxygen and nitrogen (Table 2).

The magnitude of the radiosensitization produced by methylglyoxal was often greater than that shown in the present experiments. It is possible that variation from day to day results from changes in the relative concentration of the various polymeric forms of methylglyoxal always present in an aqueous solution. Both propyl- and phenylglyoxals sensitized anoxic bacterial cells to X-ray

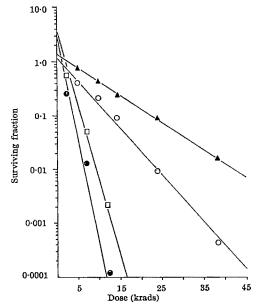


Fig. 2. Fraction of Serratia marcescens cells surviving various doses of γ -rays (cobalt-60): curves of the best fit to observed data. \bullet , 2×10^{-3} molar methylglyoxal in oxygen $(n=3\cdot03\pm1\cdot35, D_0=1,104\cdot0\pm38\cdot4 \, {\rm rads})$; \bigcirc , control in oxygen $(n=3\cdot96\pm2\cdot08, D_0=1,608\cdot0\pm110\cdot4 \, {\rm rads})$; \bigcirc , 2×10^{-3} molar methylglyoxal in nitrogen $(n=1\cdot24\pm0\cdot54, D_0=5,082\pm796\cdot8 \, {\rm rads})$; \blacktriangle , control in nitrogen $(n=1\cdot40\pm0\cdot52, D_0=8,553\cdot6\pm864\cdot0 \, {\rm rads})$.

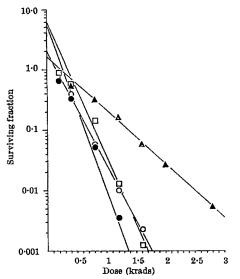


Fig. 3. Fraction of Chinese hamster cells surviving various doses of hard X-rays: curves of heat fit to peoled data from the surviving various doses of hard X-rays. Fig. 3. Fraction of Uninese hamster cells surviving various doses of and X-rays: curves of best fit to pooled data from two experiments. \bullet , 5×10^{-4} molar methylglyoxal in air $(n=5\cdot77\pm2\cdot96, D_o=158\pm15 \text{ rads})$; \square , control in air $(n=6\cdot13\pm1\cdot83, D_o=189\pm9 \text{ rads})$; \bigcirc , 5×10^{-4} molar methylglyoxal in nitrogen $(n=2\cdot01\pm0\cdot50, D_o=239\pm13 \text{ rads})$; \bigcirc , control in nitrogen $(n=1\cdot67\pm0\cdot38, D_o=476\pm24 \text{ rads})$.

induced cell death, but sufficient data are not yet available to make a detailed comparison of the effectiveness of these compounds. Glyoxal was inactive when tested in our system. It is important to note that the addition of irradiated bacteria to either non-irradiated or irradiated methylglyoxal did not result in enhanced bacterial lethality.

For Chinese hamster cells, complete uniformity was obtained throughout the experiments and no differences could be detected between them. Here therefore all the data were pooled and they are presented in Fig. 3, together with the derived values of n and D_0 in Table 3. Fig. 3 illustrates the curves of best fit obtained from the pooled data, extrapolated to zero dose. As with Serratia marcescens the effect of methylglyoxal was found to be completely dose-independent. Again the comparison of D_0 ratios showed that the effect of methylglyoxal was significant at the 0.1 per cent level in nitrogen and at the 10 per cent level in air. With the hamster cells, the radioprotective effect of anoxia (curve 4) was effectively abolished by exposure of anoxic cells to methylglyoxal (curve 3).

Table 3. RADIOSENSITIZING ACTION OF METHYLGLYONAL ON CHINESE HAMSTER CELLS IN TISSUE OULTURE

	Survival curve	Extrapola- tion No.	D_0 value (rads)	Ratio of D_0 values
Curve 1:	methylglyoxal in air	5·77 ± 2·96	158 ± 15	$\frac{189}{158} = 1.2$
Curve 2:	control in air	6·13 ± 1·83	189 ± 9	200
Curve 3:	methylglyoxal in nitrogen	2·01 ± 0·59	239 ± 13	$\frac{476}{239} = 2.0$
Curve 4:	control in nitrogen	1.67 ± 0.38	$\textbf{476} \pm \textbf{24}$	

It would be unwise to speculate at this stage on the possible mechanism of radiosensitization by methylglyoxal. It may be that methylglyoxal acts as a sensitizer in the way suggested for diacetyl by Adams and Dewey by forming a long lived stabilized radical. The reaction of methylglyoxal with thiol compounds, especially glutathione, and the possible involvement of the ubiquitous enzyme system, glyoxalase, in modifying radiosensitivity, however, cannot be excluded from consideration.

The observation that methylglyoxal at non-toxic concentrations overcomes the protective effect of anoxia when mammalian cells in tissue culture are irradiated with X-rays suggests that this compound might be effective in sensitizing to radiation tumours with anoxic foci without appreciably affecting the sensitivity of fully oxygenated cells. A preliminary study, in which methylglyoxal (4 mmoles/kg) was given to mice immediately before their exposure to an LD_{50} (780 rads) of X-rays, showed no evidence of enhancement of radiation lethality.

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Carotid Rete and Brain Temperature of Cat

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The plexus of arteries which make up the carotid rete in the cat seems to be able to modify the temperature of central arterial blood as it enters the cranial cavity.

Changes in the behavioural state of an animal are often accompanied by temperature fluctuations throughout the brain^{1,2}. Work on the rat³, monkey⁴ and rabbit⁵ has demonstrated a common factor underlying these intracranial temperature changes associated with feeding, sleeping and arousal—that is, a change in the temperature of the arterial blood perfusing the brain. The evidence that arterial blood temperature is the most important determinant of brain temperature in the monkey, rabbit and rat apparently contradicted the other findings in the

cat^{1,6-10}. When our experiments with the cat confirmed other reports that fluctuations (0·1-0·7° C) in brain temperature were independent of temperature fluctuations in the common carotid or aortic arterial blood, we began to suspect an important species difference in the regulation of brain temperature.

In the monkey and the rabbit the cerebral arterial blood at the circle of Willis is at the same temperature as the central arterial blood at the aortic arch4,5. This is to be expected because cerebral arterial blood in these

species enters the cranial cavity directly from the deeply coursing internal carotid and vertebral arteries. The cat has no internal carotid artery, however, and the circle of Willis is connected to the external carotid artery through the extracranial carotid rete. The physiological significance of this "rete mirabile"—a net-like arterial plexus present in some mammals—has remained an enigma since its description by Galen. Our studies suggest that the carotid rete in cats acts to modify the temperature of the central arterial blood as it enters the cranial cavity. We have concluded that the variations in brain temperature in the unanaesthetized cat are the result of neither local changes in neuronal heat production nor local changes in cerebral blood flow, as others have suggested, but rather, they originate in variations in the temperature of the cerebral arterial blood.

Since the comparative study by Tandler¹¹ in 1899 the arterial system of the head has been known to differ greatly between mammalian species. Daniel et al.12 have demonstrated that the rat, rabbit and dog have an intact internal carotid artery and a cerebral arterial supply similar to that of man and other primates13. On the other hand, the cat, together with the sheep, goat, ox and pig, derives most of the arterial supply to the anterior part of the circle of Willis from the carotid rete, a plexus of arteries formed principally by branches of the external carotid artery. In the cat the carotid rete lies outside the cranial cavity, near the apex of the orbit, and is termed an 'extracranial rete''12, while in the artiodactyls the rete is intracranial, lying in the cavernous sinus at the base of the skull. Neither Daniel et al.12 nor Davis and Story14, in their work on the carotid circulation of the cat, could demonstrate a patent internal carotid artery in their specimens, and both suggest that the contribution to the circle of Willis from the extracranial rete is the chief blood supply to the brain of the cat. Other studies involving the injection of dyes into live animals 15 showed that the carotid rete and its contribution to the circle of Willis supplies almost the entire brain of the cat. The rete might be haemodynamically important, as has been suggested12. Our preliminary studies indicate that the extracranial rete in the cat has a profound effect on brain temperature which may have significant thermoregulatory consequences.

We used four adult cats—male and female. Copperconstant an arc-welded thermocouples in glass tubing (outer diameter $0.7~\rm mm$) were implanted stereotaxically 16 in the brainstem at the level of the preoptic region. Two or three thermojunctions were cemented in the same glass tube for measurement of temperature at different vertical levels in the same frontal plane, and the probes were lowered through the brain into the subarachnoid space to lie on the vessels of the circle of Willis. We have shown in the monkey4 and the rabbit5 that the temperature of the cerebral arterial blood, measured in this way in the basal subarachnoid space, rapidly reflects changes in the temperature of the aortic and carotid blood. We measured central arterial blood temperature with thermocouples in polyethylene tubing (outer diameter 0.6 mm) threaded to the aortic arch through the common carotid artery (two animals) or into the common carotid through its inferior thyroid branch (two animals). Temperature at the extracranial rete was measured with thermocouples in polyethylene tubing passed retroconjunctivally from the supra-orbital process along the roof of the orbit to lie in or near the rete itself. Skin temperature was measured by a thermocouple implanted subcutaneously on the dorsal surface of the ear. Distal ends of the thermocouples were attached to miniature copper-constantan connectors cemented to a lucite platform elevated above the intact scalp on four epidural stainless steel screws, thus avoiding an artificial heat sink of dental cement over the cranium Aortic, orbital and ear thermocouples were threaded subcutaneously to the skull platform. An electroencephalogram was monitored between the two rear

screws supporting the platform. We used an Offner Type-R ink-writing oscillograph which provided continuous and simultaneous electroencephalogram and temperature records. Reference junctions in a bath of distilled water and crushed ice were used, and thermopotentials were amplified with a chopper-stabilized d.c. amplifier. The maximum sensitivity of the recording system was 0.025° C/mm pen deflexion. We recorded for more than 100 h from four unrestrained cats in a sound-attenuated, temperature controlled environmental chamber at 25° C, with one-way glass to permit observation of behaviour.

Accustomed as we had been to studying the monkey and the rabbit, in which intracranial temperature changes were related to similar changes in central arterial temperature^{4,5}, the independence of intracranial temperature from carotid temperature in the cat was very striking (Fig. 1). While the central arterial blood temperature in the cat remains rather steady, large, rapid changes in temperature related to sleep-waking cycles, occur throughout the cranium. There is no constant relationship between the temperature levels of the cranial sites and the level of the central blood temperature. As others have reported, in both anaesthetized and unanaesthetized cats1,6-10,17, at any time the central blood temperature might be higher, lower, or the same as the temperature of any site in the brain. In the rabbit and the monkey, a constant temperature gradient exists over hours, weeks and months between every brain site and both the central and the cerebral arterial blood, for there is no gradient between central and cerebral arterial blood in these species4,5. In the cat, on the other hand, while there is no constant temperature difference between brain and the central (aortic or carotid) arterial blood, there is a stable temperature gradient between brain sites and cerebral arterial blood measured at the circle of Willis (Figs. 1 and 2). It seemed thus that the behaviour-related temperature changes in the brain of the cat did originate in arterial blood temperature changes, but that the thermal state of the central arterial blood was modified on the way

The presence of the extracranial carotid rete is the most important cerebrovascular difference between the cat and the other species we have studied, and so we investigated the temperature of the rete and found that it was usually lower than cerebral arterial temperatures in the relaxed animal, but that the temperature gradient

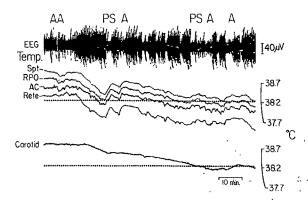


Fig. 1. Cranial and central temperature changes associated with slep and arousal in a free moving cat. Ambient temperature 25° C. The record began when the chamber door was closed after the animal was prepared for recording, and he is very excited and active (AA). Notice the drop in cranial temperatures as he falls into slow sleep (high voltage electroencephalograph, unlabelled). Rises in cranial temperatures are associated with periods of paradoxical sleep (PS) and spontaneous arousal (A). The central arterial blood temperature (carotid) shows a slow, steady decline in the 1-5 h recording, parallel to that of the cranial sites, but does not exhibit the same rapid temperature fluctuations. EEG, biparietal cortical electroencephalogram; Spt, midline septal region, 6.5 mm from the basal subarachnoid space; RPO, preoptic region, 1-6 mm from the basal subarachnoid space; AC, anterior cerebral artery, immediately rostral to optic chiaem and between the optic nerves; carotid, patent common carotid artery, at the level of the inferior thyroid artery; Rete, extracranial rete, near the apex of the orbit. The temperature, trace of the rete has been lowered 0.25° C for clarity.

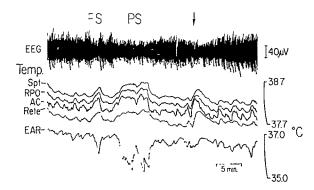


Fig. 2. Deep and peripheral cranial temperature changes in the cat in sleep and arousal. Ambient temperature 25° C. The record begins while the animal is in a period of slow sleep (unlabelled). Notice the rise in cranial temperatures and the fall in skin temperature (EAR) during episodes of paradoxical sleep (PS). At the arrow, the animal was awakened by banging on the recording chamber, and he jumped up and moved around before felling to sleep again. Labels the same as in Fig. 1 except EAR, subcutaneous temperature of the dorsal surface of the ear. The temperature trace of the rete has been lowered 0.25° C for clarity.

between intracranial structures and the extracranial rete was variable (Fig. 1). The temperature measured at the extracranial rete showed fluctuations similar to those observed at the anterior cerebral artery and in the brainstem (Figs. 1 and 2). Thus the temperature fluctuations in the brain of the cat which occur with arousal and with sleep cycles occur in parallel with similar fluctuations both at the circle of Willis at the base of the brain and outside the skull at the extracranial rete.

Upon arousal from slow sleep (high voltage electroencephalogram), during paradoxical sleep⁸ or when the animal is disturbed by noises, the temperatures of the cranial structures rise (Figs. 1 and 2), while the central arterial blood temperature remains steady (Fig. 1). Longterm changes in central blood temperature may parallel those of the cranial sites (Fig. 1), but the rapid changes in cerebral arterial blood and brain temperature which occur with arousal and with paradoxical sleep are not present in the central arterial blood. Subcutaneous temperature of the ear usually showed an inverse relationship to the cranial temperature changes (Fig. 2). We have described a peripheral vasconstriction and a rise in arterial blood and brain temperature during paradoxical sleep in the rabbit⁵.

In the cat^{6,9,10}, monkey and rabbit^{4,5} there is a gradient of increasing temperature from the arteries in the basal subarachnoid space toward the centre of the brain. The cerebral arterial blood is cooler than the heat-producing nervous tissue in all species, and so this gradient is expected4,5,17,18. Brain temperatures decrease towards the surface of the hemispheres, presumably because of the hemispheric subarachnoid arterial net and the influence of environmental cooling on the superficial brain sites4,6,19.

Our studies suggest that the changes in brain temperature of the cat in sleep and arousal originate in temperature changes in the cerebral arterial blood. Our finding that parallel changes in temperature occur outside the cranium at the extracranial rete, and inside the cranial cavity at the basal arteries and in the brain, with no relationship to arterial blood temperature high in the common carotid artery, suggests that the temperature of the central arterial blood in the cat is modified across the extracranial rete.

Both the configuration and the location of the rete suggest that it is vulnerable to various thermal influences. The extracranial rete lies within a venous lake12,14, and the large surface area of the interlacing network of vessels may permit a "countercurrent" exchange of heat between the arterial blood of the rete and the venous return from various regions of the head. Our observations of inverse temperature changes occurring between ear skin and rete (Fig. 2) suggest that cranial peripheral vasomotor changes and changes in heat loss from the head influence the temperature of arterial blood in the extracranial rete by such a countercurrent mechanism. Furthermore, the close relationship of the rete to the cranial, oral and nasal cavities and to the muscles of mastication suggests that the temperature of the blood in the plexus of arteries might be altered by cerebral venous blood, the temperature of the food or of the respiratory gases19, muscular heat production, or vascular changes in the mucosa of the oral or nasal passages.

The full significance of these findings for thermoregulation in the cat remains to be studied. Since the localization of thermosensitive regions in the hypothalamus²⁰, it has been assumed that information about the thermal state of the body core may be carried rapidly to central thermoreceptors by the arterial blood²¹. In the cat, however, because of temperature changes across the carotid rete, large thermal changes occur in the cerebral arterial blood and in the hypothalamus without concomitant changes in deep body temperature. The hypothalamus of the cat is thus exposed to temperature shifts which do not appear to be of primary thermoregulatory significance. Any theory of temperature regulation in the cat must take into account this dissociation between the thermal changes occurring in the hypothalamus and those of the body core.

Reports of thermosensitive regions in the medulla of the cat^{22,23} suggest that lower brainstem regions within the area of supply of the vertebral-basilar system¹⁵ may be involved in thermoregulation in this species. expect that the behaviour related temperature fluctuations in the rostral brainstem would not be present in the lower medulla, for the vertebral arterial blood comes directly from thoracic arteries and does not traverse a rete. In the cat, the presence of dual thermosensitive zones bathed by blood from thermally divergent sources suggests a "two-tiered"21 control of homeothermy. There may be a basically different mode of thermoregulation in the internal carotid animals in which the cerebral arterial blood is thermally homogeneous4,5. We conclude that the species differences in the cerebral circulation may be important in the central neural organization for the regulation of body temperature.

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LETTERS TO THE EDITOR

PLANETARY SCIENCE

Observation of Raman Scattering from the Atmosphere using a Pulsed Nitrogen Ultraviolet Laser

This communication reports the experimental observation of optical Raman scattering from oxygen and nitrogen in the atmosphere using a pulsed nitrogen ultraviolet laser^{1,2} as a light source. Previous atmospheric laser scattering experiments have been reported^{3,4} which utilized Rayleigh scattering or scattering from particulate matter such as aerosols and dust particles. With Raman scattering, the wavelength of the scattered light is shifted, the amount of the shift being specific to the scattering molecule. The importance of the Raman scattering technique is that it enables a range resolved measurement of atmospheric constituents, with respect to both species and concentration, from a single remote location. This type of measurement could be useful in such fields as meteorology, atmospheric physics and air pollution control.

The apparatus used in the Raman scattering experiments is shown in Fig. 1. The transmitter consisted of an uncollimated 100 kW peak power, 10 nsec, 3371 Å pulsed nitrogen laser (Avco model C-102). A 20 cm diameter, 1.6 m focal length telescope and an RCA 7265 photomultiplier with an S-20 cathode response served as the receiver. Continuous wavelength selection was obtained by tilting interference filters away from normal incidence. Two interference filters were used, each with a transmission band width of 35 Å at half maximum. The transmissions for these filters were centred at 3557 Å and 3658 Å for normal incidence. A maximum filter tilt angle of 35° was possible with the apparatus, yielding a maximum wavelength shift of approximately 200 Å. The experiment was conducted at Everett, Massachusetts, at times shortly after sunset during July 1967.

A spectral analysis of the experimentally obtained air scattering return at zero elevation is shown in Fig. 2. In addition to the strong return at the 3371 Å transmitter wavelength, signals, weaker by about a factor of 1,000,

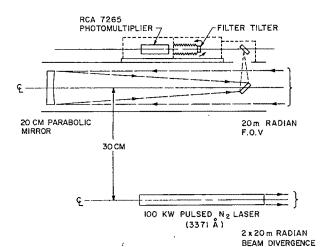


Fig. 1. Schematic diagram of the apparatus used to observe atmospheric Raman scattering.

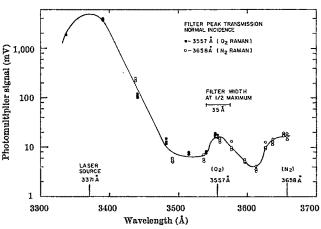


Fig. 2. Photomultiplier signal from atmospheric backscatter as a function of wavelength. Each data point represents a single measurement obtained with one laser pulse.

were observed at 3557 Å and 3658 Å, corresponding to the vibrational Raman shift for oxygen and nitrogen, respectively. The magnitude of the observed Raman signals was consistent with the expected signal calculated on the basis of an estimated Raman cross-section of 10^{-29} cm². Measurements of this type can be used to obtain directly the oxygen to nitrogen concentration ratio as a function of range.

The apparent spectral width of the experimental back-scatter signal in Fig. 2 near the laser line at 3371 Å is thought to result from the rejection properties of the interference filter. This was determined by comparing the air backscatter near 3371 Å with the return from a solid target. No significant difference, as a function of wavelength, was observed when the target signals were normalized to the air backscatter.

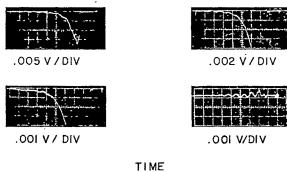
It is hoped that, with improvements in both resolution and rejection, Raman signals can be observed in this wavelength region from other sources. Signals originating from rotational transitions in oxygen and nitrogen, infrared transitions in various atmospheric contaminants, and vibrational transitions in small solid air-borne particles are of particular interest.

Typical oscillograms of the photomultiplier signal, obtained at the 3658 Å nitrogen Raman line, are shown in Fig. 3. At the beginning the observed pulse shape is dominated by the non-coaxial transmitter-receiver geometry. When the fields of view of the transmitter and receiver have sufficiently overlapped, the signal decay can be fitted to an inverse squared law multiplied by an exponential extinction. The signals shown in Fig. 3 yield useful data for up to 8 µsec, which corresponds to a range of 1·2 km. The application of photon counting methods, with pulses averaging 100/sec for several seconds, should greatly extend the useful range with the same laser transmitter power.

A range resolved measurement of backscattered power in the Raman echo pulse in Fig. 3 can be shown⁵ to yield directly the atmospheric transmission at the range of interest. Range-gated atmospheric Raman scattering may thus be useful in single ended transmissometer applications. Previously proposed single ended transmissometers⁶ suffered from a lack of complete knowledge of the relationship between the backscatter from the suspended particles, causing the reduced visibility, and the transmission through them, and relied therefore on an analysis of the backscatter pulse shape, rather than a simple power measurement.

The 3371 Å pulsed nitrogen laser was chosen from among the available strong laser sources for the following reasons. Raman scattering cross-sections vary inversely with the fourth power of the incident wavelength. Thus the pulsed nitrogen laser is more effective than a ruby laser by a

No RAMAN SCATTERING (λ= 3658 Å)



0.5 µ S/DIV

Fig. 3. Oscillograms showing a typical photomultiplier signal obtained at 3658 Å. The trace at the lower right is the background noise observed when the laser is fired with the photomultiplier masked.

factor of $(6940/3371)^4 = 17.96$. The photoefficiency of photocathodes is much higher in the ultraviolet than in the near infrared. With a 6940 Å ruby source the nitrogen Raman line is shifted to 8280 Å where the best photoefficiency (S-1) is about 0.4 per cent compared with 10 per cent for the S-20 surface at 3658 Å. Thus a factor of 25 in favour of the pulsed nitrogen laser results.

The product of the foregoing two factors is $17.96 \times 25 =$ 449. When the difference in energy for each photon is taken into account, it is found that the 100 kW pulsed nitrogen laser is as effective in producing Raman scattered photoelectrons as would be a ruby laser of 21.8 MW with the same pulse width. The nitrogen laser has the further advantage of a high repetition rate capability (100 pulses per second) which permits rapid scanning or signal averaging.

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Graphical Representation of Mantle Convection

COMMUNICATIONS which deal with mantle convection usually fall into one of two groups. The geological approach is to assume the East Pacific rise and Atlantic ridge systems to be loci of rising mantle material, which, after flowing parallel to the Earth's surface, sinks again in the vicinity of the East Indian and Caribbean trenches or the young mountain systems of western America and Eurasia. Good examples are given by Girdler¹ and Wilson². No attempt is made to portray the forms of convection cells within the mantle.

The theoretical approach was well illustrated in Licht³ and Runcorn4. Symmetrical sets of flow cells, usually of three- to five-fold symmetry, are displayed on a global There is no particular correlation between the cell locations and surface geology.

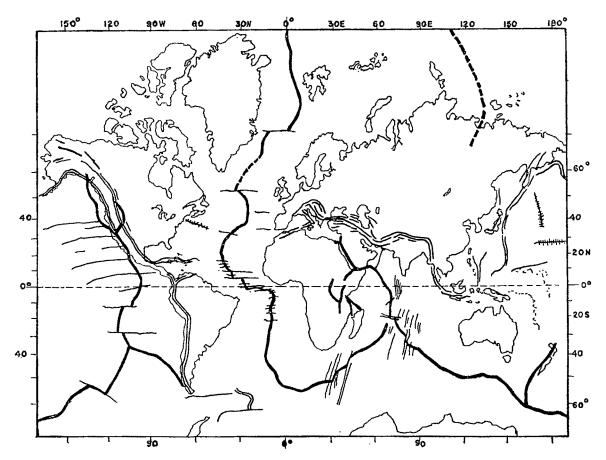


Fig. 1. Mercator's projection showing rifts (----), trenches and tertiary mountain systems (=----)

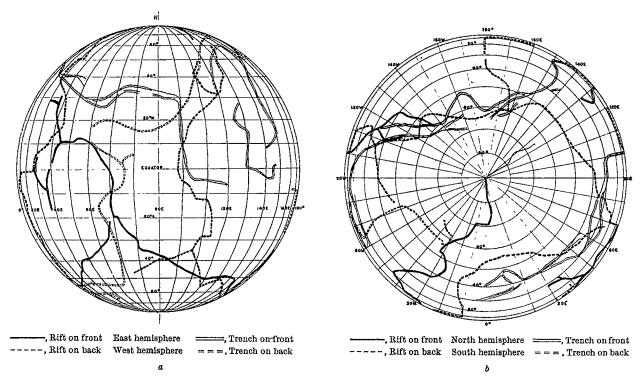


Fig. 2. a, Equatorial projection of surface features indicating convection. b, Polar projection of surface features indicating convection.

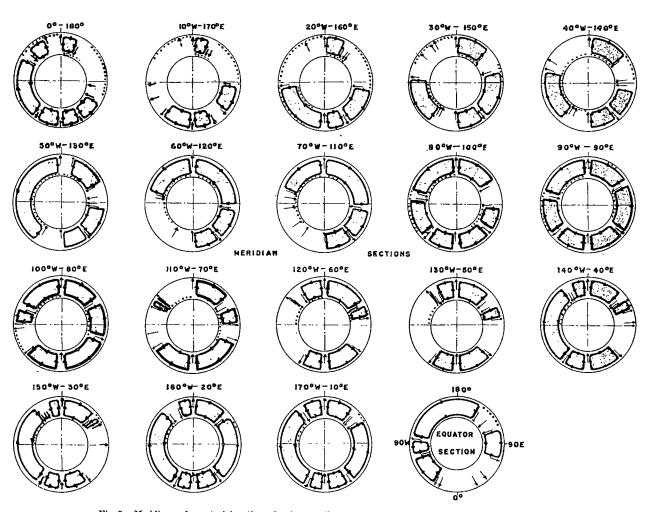


Fig. 3. Meridian and equatorial sections showing mantle convection cells. ..., Flow direction out of paper.

It seems attractive to attempt a matching of the theoretical mantle cells with the significant surface geological features. The procedure, of course, is quite simple: after outlining the rises and trenches on a global projection, one slices the appropriate sections to expose the mantle. The convection cells should then be contained between successive "up" and "down" points on the circumference.

Fig. 1 is a Mercator's projection showing the location of known rift, tertiary mountain and trench systems^{2,5}. These are transformed to equatorial and polar projections in Fig. 2a and b. The sections shown in Fig. 3 are obtained either by cutting through the equator in Fig. 2a, or by slicing meridians at successive 10° angles from Fig. 2b. For clarification, the cells are drawn to fill the entire mantle. The small dots indicate flow components normal to the section.

In the equatorial section of Fig. 3, only four asymmetrical cells appear. The larger empty area encompasses Africa, which is partially enclosed by a rift system; the smaller corresponds to the East Indian trench.

A descending column has been introduced at the South Pole in all the meridian sections. The justification for this is the presence of the Antarctic mountain system, connected to the Patagonian Andes by the Scotian arc. The meridian slices consequently show some symmetry at the poles, although the cells are far from uniform in size.

Theoretical studies, involving spherical harmonic analysis of the topography of the Earth's surface and of the geopotential^{4,7,8}, indicate that the pattern of mantle convection has changed, with the growth of the Earth's dense core, from a first order harmonic mode to the fourth or fifth at present. Thus there would now be eight or ten convection cells in the mantle. None of the sections shown in Fig. 3, however, has more than six cells, while

eight of them have only four cells. The small number of cells and the many gaps between them do not fit any symmetrical theoretical model. It is unrealistic to assume that the somewhat random distribution of rift and trench systems—and of land masses—is controlled by a perfectly regular system of convection cells in the mantle.

There is some three dimensional character to the convection systems displayed in Fig. 3. They resemble sectorial and tesseral harmonics, lying north south in the western hemisphere, less complete and principally east—west in the eastern.

Heat flow measurements ought to provide significant evidence of mantle convection, for rising currents are marked by high heat flow and vice versa. Fig. 4 shows heat flow zones which are known, based on the work of Lee and Uyeda 10, plus later data. The world mean heat flow is given as 1.5 ± 10 per cent $\mu \text{cal/cm}^2/\text{sec}$. Because 80 per cent of the measurements have been made in ocean bottoms, many of these in rift—rise areas, this figure may be high.

Average heat flow of the rift—rise systems (50 areas of 186) is 2·4 µcal/cm²/sec, while the average in trench and young mountain regions is 1·36 (32 of 180). Thus the former areas are anomalously high, but the latter are only slightly below average. The zones of lowest heat flow (average 1·0 or less) appear to be adjacent to the rifts, chiefly in ocean basins. A definite pattern of heat flow anomalies will require many more measurements, particularly in land areas.

If convection is taking place in the mantle, the rising streams appear to be located beneath the rifts and ridges, based on high heat flow and, to a lesser degree, on low gravity in these areas. Equivalent indications of the descending streams, however, are not very clear. The cell system is generally incomplete, irregular and of

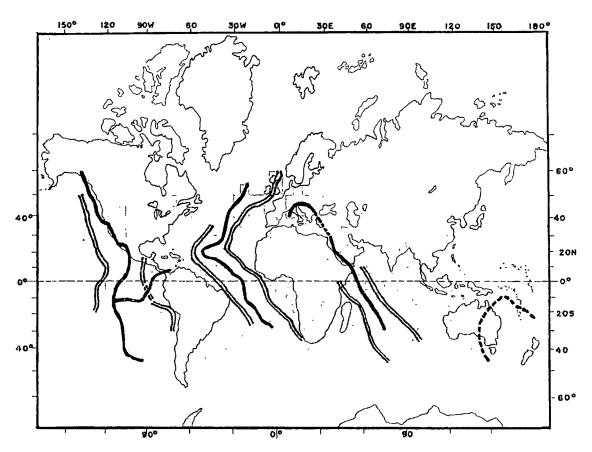


Fig. 4. Mercator's projection showing zones of anomalous heat flow. —, High heat flow; —, low heat flow.

limited angular extent. Consequently, convection would be confined to the upper few hundred kilometres of the mantle^{11,12}.

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Photogeological Investigation of the Mozambique Front in Tanzania

THE boundary between the Tanganyika Shield and the Mozambique Orogenic Belt in north-central Tanzania is being investigated by means of an initial photogeological study of an area of about 70,000 square miles, making use of the extensive reconnaissance mapping by the Tanzania Geological Survey.

Seventeen "tectonic domains" were established photogeologically and their distribution agrees with the known extent of the Tanganyika Shield and the Mozambique Orogenic Belt. The interpretation showed that it was possible to recognize photogeologically the following major features, most of which were already well known²⁻⁶: the general north-south trend of the Mozambique Belt and, in contrast, the east-south-east trend of many of the folds within it which strike across the Belt: the identity of the Tanganyika Shield, a vast extent of granite, with steeply dipping Nyanzian metasediments and a flat-lying cover of Bukoban (Proterozoic) rocks in the north; the boundary between the Shield and the Mozambique Orogenic Belt defined by steeply dipping marginal quartzites with northerly strike in the Serengeti; the ancient Dodoman and Nyanzian orogenic belts which strike east-south-east within the Shield; and the occurrence of probable basement rocks older than the Usagaran metasediments within the Mozambique Belt.

To amplify these observations and to make possible further photogeological and tectonic interpretations, we carried out field work at the end of 1966. Observations were made over 6,000 miles of traverse, and five areas lying across the boundary were examined in some detail (Fig. 1).

A transition zone, which lies between the (cratonic) Tanganyika Shield and the (orogenic) Mozambique Belt, takes a variety of forms which must reflect different tectonic environments along the margin. Thus in the north, flat-lying nappes of Usagaran metasediments approach the cataclastically deformed margin of the Further south, around Kondoa, a transition through zones of cataclasis, thrusting and of plastic deformation, shows beyond doubt that many of the gneisses previously thought to belong to the Usagaran System are tectonically derived from the pre-Usagaran basement.

The charnockites which occur at the margin of the Shield at Msagali7 were intruded by, and partially converted to, granite. This may correspond to the pre-Mozambiquian event which has tentatively been equated2 with the epoch of granitization in which the Shield developed as a megastructural unit. Our field work showed that the resulting complex was tectonized and converted into well foliated and rodded gneisses in the Mozambique orogeny. Farther south the Orogenic Belt consists entirely of Usagaran metasediments flanked by the much-discussed Konse System which may be analogous to the Umkondo System of Rhodesia,10.

Cahen¹¹ and Harpum³ have suggested that the Mozambique Belt may contain, and partly consists of, relics of rocks metamorphosed in earlier cycles, and this is confirmed by the recognition both photogeologically and on the ground of tectonized relics of basement rocks for up to 50 miles within the Belt. We do not consider that any of the charnockitic rocks we saw in the Mozambique Belt owe their high grade to metamorphism during the Mozambiquian orogeny.

The Mozambique Belt in Tanzania is largely a tectonic unit which owes its common characteristics to the effects of one dominant orogenic deformation. In view of the different opinions which have been expressed about the status of small scale structures near orogenic fronts, it is

interesting to observe that the main effect of the deformation is a preponderance of strongly developed linear structures such as tight, rodded, reclined folds, mullions and stretched conglomerates which plunge to the east-southeast and south-east. This axial direction, which is transverse to the general northerly trend of the orogenic belt, is

the main direction of transport and stretching. It is the

Usagaran metasediments Psammiles, petites and semi-petites Area in which some detailed mapping was done Quartzo-feldspathic gneiss Margin of volcanics Usagaran Reworked basement Granitoid Shield Konse Series Granite **a** 100 150 kms Same Market ťη $\boldsymbol{\omega}$ $\boldsymbol{\pi}$

Fig. 1. The Mozambique Front in central Tanzania, showing the relationship between the Usagaran metasediments, the Tanganyika Shield and the pre-Usagaran basement, which consists of tectonized gneisses of various origins.

common axis of the majority of small scale structures not only within this part of the Mozambique Belt but also within the cataclastically and plastically deformed margin of the granitoid Shield. This precludes the possibility that the east-south-east plunging structures belong to a pre-Mozambiquian east-south-east trending orogeny. These structures are mainly expressed on a mesoscopic scale, but some can be recognized photogeologically.

Folding on north-north-east axes overturned to the west occurs in comparatively small areas near to the orogenic front. This may be later than the structures

trending east-south-east.

There are noteworthy resemblances between the relationships which we have observed in Tanzania and those described by Sanders¹² in western Kenya at the junction of the Mozambique Belt and its foreland.

Photogeological interpretation of the selected areas and extrapolation of the results with the intention of constructing a tectonic map of the boundary region is continuing. We hope to be able to show the variations along, and to draw tectonic sections across, a larger section of an orogenic front than has previously been possible.

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ASTRONOMY

Possible Circular Polarization of Compact Quasars

WE wish to point out in this communication that both the radio and optical radiation from compact quasars may be circularly polarized. We consider here the situations in which this polarization is most likely to be detectable, and discuss the theoretical implications if it should be found.

The synchrotron radiation from a system of relativistic electrons moving in a uniform magnetic field of strength H gauss, with an isotropic distribution of pitch

angles, will be circularly polarized by $\sim 100 \left(\frac{v_L}{v_L}\right)^{\frac{1}{2}}$ per

cent^{1,2}, ν_L being the electron gyrofrequency (~3 H Mc/s): the precise value depends on the electron energy spectrum and on the observer's orientation relative to the field. Non-uniformities and self-absorption in the source would naturally diminish the observed degree of circular polarization.

In a typical radio galaxy with $H \sim 10^{-5}$ gauss, we would expect an unobservably small amount of circular polarization—≤ 0.1 per cent at ~100 Mc/s, and even less at higher frequencies. The percentage of polarization may, however, be much larger for compact quasars, the magnetic field of which (at least within the variable component) may be as great as between 1 and 100 gauss^{3,4} ($\nu_L \sim 3-300$ Mc/s). The radiation from these objects at, say, $\sim 3,000$ Mc/s could thus, in principle, he 3-30 per cent circularly polarized. The observed net polarization would, however, be reduced for three reasons. (a) The magnetic field in the compact component might be (b) Synchrotron self-absorption would non-uniform. destroy circular polarization within the compact component if the optical depth were large. (c) A contribution to the flux might come from a non-varying "halo" with a weaker magnetic field.

As regards (a), we note that the variable radio flux from 3C 273 is linearly polarized, so that the magnetic field in that case is probably fairly uniform. Moreover, the absence of linear polarization in other compact sources may be due to differential Faraday rotation, which would not affect circular polarization. To minimize the effects of self-absorption we suggest exploiting the proposal of several authors that the variable flux comes from a component which is expanding. If this idea is correct, the maximum flux is attained just when the expanding component starts to become transparent at the frequency of observation. Thus the consequences of (b) and (c) would simultaneously be minimized if observations were made at, or soon after, the time when the flux reaches a peak.

We conclude that the existence of at least about 1 per cent circular polarization in the radio emission from compact quasars is by no means unlikely. The chances of detecting it would be greatest if observations were made at a frequency where large amplitude intensity variations are known to occur (which restricts us to frequencies \gtrsim 1,000 Mc/s), at a time when the flux is just starting to decrease. If circular polarization were indeed observed it would provide evidence for the operation of the synchrotron process, and enable us to estimate the magnetic field strength directly. We would not, however, expect the observed degree of circular polarization to be exactly proportional to v-1 for the compact component would not contribute the same fraction of the total flux at different frequencies. Plasma radiation might also be circularly polarized in the presence of a magnetic field, but only in a narrow range of frequencies10.

We note finally that the optical continuum would not be circularly polarized if it is the result of synchrotron emission (unless the velocity distribution of the electrons had a very unlikely and anisotropic form). There is the alternative possibility, however, that the optical continuum results from inverse Compton scattering of radio synchrotron radiation^{3,4,11}, which may, as we have seen, possess significant circular polarization. Because inverse Compton scattering preserves polarization12,13, the optical continuum could then be circularly polarized by about 1 per cent. If the light from quasars were found to exhibit detectable circular polarization, this would therefore indicate that it arose from the inverse Compton process, and not from synchrotron emission.

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Possible Instability in the Self-closure Phenomenon in Gravitational Collapse

PRESENT ideas on the nature of relativistic collapse are largely derived from the study of spherisymmetric models, the only ones readily accessible to mathematical analysis. The most striking feature of spherical collapse—the formation of a critical surface ("event horizon"), characterized by infinite gravitational red-shift, which divorces the collapsing object from the external observer—is believed also to typify realistic situations, at least if the asymmetries are not too large. The purpose of this communication is to point out that, on the contrary, models with exact spherical symmetry possess idiosyncrasies which render them dangerous, and perhaps misleading, as a basis for induction.

Ten years ago, Regge and Wheeler analysed small perturbations of Schwarzschild's spherically symmetric field of a point mass m. A curious result emerged from their formulae. Static perturbations exist which remain finite down to the event horizon r=2m, but these are all induced by the presence of exterior masses^{2,3}. All asymmetric static perturbations caused by sources within r=2m (such as a quadrupole moment) become singular on r=2m. This result has recently been codified as a rigorous theorem4: among all static, asymptotically flat vacuum fields with closed simply connected equipotential surfaces $g_{00} = \text{constant}$, Schwarzschild's solution is the only one which has a non-singular event horizon $g_{00} = 0$. It would seem that other fields with event horizons similarly have an exclusive status. For example, we have proved that the Reissner-Nordström solution for a charged particle is the only static, asymptotically flat electrovac field with a regular event horizon, and there is reason to suspect that Kerrs' rotating particle solution is similarly distinguished in the class of stationary fields.

Let us now consider the collapse of an asymmetric (non-rotating) star, on the assumption that an event horizon is formed. Because of the slowing-down of processes for the external observer, it seems reasonable to suppose—and this can be supported by arguments based on small-perturbations analysis—that it is permissible to treat the limiting external field as static. The foregoing theorem on singular event horizons then applies. Before reaching a singularity at the event horizon, the star will respond to the precursory large tidal forces in such a way as to subdue, if possible, the rise of the singularity itself. There are two possibilities.

The first is that the star shakes off its quadrupole and higher moments and is, so to speak, patted into spherical shape before crossing the event horizon. Some such idea has been suggested previously³. The chief difficulty here is to find a plausible mechanism which acts locally (to avoid the delay resulting from retarded-potential effects) and which will accomplish this task in the finite proper time available.

The second possibility, which has not been considered before, is that the event horizon becomes smudged out and obliterated. One is inclined at first to dismiss this alternative because one does not normally expect the qualitative character of a phenomenon to be destroyed by an arbitrarily small perturbation. To entertain it seriously, one would first have to be convinced that there exists a fully general class of collapse regimes which initially deviate arbitrarily little from spherical symmetry and which do not exhibit self-closure. Now there is actually a theorem which, although more limited in scope, does lend considerable support to this idea. We confine ourselves to the class of static, axi-symmetric vacuum ("Weyl") fields. It can be proved that there exists a general class of Weyl fields which deviate arbitrarily little from Schwarzschild's solution for $r \ge (1 + \sqrt{(2)})m$, which are analytical except for a particle like singularity (well within the gravitational radius r=2m) and which are free of event horizons.

The proof is simple. We write the metric of a Weyl field in the standard form⁵

$$ds^{2} = e^{2(\nu-\lambda)} (d \rho^{2} + dz^{2}) + \rho^{2} e^{-2\lambda} d\phi^{2} - e^{2\lambda} dt^{2}$$
 (1)

Einstein's vacuum field equations then require that $\lambda(\rho,z)$ satisfy

$$\partial^2 \lambda/\partial \rho^2 + \rho^{-1} \partial \lambda/\partial \rho + \partial^2 \lambda/\partial z^2 = 0$$

which is formally Laplace's equation in cylindrical coordinates. (The function $\nu(\rho,z)$ is derivable from λ by a quadrature.)

We first choose for λ the formal expression for the Newtonian potential of a uniform thin rod of mass m placed along the axial segment $\rho = 0$, $-m \le z \le m$. In this case, equation (1) is known to be a disguised form of Schwarzschild's line-element², the Schwarzschild radial co-ordinate r being given implicitly by

$$\rho^2/r(r-2m)+z^2/(r-m)^2=1$$

Expanding λ in spherical harmonics, we have

$$\lambda = -\sum_{n=0}^{\infty} (m/R)^{2n+1} P_{2n}(\cos \theta)/(2n+1)$$
 (2)

with R and θ defined by $\rho = R \sin \theta$ and $z = R \cos \theta$. The infinite series in equation (2) diverges logarithmically on the "sphere" R = m (not really a sphere, for r is variable over it, ranging from 2m at the poles to $(1 + \sqrt{(2)})m$ at the equator). For R < m, equation (2) has therefore to be replaced by

$$\lambda = -\sum_{n=0}^{\infty} (R/m)^{2n} P_{2n} (\cos \theta)/(2n+1)$$
 (3)

It is the analytic continuation of equation (1) cum equation (3) within the "rod" (in reality the sphere r=2m) which gives rise to the peculiarities of the extended Schwarzschild manifold—in particular, the appearance of an event horizon $g_{00}=-\mathrm{e}^{z\lambda}=0$ for r=2m.

We consider next the potential function λ_N obtained by truncating the series in equation (2) at the Nth term (or, more freely, by changing the coefficients, from the Nth term onwards, in any manner such that the modified power series in (m/R) has an infinite radius of convergence). By taking N sufficiently large, we can make the Weyl field associated with λ_N differ as little as we please. from spherical symmetry for R > m. The extension within R=m, however, differs utterly from equation (3). modified series in equation (2) representing λ_N continues to be valid for R < m, and it describes a manifold which is regular down to a multipole singularity at R=0. The theorem is thus established, for there is no infinite redshift surface in this solution. Inward-falling probes and light-rays penetrate well inside the gravitational radius in a co-ordinate time t comparable with Newtonian predictions. In the immediate vicinity of R=0 their behaviour is more complicated. It is easy to verify that equatorial probes aimed at the singularity reach it in finite proper and co-ordinate time. On the other hand, the corresponding co-ordinate time for a probe falling down the axis is infinite; but it is very possible that the axis is unique in this respect, and that this anomaly will vanish entirely when the assumption of exact axial symmetry is relaxed.

If, now, we are prepared to admit that the exterior field of a collapsing, nearly spherical star might be broadly similar in structure to such a static field, then we are led to an unexotic picture of collapse and re-expansion, to which Newtonian theory should provide a tolerable first approximation. The multipole singularity of the static vacuum field should be precluded in the dynamical case by the asymmetry of the motion. (Penrose-type theorems? on the inevitability of singularities are inapplicable here, because "trapped surfaces" are absent, that is, light signals can be sent outwards to infinity from any point of space.) From the astrophysical point of view, such a picture would, of course, provide a natural explanation of several otherwise puzzling features (extended

life-times, quasi-periodic variability, compactness) of the quasi-stellar sources, and might also explain the continued activity of the supernova remnants in the Crab

The principal question left open is the extent to which results established for static fields can provide information at all relevant to an essentially dynamical phenomenon. For nearly spherical situations, it can be argued that, as far as the exterior vacuum field is concerned, the output of gravitational radiation is necessarily small, and therefore the non-static aspects cannot be important. It is well, however, to be cautious: recent results in the theory of gravitational waves8 have hinted at unsuspected subtleties in the transition between stationary and nonstationary states. A full dynamical analysis of asymmetric collapse remains a problem for the future. Only such an analysis can determine how far the considerations presented here are true.

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THE SOLID STATE

Permanent Magnet Properties of Platinum-Cobalt Alloys

Alloys of platinum-cobalt near the equiatomic composition can, by suitable heat treatment, undergo a disorder to order phase transformation which produces extreme magnetic hardness. Coercivities up to 6,800 oersteds have been observed at room temperature. Walmer¹ has recently given a comparative table of magnetic properties since the discovery of the alloys in 1936 by Jellinghaus2, in which the coercivities obtainable have risen from 4,000 to 4,300 oersteds (1961) and the energy product $(BH)_{\text{max}}$ from 4.6×10^6 to 9.5×10^6 gauss-oersteds. Walmer also includes a polar diagram which shows how the coercivity varies with crystallographic direction. A maximum of 6,800 is observed in <111> and 4,300 oersteds in <100>. Table 2 of Walmer's article lists the optimum magnetic properties as follows:

These properties have been further improved by Chaston³, who obtained an energy product of 10.3×10^6 gauss-oersteds, a remanence of 7,125 gauss and a coercivity of 4,430 oersteds.

The purpose of the present communication is to report work which has produced many more detailed results and considerably improved permanent magnet properties.

An alloy of platinum-cobalt containing 52.12 atomic per cent cobalt was prepared by melting the pure constituents in an argon arc furnace. The ingot was then homogenized in a vacuum furnace at 1,200° C for 12 h. The measured density of the resulting alloy was 15.55 g/cm³ which was in good agreement with that calculated by X-ray analysis.

A thin disk of the alloy (about 0.5 cm diameter \times 0.03 cm thick) was disordered by holding at 1,000° C for 0.5 h. It was then quenched and aged for 20 min at 695° C. The resulting magnetic properties are shown in the table below.

> $\begin{array}{lll} \mbox{Coercivity} & H_c & = 4,900 \mbox{ oersteds} \\ \mbox{Remanence coercivity} & H_R & = 5,200 \mbox{ oersteds} \\ \mbox{Remanence} & B_r & 5,280 \mbox{ gauss} \\ \mbox{Maximum energy product} & (BH)_{\rm max} & = 14.1 \times 10^{9} \mbox{ gauss-oersteds} \\ \end{array}$ All the measurements were made at 24° C.

(The remanence coercivity is the reverse field required to reduce the remanence to zero, whereas the intrinsic coercivity is the reverse field required to reduce the magnetization to zero while the field is still on.)

As far as I know this value of $(BH)_{\max}$ is the highest ever recorded for any permanent magnet material.

It should be emphasized that these properties were obtained by using a maximum magnetizing field of 15,000 oersteds. Because the uniaxial magnetocrystalline anisotropy of the ordered tetragonal phase is at least1 of the order of 2×10^7 erg/cm³ it is clear that this field would not be sufficient to produce saturation. (More recent measurements by Brissoneau et al.4 have shown that $K_1 \sim 5 \times 10^7 \text{ erg/em}^3$.)

Investigation of the properties of single crystals of the alloy which has been carried out in some detail⁵ has shown that there is considerable anisotropy of properties even when the specimen consists of a mixture of the cubic and tetragonal phases. Initially, of course, the specimen is a face centred cubic single crystal. Subsequent heat treatment produces platelets^{1,5,6} of the high anisotropy ordered tetragonal phase on (110) planes. The optimum permanent magnet properties obtained with the crystal ((112) orientation) are given in Table 1.

Table 1 Coercivity, H_σ 1111 1 110 1100 Coercivity, H_σ 3,500 oersteds 3,000 oersteds Remanence coercivity, H_R 3,800 oersteds 3,300 oersteds Remanence, B_r 6,065 gauss 4,050 gauss Energy product $(BH)_{\max}$ 11.0 × 10° gauss-oersteds 6.9 × 10° gauss-oersteds

More details of these properties, and in particular the temperature variation, from both an experimental and a theoretical standpoint have recently been discussed by McCurrie and Gaunt⁵ and Gaunt⁶.

From this work it seems very probable that the potential permanent magnetic properties of platinum cobalt alloys have not yet been fully realized. Further work will almost certainly lead to increases in the coercivity, but the most difficult problem is that of increasing the remanent magnetization-the absolute maximum is, of course, defined by the saturation magnetization of the cubic phase, that is, about 8,800 gauss depending on the cobalt content.

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Pre-yield Relaxation and the Yielding of Single Crystals of Iron

In tensile tests on polycrystalline iron at the usual rates of strain, if the process of strain is repeatedly interrupted for intervals of three minutes at stresses below the usual upper yield stress, the load immediately falls because of relaxation. If, after this, the process of strain is halted at roughly the upper yield stress of an uninterrupted test, there is no immediate relaxation; instead the load is maintained for a delay period of 2·3-90 sec, when there is sudden yielding and relaxation to the lower yield stress¹. I accounted for such a period without load drop by a reduction of the number of mobile dislocations (or of operative dislocation sources) by the series of relaxations. This explanation was strengthened by the observation that if after relaxation from a pre-yield stress, the stress is brought back to its original value by further straining, a further relaxation gives a smaller load drop. It was expected that single crystals would behave differently from polycrystalline material, and this has now been verified.

Results for two single crystals of iron, a crystal with 3.25 per cent silicon and a specimen of polycrystalline iron from the same heat as the iron crystals, are given in Fig. 1. The iron crystals (iron 99.97, carbon 0.0012, nitrogen 0.0016 per cent by weight) were grown by strain annealing and were cooled from 890° C in a furnace. The polycrystalline iron was furnace-cooled from 790° C. Both kinds of specimens were from stock and had been stored at room temperature for 4 yr. The iron-silicon crystal was of similar purity and was grown by cooling from the melt at 200° C/h.

The actual strain rate¹ in the pre-yield region was 1.7×10^{-5} sec⁻¹. Test pieces had an electrolytically formed gauge section with 4BA threads at each end. The relaxation of the machine is negligible, as is that of the threads of polyerystalline test pieces near the yield stress. It is unlikely that the relaxation of the threads with single crystal specimens is significantly different.

In Fig. 1 the stress drop resulting from relaxation is plotted against the increasing stress at which the tensile machine was stopped; where straining to the same

stress was used, the numbers indicate the sequence in which load drop values were obtained. Unlike polycrystalline specimens, single crystals do not show a period without load drop followed by sudden yielding; instead, the load drop on relaxation increases up to and through the usual yield stress (with a rapid increase near yield for iron-silicon). Repeatedly reloading crystals to the same stress just below the yield point only slowly reduces the stress drop on relaxation, in contrast with the behaviour of polycrystalline material, where the stress drop rapidly decreases (until the post-yield Lüders region is reached).

The behaviour of the single crystals accords with the view that: (i) in high purity iron and iron—silicon crystals, there are always dislocations which can move at stresses below the yield stress and which are either lightly pinned dislocations or dislocations produced at some favoured source; and (ii) at the yield stress these dislocations are able to multiply rapidly and produce general yielding. The results suggest a large increase in mobile dislocation density in the pre-yield region and this is borne out by normal tests on iron crystals, which demonstrate a decrease of the slope of the stress-strain curve before the abrupt decrease at the yield point. Because pre-yield relaxation is little reduced on reloading after relaxation to the same stress, it follows that although dislocations effectively come to rest during relaxation, the movement of dislocations readily starts again with increasing stress. By contrast, mobile dislocations in polycrystalline material are probably prevented from multiplying rapidly by grain boundaries, and multiplication is difficult to restart after relaxation, so that the movement of dislocations is much reduced when loading is begun again.

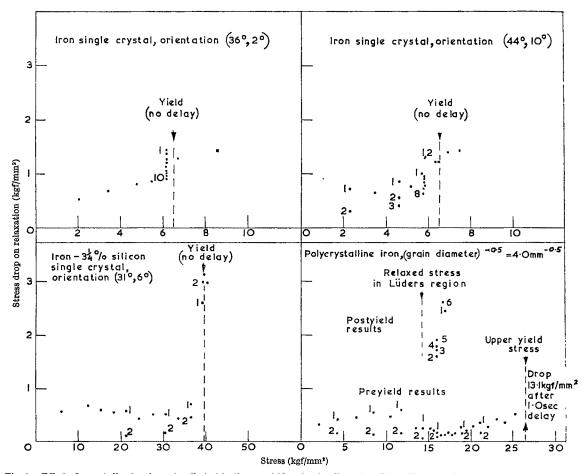


Fig. 1. Effect of repeatedly stopping a tensile test in the pre-yield region to allow relaxation. The stress drop resulting from relaxation is plotted against the increasing stress at which the tensile machine was stopped. Sometimes, after relaxation, the stress was increased to the same value as before relaxation; this was done as many as ten times, and the figures indicate the sequence of stress drop values for these cases.

repeated pre-yield relaxation in polycrystalline material, general yielding at the usual upper yield stress only obtains if either new dislocations (or dislocation sources) are unlocked, or if some critical amount of movement is attained in part of the dislocation network in yielded grains. This requirement leads to the observed delay before sudden yield.

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CHEMISTRY

Absolute Configuration and the Structure of Chlorophyll

THE structure of chlorophyll is known in considerable detail. The correct gross structure was put forward by Hans Fischer¹ and confirmed in a beautiful synthesis by R. B. Woodward^{2,3}; the relative configuration of the methyl and propionic ester groups on ring D was shown to be trans by Ficken, Johns and Linstead4; the stereochemistry and absolute configuration of the phytyl group was shown by Burrell, Jackman and Weedon^{5,6} to be 2^{r} -trans-7'R,11'R; and the relative configuration at C₁₀ was shown very recently by Wolf, Brockmann, Biere and Inhoffen^{7,8} to be that in which the methoxycarbonyl group is trans to the propionic ester side chain on C₇. (A recent review mentions the first X-ray crystallographic structure determination of a chlorophyll derivative, phyllochlorin ester. Success has come late in this field, because of disordered structures in the crystals of chlorophyll derivatives. The review gives no information about the absolute configuration.) The absolute configuration at C_7 and C_8 , however, was not known and there were therefore two diastereoisomeric structures still possible for chlorophyll. This report provides the missing information and completes the structure of chlorophyll-a (and therefore of chlorophyll-b) the configuration of which has been shown to be the same as that of chlorophyll-a).

In their work on the relative configuration of the groups on ring D, Ficken, Johns and Linstead⁴ oxidized phacophorbide-a and obtained (-)-trans-dihydrohaematinimide, (1) or (2), with $[\alpha]_{0}^{25^{\circ}}-46^{\circ}$, which they characterized as its benzylamine salt.

I have prepared the optically active imides (1) and (2) by resolution of synthetic, racemic imide¹¹ using the salt with (-)- α -methylbenzylamine¹² (for the (+)-imide) and with (+)- α -methylbenzylamine (for the (-)-imide). The (+)-imide had $[\alpha]_D^0+67^\circ$, which indicates that Linstead's sample had suffered a little racemization. The (+)-imide was hydrolysed to (+)-trans-dihydrohaematinic acid which crystallized (Linstead, no doubt partly because of the slight racemization, failed to obtain crystalline, optically active acid). The (+)-acid was converted into its tri-p-bromophenacyl ester, melting point $108.5^\circ-109^\circ$ C which was laevorotatory. I have prepared the enantiomer of this tri-p-bromophenacyl

ester from (-)- α -santonin (3) by the route illustrated, the chemistry of which need not be elaborated here. was because the acid (-)-(9), as prepared, was too impure to crystallize that the preparation of the tri-p-bromophenacyl esters was necessary. The derivative was separated from the other products of the ozonolysis by careful preparative thin-layer chromatography and obtained in very low yield but pure after one recrystallization. It was identical in melting point, infrared spectrum, thin-layer chromatographic behaviour and magnitude but not sign of rotation with the ester obtained before. When the esters (+)-(10) from santonin and (-)-(10) from the (+)-imide were mixed in equal quantities and recrystallized, pure racemic ester was produced, melting point 119°-120° C, identical in melting point, mixed melting point and infrared spectrum with an authentic sample prepared from racemic trans-dihydrohaematinic acid.

Because (-)- α -santonin has been shown with unusual thoroughness13-18 to have the structure and absolute configuration illustrated in (3), it follows that the (+)-imide has the absolute configuration (2). Because the (-)-imide is obtained from chlorophyll the absolute configuration of chlorophyll corresponds to that in the imide (1).

Chlorophyll-a therefore has the configuration 7S, 8S, 10R, 7'R, 11'R, and the structure (11).

Experimental details and a proof that two separate inversions, which could have led from santonin to the mirror image of (10), had not taken place will be published elsewhere.

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BIOCHEMISTRY

Evolution of Protamine: a Further Example of Partial Gene Duplication

Partial gene duplication has been suggested as an important mechanism in the evolution of proteins1 on the basis of a study of the repeating structure of the light $(\alpha -)$ chain of the human haptoglobin molecule controlled by the Hp^2 gene. Support for this hypothesis has come from the discovery in several unrelated proteins of homologous sequences of amino-acids. In ferredoxin from Clostridium pasteurianun, portions of the N-terminal and C-terminal halves are homologous2 and these halves of the molecule may have arisen by partial gene duplication from an intermediate nonadecapeptide which in turn could have been derived by the same processes of duplication from an archetypal tetrapeptide^{2,3}. Other examples of internal homology have been postulated in cytochrome C (ref. 3), the haemoglobin chains4 and the light and heavy chains of immunoglobins.

We wish to propose a mechanism for the evolution of the clupeines. the protamines of the Pacific Herring (Clupea pallasi), through a series of partial gene duplications indicated by the observed internal homologies in the molecules. When the three polypeptides (YI, YII and Z) are aligned as in Table I, they are seen to be closely related. The differences can be explained by the genetic mechanisms of amino-acid insertion, deletion and replacement by single base change⁹. Clupeines Z and YII are related by three mutational events, Z and YI by eight, and YI and YII by eleven. In the evolution of the clupeines an initial divergence of the Z and YI sequences after a complete gene duplication appears to have been followed by a more recent divergence between the Z and YII sequences by the same mechanism10.

Examination of the sequences of the clupeines shows a repeating occurrence of the sequence Neu-Arg-Arg-Arg-Arg where Neu is a neutral amino-acid. If protamine has evolved from such an ancestral pentapeptide the codons' of the neutral amino-acids occurring in the first position favour the ancestral sequence Ala-Arg-Arg-Arg-Arg. A more comprehensive examination for internal homology was carried out using a computer programme similar to that described by Fitch¹¹. By this means all pentapeptide sequences which are related by one or less base changes were obtained. This value is considerably lower than that required in a similar examination of the haemoglobin chains because the clupeines are small molecules with a

Table 1. SEQUENCES OF HERRING SPERM PROTAMINES (CLUPEINES)6-8 Clupeine YI Ala-Arg-Arg-Arg-Arg-Ser - - Ser-Ser-Arg-Pro-Ile-Arg-Arg-Arg-Arg Clupeine Z Ala-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Ala-Ser-Arg-Pro-Val-Arg-Arg-Arg-Arg Clupeine YII Pro-Arg-Arg-Arg — Thr-Arg-Arg-Ala-Ser-Arg-Pro-Val-Arg-Arg-Arg-Arg ${\bf Pro\text{-}Arg\text$ \mathbf{z} Pro-Arg-Arg - Val-Ser-Arg-Arg-Arg-Arg-Ala - Arg-Arg-Arg-Arg

high arginine content. For elupeine \mathbf{Z} , the average number of base changes for a random comparison of pentapeptide sequences is 3.5, whereas the corresponding figure for the Of the three clupeines, haemoglobin chains is 7.25. clupeine Z showed the greatest degree of internal homology and these homologous sequences, represented as two series of overlapping pentapeptides, and their relationship to the complete clupeine Z polypeptide chain are indicated

On the basis of these findings it is possible to propose several models for the evolutionary pathway from the ancestral polypeptide Ala-Arg-Arg-Arg-Arg to clupeine Z, and the one which seems to involve the minimum number of mutational events is presented in Fig. 2. We propose that there have been four successive partial gene duplications of different lengths. The first duplication was of five residues (Ala-Arg-Arg-Arg-Arg) leading to a decapeptide, and the second duplication involved a sequence of eight residues (Ala-Arg-Arg-Arg-Arg-Ala-Arg-Arg) and resulted in an octadecapeptide. In the next step the chain is shown in Fig. 2 as being lengthened by a

single Ala at position 9 or 10 as a result of a partial duplication limited to one residue. There is another alternative for this step, that is the second duplication could have been of nine residues leading to a nonadecapeptide followed by a two step mutation at position 9 from Arg to Ala (ref. 9). It is not possible to assess the relative probabilities of these two mechanisms. After this Ser could be obtained from Ala at position 10 by a single base change. A fourth duplication, this time of twelve residues (Ala-Arg-Arg-Arg-Arg-Ala-

Arg-Arg-Ala-Ser-Arg-Arg), could then have occurred and the full length (thirty-one residues) of present day clupeine Z would have been reached. The resultant polypeptide differs by only five single point mutations from elupeine Z, at positions 6, Ala-Ser, 12, Arg-Pro, 13, Ala-Val, 18, Ala→Pro and 21, Ala→Val, all of which can occur by a

single base change within the coding triplet.

Before the occurrence of this last single base change (position 21) a complete gene duplication could give rise to the ancestral clupeine YI which then evolved by single base changes at position 9, Ala—Ser, 13, Val—Ilc, 21, Ala-Thr, the deletion of Arg-Arg, 7 and 8, and the insertion of Arg between positions 20 and 21 and Gly between positions 27 and 28 to give rise to present day clupeine YI. Clupeine YII can be derived from clupeine Z by the deletion of an Arg between positions 2 and 5 and two single point mutations at positions 1, Ala-Pro, and 6, Ala-Ser.

Three of the possibly significant comparisons shown in Fig. 1 have not been used in this evolutionary pathway. These are considered to be a consequence of the high arginine content and small size of the clupeines, which

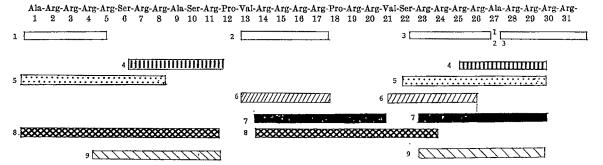


Fig. 1. The relationship of segments of amino-acid sequence of clupeine Z which were determined by a computer comparison to show significant homology. Segments indicated by the same number (and similar shading) are related by less than 0·2 base changes/amino-acid codon. Segments 4, 6 and 9 were not used in deriving the evolutionary pathway proposed in Fig. 2 for the reasons discussed in the text.

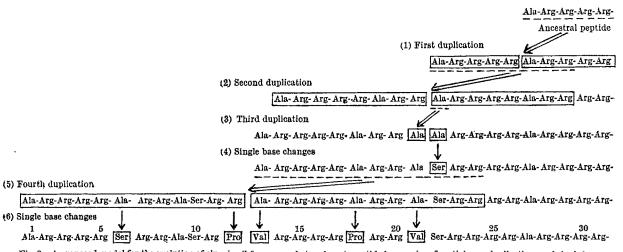


Fig. 2. A proposed model for the evolution of clupsine Z from an archetypal pentapeptide by a series of partial gene duplications and single base changes in the structural gene for this protein. The segment of polypeptide underlined corresponds to that portion of the structural gene which is partially duplicated and the resulting new polypeptide sequence is enclosed in a box. Single arrows represent single base changes while the double arrows show the partial gene duplications.

mean that a statistical search for homology will yield a proportion of comparisons which are not in accordance with any proposed pathway. The evolutionary pathway which we propose is based not only on the small number of base changes involved in each comparison but also on the minimal number of mutational events required to obtain the present day clupeines from the ancestral pentapeptide Ala-Arg-Arg-Arg-Arg.

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Kinetics of RNA Labelling in Fractions enriched with Neuroglia and Neurones

THERE are now methods of separating in bulk neuronal and neuroglial cells1,2, and we have used them to study the in vivo synthesis of RNA in these cellular fractions. There seem to be significant differences between the kinetics of labelling of the RNA of the two cell types.

6-14C-orotic acid (44.5 mc./mmole) was given subarachnoidally to adult rabbits weighing 3-4 kg in a dose of 100 $\mu c.$ in 0.2 ml. RNA was extracted with a mixture of sodium dodecyl sulphate and phenol at 4°, followed by re-extraction of the phenol phase and interphase at 45° C, digested with DNase and analysed by centrifugation in 5-20 per cent sucrose gradients containing 0·1 molar sodium chloride and 4 molar urea (15 h at 20,000 r.p.m. in the SW25 rotor of a Spinco ultracentrifuge at 0°-4° C). The results are summarized in

One hour after the injection of the precursor neuronal RNA had a lower specific activity than neuroglial RNA, while after 3-6 h it was much more heavily labelled. These differences were more clearly seen in the cellular fractions prepared by the method of Satake and Abe² (Fig. 1a and b). Some of the most obvious differences were observed in the RNA fractions which sedimented before the 28S peak. These fractions represent a mixture of pre-ribosomal components and of DNA-like nuclear RNA of unknown function³ which is synthesized more rapidly than the other types and is more rapidly degraded4,5. The relative composition of neuronal RNA rich in the ribosomal species and of glial RNA rich in the nuclear components may be the basis of the different kinetics of labelling which we observed. Other explanations, however, cannot be excluded.

Fourteen hours after administration of labelled orotic acid the specific activity of neuronal RNA prepared by the method of Satake and Abe² decreased considerably with little concomitant change in the specific activity of the RNA extracted from the heterogeneous non-neuronal fraction (Fig. 1b). This might be explained by migration of neuronal RNA into cellular compartments not recovered with the nerve cell bodies, such as axons and nerve endings. The corresponding increase in the

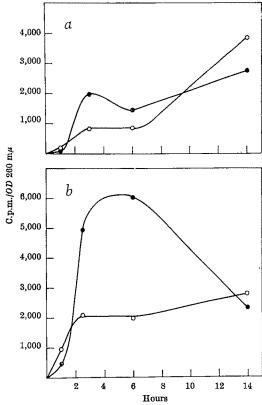


Fig. 1. Kinetics of labelling of total RNA in fractions enriched with neurones (a) and neuroglia (5). (a) Method of Rose; (b) method of Satake and Abe. Optical densities and radioactivity refer to data obtained by integration of sucrose gradient patterns.

specific activity of neuroglial and to a smaller extent neuronal RNA prepared by the method of Rose¹ (Fig. 1a) might reflect contamination of these fractions with such particulates.

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Evidence for the Random Aggregation of Sub-units to produce Multiple Forms of Lactate Dehydrogenase in the Brains of Rat and Man

LACTATE dehydrogenase (LDH) activity is present from the earliest stages of animal development and has been detected in the oocytes of both vertebrates1 and invertebrates2. It is now recognized that the enzyme is composed of two types of sub-unit3, with separate structural genes4, which probably combine randomly into tetramers forming the five electrophoretically distinct forms, H_4 , H_3M , H_2M_2 , These have been numbered LDH-1 to 5, respectively, with LDH-1 being the most electropositive.

The proportions of the LDH forms have been observed to change during the development of various tissues from rat⁵, chick^{6,7}, rabbit⁸ and human^{5,9}, which suggests that the two structural genes are under independent although possibly linked control. Attention has been directed chiefly towards liver and skeletal muscle in which the proportions of the more slowly migrating, M-containing enzyme forms increase during development, and towards heart where the proportion of H-sub-units increases during development.

In adult brain LDH-1 to 3 appear to be the dominant forms^{7,10-13} although a comparison of various primates shows that this is not always the case¹⁴. We have studied the developmental changes in the lactate dehydrogenases of rat brain between day 16 of gestation and 18 days post-partum, and human brain between 5 weeks and 20 weeks of gestation. The human foetuses were obtained either by surgical induction or from spontaneous abortions. As soon as the tissues became available they were homogenized in ice cold isotonic saline (0.5 g/2 ml.) and analysed by horizontal starch gel electrophoresis using the discontinuous buffer system of Poulik16. The enzyme was stained with a mixture of NAD+, sodium lactate, phenazine methosulphate and nitro blue tetrazolium16 and quantitative estimates of the colour intensity in the gel were made with a Joyce 'Chromoscan' reflectance densitometer with integrator using a yellow-brown filter (Joyce 5021). Preliminary experiments determined the range over which intensity of reduced nitro blue tetrazolium in the gel was proportional to lactate dehydrogenase activity. In both rat and human, the enzyme patterns in cerebrum and cerebellum were the same at all the stages of development we investigated.

Rat brains from foetuses of 16 days gestation and older showed all five LDH forms (although under the conditions of electrophoresis used LDH-5 often lay partly within the origin). At 16 days LDH-5 is the most prominent form and LDH-1 the least prominent. Up to parturition LDH-5 progressively decreases to about 10 per cent of the original value while LDH-1 increases slightly (Fig. 1). LDH-2 and 3 also increase but LDH-4 decreases slightly. By about 14 days post-partum the proportion of the five forms is close to the 1:3:6:3:1 ratio expected from random association when H and M-type sub-units are being synthesized in equal amounts (Fig. 1). In the adult rat brain (Fig. 2), there is slightly more LDH-1 and 2 and less LDH-5, suggesting that the H-type sub-units are being synthesized faster than the M-type sub-units.

In contrast to the rat, the human brain presents a very static pattern of LDH forms from 5 weeks gestation, at which point the neural tube is not completely closed, up to 20 weeks—the maximum age at which foetuses were obtainable—and is quite similar to that of the adult (Fig. 3). Over this period the ratio of the five forms is very close to 1:3:6:3:1 (Table 1) suggesting that both sub-units are being produced in equal amounts and then

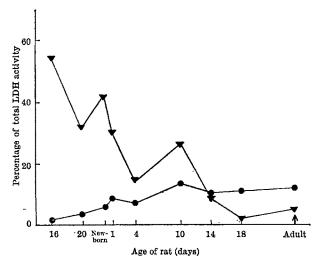


Fig. 1. Time course of the change in concentrations of LDH-1 (●——●) and LDH-5 (▼——▼) in the developing rat brain.

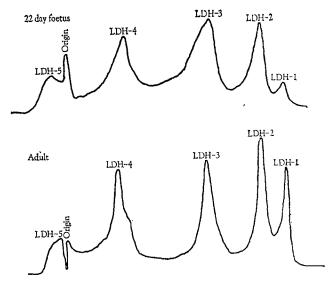


Fig. 2. Comparison of densitometer tracings of the multiple forms of lactate dehydrogenase from adult and 22 day foctal rat brain to show the relative increase in LDH-1 with increasing maturity.

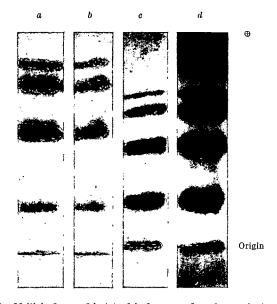


Fig. 3. Multiple forms of lactate dehydrogenase from human brain at different stages of development showing the similarity in general overall pattern between adult (a) and 15 week gestation (b), and between 5 weeks gestation (c) and adult (d).

combining in a random manner. There seems to be a slight change between 20 weeks and adulthood, however, when the concentration of LDH-1 increases about two-fold and LDH-5 and 4 fall to lower levels. This latter change was found to be rather variable and may possibly reflect the inevitable delay between death and obtaining samples. LDH-1 is very labile and considerable activity

Table 1. PROPORTIONS OF THE MULTIPLE FORMS OF LACTATE DEHYDROGENASE IN DEVELOPING HUMAN BRAIN

No. of weeks of	Per			DH for				f LDI		
gestation	1	2	3	4	5	1	2	3	4	5
.5	8.5	29.5	51	11	-	1.0	3-4	6.0	1.3	
10	10	21.5	35.5	28	5	1.4	3.1	5-1	4.0	0.7
12	9.2	17.2	36.8	21.8	15	1.4	$2 \cdot 8$	5.8	3.5	24
13	7.4	23.8	39.2	24.6	5	1.0	$3 \cdot 3$	5-6	3.4	0.7
13	8	22	38	27	5	1.1	$3 \cdot 1$	5.3	3.7	0.7
14	5	23	42	22	8	0.7	3.2	5.8	$3 \cdot 1$	1.1
16	9	24	42	22	3	1.3	3.3	5.8	3.1	0.4
20	12.5	26	40	21.5	******	1.5	3.2	5.0	2.7	-
20	5.9	20	40.1	27	7	0.8	2.7	5.6	3.7	1.0
					Mean	1.13	3.1	5-6	$3 \cdot 2$	1.0
Adult	17	28	42.5	11.5	1	2.4	3.9	5.9	1.6	0.1
Adult	15	30	40	15	0	$2 \cdot 1$	$4\cdot 2$	5.5	$2 \cdot 1$	0

is lost by overnight storage in the refrigerator. Indeed, Latner and Skillen⁹ were unable to detect any LDH-1 and 2 in foetal brain while Pfleiderer and Wachsmuth¹² found a higher proportion of LDH-1 in adult and foetal brains than any recorded in our experiments.

In conclusion, the developmental patterns of brain lactate dehydrogenase are quite different in the rat and the human, and the human resembles the chicken' more than it does the rat. Whether or not there is any significance in the developmental patterns in terms of enzyme function, as has been suggested in relation to muscle activity¹⁷, is not at present clear. The results are in accord with the inference⁵ that the multiple forms of lactate dehydrogenase arise by random aggregation of the H- and M-type sub-units.

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Effect of Alloxan Diabetes, Starvation and Refeeding on Glycolytic Kinase Activities in Rat Epididymal Adipose Tissue

THE levels of glucokinase, phosphofructokinase (PFK) and pyruvate kinase (PK) in the liver are decreased in alloxan diabetes and starvation, and are increased on refeeding¹⁻³. Weber $et\ al.$ have postulated that glycolysis may be, at least partially, controlled by the synchronous induction and repression of these regulatory enzymes through the action of hormones on a "functional genome" 4. Changes of similar magnitude have not been observed in the heart with hexokinase (HK) (ref. 5) and PFK (ref. 6), but in epididymal fat pad, HK activity is markedly affected by these conditions^{7,8}. The observed sensitivity of the PK in the fat pad to the allosteric effector fructose 1,6-diphosphate (Pogson, unpublished results), and the known kinetic properties of the PFK in the fat pad, support the suggestion that these enzymes are of significance in the control of fat pad glycolysis. In view of these findings, it seemed important to determine whether the levels of all three kinases were responsive to conditions of alloxan diabetes, starvation and refeeding.

Chemicals were obtained from sources described previously^{10,11}. All enzymes were obtained from Boehringer Corporation (London), Ltd. Male albino Wistar rats (150-200 g) were used throughout and the diet, unless otherwise stated, was as given by Newsholme and Randle¹². Acute alloxan diabetes was induced by intravenous injection 48 h previously of 60 mg of alloxan/kg. High blood sugars (>300 mg per cent) were confirmed with

'Dextrostix' (Ames Co., Slough). In the starvationrefeeding experiments, animals were starved for 3 days, then fed for a further 3 days on a powdered diet consisting of one part fat free milk extract (J. Sainsbury, Ltd.) and two parts castor sugar; controls were fed a powdered normal diet for the first 3 days and then high carbohydrate diet.

Rats were killed by decapitation. The pairs of epididymal fat pads were rapidly excised, washed briefly in the extracting buffer at 22° C, blotted gently, weighed and extracted once with 3 vol./g of 50 mmolar tris-acetate buffer, pH 7.4, containing 50 mmolar magnesium sulphate and 2 mmolar EGTA (ethylene glycol-bis (2-aminoethyl)tetraacetate) in a hand-operated glass Potter-Elvehjem homogenizer at 0° C. The homogenates were centrifuged at 2,800g for 20 min at 2° C, and the supernatants were then decanted and kept at 0° C.

All enzymes were assayed in the supernatants within 8 h of extraction by following the changes in extinction at 340 mµ through a 1 cm light path in a Hilger-Gilford recording spectrophotometer at 25° C. In all cases, activity was measured in 50 mmolar tris-acetate buffer, pH 7·4, containing 50 mmolar potassium chloride, 15 mmolar potassium sulphate and 5 mmolar magnesium sulphate; final volume 3.0 ml. HK was assayed by following NADP reduction in the presence of 10 mmolar glucose, 5 mmolar ATP and excess glucose-6-phosphate dehydrogenase; PFK by following NADH oxidation in the presence of 1 mmolar glucose-6-phosphate, 2 mmolar ATP and excess phosphoglucose isomerase, aldolase, triosephosphate isomerase and α-glycerophosphate dehydrogenese; and PK by following NADH oxidation in the presence of 1.5 mmolar phosphoenolpyruvate, 2 mmolar ADP, 0.3 per cent bovine serum albumin and excess lactate dehydrogenase. In addition, as a control activity, aldolase was assayed in a similar medium containing 2 mmolar fructose-1,6-diphosphate and excess triosephosphate isomerase and α-glycerophosphate dehydrogenase. One unit of kinase is defined as that amount catalysing the formation of 1 µmole of product/min at 25° C. One unit of aldolase is taken as the amount catalysing the disappearance of 1 µmole of fructose-1,6diphosphate/min at 25° C.

Preliminary control experiments demonstrated that all activity is extracted by this procedure, that enzyme activities in supernatants remain unchanged during the course of experiments and that maximal activities are obtained with the substrate concentrations stated previously. Simultaneous blank determinations were carried out for PFK (minus glucose-6-phosphate) and aldolase (minus fructose-1,6-diphosphate); NADH oxidase activities were about 10 per cent and 1.5 per cent, respectively. PK blanks were insignificant; in HK assays added NADPH was not oxidized. For calculation of the HK activity, we assumed that 2 moles of NADP were reduced for each mole of glucose phosphorylated, because (a) extracts contained 6-phosphogluconate dehydrogenase in excess of HK activity, and (b) rates in HK assays were not increased by addition of a 1,000-fold excess of purified 6-phosphogluconate dehydrogenase. Protein concentrations were determined by the method of Lowry et al.13, with bovine serum albumin as standard.

The results of these experiments are given in Table 1. The differences shown in enzyme activities between control rats in alloxan diabetic and starvation-refeeding experiments may be attributed to variations between batches of animals from different sources. Activities are expressed in terms of units per fat pad pair and per mg protein; results expressed in terms of tissue weight are misleading in that the reduction in fat pad weight in alloxan diabetes and starvation may be largely attributed to fat mobilization under these conditions. The lowest activities in the normal rats are those of HK and PFK, which varied in different experiments from 9.7 to 36.4 mu/mg protein and 15.8 to 48.4 mu/mg protein, respectively.

Table 1. Effects of alloxan diabetes, starvation and refereding on the activities of hexokinase, phosphofructokinase, pyruvate kinase and aldolase in rat epididymal adipose tissue

Experiment	No. of obser- vations		kinase ndard error mu/mg of protein		ructokinase .ndard error mu/mg of protein	Pyruvat mean±star mu/fat pad pair			olase ndard error mu/mg of protein
Control rats, normal diet Alloxan diabetic rats, normal diet Control rats, powdered normal diet 3-day starved rats Control rats, powdered high carbohydrate	8 9 8 8	150 ± 12 93 ± 4* 126 ± 10 78 ± 9*	23·5±2·4 13·6±1·3* 18·0±1·8 9·7±0·9*	$\begin{array}{c} 141 \pm 12 \\ 123 \pm 19 \\ 219 \pm 17 \\ 190 \pm 18 \end{array}$	$\begin{array}{c} 21 \cdot 6 \pm 1 \cdot 5 \\ 17 \cdot 9 \pm 1 \cdot 1 \\ 31 \cdot 3 \pm 3 \cdot 3 \\ 23 \cdot 5 \pm 1 \cdot 9 \end{array}$	$\begin{array}{c} 1,637 \pm 159 \\ 1,521 \pm 263 \\ 3,220 \pm 202 \\ 3,644 \pm 293 \end{array}$	$\begin{array}{c} 250 \pm 21 \\ 212 \pm 23 \\ 470 \pm 45 \\ 454 \pm 32 \end{array}$	324 ± 26 265 ± 26 495 ± 37 443 ± 33	50.5 ± 4.0 40.3 ± 5.2 70.1 ± 6.9 55.2 ± 3.6
diet (3 days) Starved rats, refed powdered high	8	206 ± 13	21·9 ± 1·7	285 ± 19	30·0 ± 1·9	$6,322 \pm 633$	663 ± 62	755 ± 66	79·7 ± 7·4
carbohydrate diet (3 days)	8	180 ± 22	19.8 ± 2.2	261 ± 22	29.0 ± 2.5	$5,755 \pm 555$	643 ± 66	675 ± 63	74.6 ± 6.1

Conditions of diet and treatment are given in the text. * P < 0.005. For other differences, P > 0.025.

compare with maximum uptakes of glucose in fat pads from rats fed normally on approximately 10 mumoles/mg protein/min, as calculated from known data¹⁴. Total HK activity is decreased approximately 40 per cent in alloxandiabetes and starvation. These figures are in close agreement with previously published data7,8. In contrast to the changes in HK, levels of PFK, PK and aldolase are not depressed to any statistically significant extent. Refeeding after starvation restores HK activities to control values in experimental animals. The levels of both HK and PK in the controls fed the high carbohydrate diet are markedly increased over those of the controls on the normal diet, while smaller changes are apparent in PFK and aldolase activities. The physiological importance of the changes in HK activity in epididymal fat pad have been considered previously^{7,15}. Ballard et al. 16 have recently reported the occurrence of phosphoenolpyruvate carboxykinase in this tissue, and have demonstrated the conversion of pyruvate to glyceride-glycerol through the dicarboxylate shuttle. It is most probable therefore that PK has a regulatory role in determining the fate of phosphoenolpyruvate in fat pad. The changes observed with high carbohydrate diet are consistent with this hypothesis, and may thus be of some physiological significance.

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Fatty Acid Ester-Hydrocarbon Correlation Trial: a Comment

DURING the sixth International Symposium on Gas Chromatography in Rome¹, Dr P. A. T. Swoboda, on behalf of the Data Sub-committee of the Gas Chromatography Discussion Group, presented a preliminary report on an inter-laboratory trial on the reproducibility of retention data, which gave the following conclusion: "... the use of the carbon number scale leads to a high degree of agreement between laboratories for the methylesters of unsaturated fatty acids. In contrast there is a wide variation for the hydrocarbons. Thus the use of a secondary series of standards (such as the methylesters of the n-alkanoic acids) as the basis for a logarithmic scale of retention gives a higher inter-laboratory precision for measurement of compounds of similar structure than would be obtained by using the Retention Index based on the n-alkanes".

In my opinion the data presented do not justify this conclusion. Only esters have been compared with hydrocarbons and extension of the generalization to other classes of compounds does not seem justified without more data.

Another point to be considered is the stationary phase used (polyethylene glycol adipate, PEGA). Each participant used his own batch of PEGA and the reporter observed that the results of participants whose PEGA was either too polar or too apolar were not included in the analysis. Moreover, no mention is made of the amount of stationary phase nor of the support used. I feel that this indicates that different batches of PEGA differed in polarity or at least that the total columnpackings differed in polarity. Furthermore, PEGA is also an ester and hence similar to the compounds chromatographed.

In my opinion, the wide range of carbon numbers, as displayed by the n-alkanes, reflects the differences in polarity between the column-packings, this reflexion being less for the esters because of the similarity between the solutes and solvent (PEGA).

I should also like to comment on the way the carbon numbers were calculated. It is formally stated that "the algebraic equation defining the scale is the same as that used for the calculation of Retention Index". For the calculations, however, a straight line was fitted statistically instead of using the actual measurements. In the case of the n-20-alkane even extrapolation was used. This method of calculation introduces an error unnecessarily. Although the statistical procedure permits confidence limits to be drawn, no such limits are mentioned. In view of the fact that in the equation given in the report, both constants are related, I believe that in fact $r_{x_{17}}$ values, not carbon numbers, were determined.

Apart from the comment on the calculation, my principal point is that there is a need for some kind of characterization of columns with respect to "polarity", especially in trials of this kind.

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¹ Sixth Intern. Symp. Gas Chromatog., Rome 1966 (edit. by Littlewood, A. B.) (1967).

Influence of Acute and Chronic Administration of Alcohol on Carbohydrate Breakdown and Energy Metabolism in the Liver

AFTER a single dose of ethanol, changes in the contents of metabolites of the Embden-Meyerhof glycolytic pathway in the liver, which indicate that the degradation of carbohydrates through the first steps of this pathway is accelerated, can be observed. These changes are caused partly by the effect of catecholamines (unpublished work), which are liberated in excess from the adrenals1-4, and partly by the enhanced formation of reduced nicotinamide-adenine dinucleotide (NADH₂) during the oxidation of ethanol⁵. Until now the influence of a prolonged intake of alcohol on the metabolites of carbohydrate breakdown and energy metabolism has not been investigated. Because we thought that there might be differences in the effects of ethanol after acute and chronic administration, as a consequence of adaptational processes, we investigated the contents of glycogen, glucose, glucose-6-P, fructose-1·6-P₂, dihydroxyacetone-P, glycerol-1-P, pyruvate, lactate and ATP in the livers of animals which over a period of 6 months had received a 15 per cent solution of alcohol instead of drinking water.

To study the effect of a sudden withdrawal of ethanol on the metabolite contents of the liver of animals which have been adapted to alcohol, we treated animals with ethanol for 6 months and determined the metabolite contents of the liver 4 h after the alcohol had been withdrawn. At this time alcohol in the blood was no longer detectable.

detectable.

All experiments were carried out at an environmental temperature of 24° C on female NMRI-mice which had free access to a standard diet (Altromin R; Altromin GmbH, Lage/Lippe, Germany) before and during the experiments.

One group of animals was intravenously injected with 1.5 mg/g ethanol and the metabolite contents in the liver were estimated 30 min later. A second group of animals received a 15 per cent (w/v) solution of alcohol ad lib. instead of drinking water for 6 months. The animals were then killed to determine the metabolites in the liver. A third group of animals was treated with ethanol in the same manner as the second group, but 4 h before the animals were killed the alcohol was withdrawn and replaced by tap water. Groups of untreated animals of the same age served as controls.

In order to investigate the metabolites in the liver, the animals were killed by immersing them in liquid air, and the livers were removed while still frozen. Blood was collected from animals which were killed by decapitation. The analytical procedures for the assay of bloodalcohol, glycogen, glucose, glucose-6-P, fructose-1-6-P₂, dihydroxyacetone-P, glycerol-1-P, pyruvate, lactate and ATP were the same as described in previous papers^{5,6}. Mean values and their standard errors were calculated from 10–15 individual values. The results were checked by the Student's t test and differences between two mean values were regarded to be significant if $P \leq 0.05$.

Thirty minutes after the intravenous injection of 1.5 mg/g ethanol, the blood alcohol level was 2 %/00. Glycogen, glucose and pyruvate contents in the liver had decreased; fructose-1.6-P2, dihydroxyacetone-P and glycoerol-1-P had increased, while the contents of glucose-6-P and lactate had not changed significantly. The decrease of the glycogen content is regarded as the result of an enhanced glycogen breakdown caused by the effect of catecholamines which are mobilized from the adrenal medulla by alcohol¹⁻⁴. The increase of fructose-1.6-P2 and dihydroxyacetone-P points to an accelerated degradation of carbohydrates through the first steps of the glycolytic pathway. The enhanced formation of NADH2, resulting from the dehydrogenation of ethanol, promotes

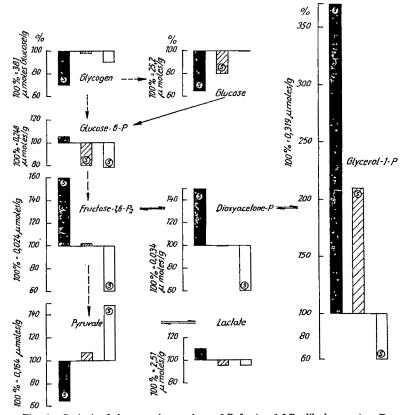


Fig. 1. Contents of glycogen, glucose, glucose-6-P, fructose-1-6-P₂, dihydroxyacetone-P, glycerol-1-P, pyruvate and lactate in the liver of white mice: \blacksquare , 30 min after intravenous injection of 1-5 mg/g alcohol; H, 6 months after alcohol instead of drinking water; \square , 4 h after withdrawal of ethanol. Encircled 'S', $P \le 0.05$.

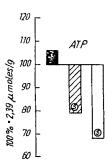


Fig. 2. Contents of ATP in the liver of white mice: \blacksquare 30 min after intravenous injection of 1.5 mg/g alcohol; //, 6 months after alcohol instead of water; \square , 4 h after withdrawal of ethanol. Encircled 'S', $P \le 0.05$.

the conversion of dihydroxyacetone-P to glycerol-1-P, a reaction in which NADH, is oxidized. On the other hand, the oxidation of 3-phosphoglyceric aldehyde to 1.3diphosphoglyceric acid, which is linked with the reduction of NAD to NADH2, is probably reduced. This is indicated by the low level of pyruvate, which forms the final product of this metabolic chain. It must be remembered, however, that the pyruvate content may also be lowered as a result of the raised ratio of NADH2 to NAD in the cytoplasm, which promotes the conversion of pyruvate to lactate. According to Bücher and Klingenberg⁷, shifts in the NADH₂ to NAD ratio are indicated by the ratios of glycerol-I-P to dihydroxyacetone-P and lactate to pyruvate.

The excess glycerol-1-P that is formed is required for the synthesis of triglycerides, which is enhanced under the influence of alcohol⁸, as well as for the transport of hydrogen, which is derived from the oxidation of ethanol, from the cytoplasm into the intramitochondrial compartment7. The more than 2.5-fold increase of glycerol-1-P shows that the synthesis of glycerol-1-P by far exceeds the requirements.

After prolonged intake of alcohol for 6 months, the food consumption of the mice decreased from 137 ± 9 to 84 ± 8 mg/g body weight/day and liquid consumption decreased from 0.139 ± 0.005 to 0.079 ± 0.003 ml./g body weight/day. Blood alcohol levels were found to be between 0.8 and $1.8~^{\circ}/_{\circ \circ}$ depending on the hour of the day. In the liver only the glycerol-1-P content was elevated and only glucose and glucose-6-P contents were decreased. contents of the other metabolites investigated did not differ significantly from their control values. The decrease of glycogen content, found after a single dose of alcohol, cannot be observed in the chronically treated animals. This finding, which contrasts with the results of Mirone⁹, may be caused by an adaptation of the rate of glycogen formation to glycogen breakdown. The decrease of glucose and glucose-6-P content in the liver, while fructose-1.6-P2 and dihydroxyacetone-P remain unchanged, suggests that the first steps of the glycolytic pathway are accelerated in chronically treated animals as well as in animals which have had a single dose of ethanol. Though there was no rise in the fructose-1.6-P2 and dihydroxyacetone-P contents, the glycerol-1-P content was still elevated. Just as in the acute experiments this is probably due to the increased supply of NADH₂.

In contrast to the results in acutely treated animals, there is no evidence that in chronically treated mice the rate of glycolysis is slowed down between dihydroxyacetone-P and pyruvate. This suggests that in the enzyme systems involved adaptative changes have taken place which ensure a sufficient supply of pyruvate in spite of the increased formation of glycerol-1-P and a high NADH₂ to NAD ratio.

Four hours after the withdrawal of the ethanol it was found that the ethanol had disappeared from the blood. This causes a fall of the elevated NADH2 to NAD ratio

as indicated by the glycerol-1-P to dihydroxyacetone-P and lactate to pyruvate ratios. Thus the formation of glycerol-1-P is greatly reduced and the glycerol-1-P content falls to about 60 per cent of the control values. At the same time excess pyruvate is formed probably on account of glucose-6-P, fructose-1.6-P2 and dihydroxyacetone-P, the content of which is decreased. This may be due to a rebound of the glycolytic enzyme systems which were formerly under the control of the high NADH2 to NAD ratio. In this way, our results show once more the important part that is played by the redox status of the cytoplasmatic NAD for the regulation of the glycolytic pathways.

After a single intravenous injection of 1.5 mg/g ethanol there was a small but insignificant rise in the ATP content of the liver, whereas the ATP content decreased after chronic administration of alcohol. The ATP content remained low after the withdrawal of the alcohol. It appears that chronic ethanol intake leads to a derangement of ATP synthesis, which persists at least 4 h after the withdrawal of the alcohol. The derangement of ATP synthesis is believed to be an expression of a functional cellular damage which outlasts the direct toxic effect of ethanol itself.

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BIOLOGY

Optimization of the Genetic Code

THE Atlas of Protein Sequence and Structure compiled by Eck and Dayhoff¹ provides an opportunity for preliminary statistical examinations of aspects of the genetic code.

Taking twenty-five different proteins—admittedly not a random sample representing their natural occurrence-I have found the following frequencies for the twenty amino-acids

Asn 108 Arg 104 Pro 99 Asp 94 Ilu 90 Glu 86 Phe 86 Gln 78 Cys 76 Ser 218 Ala 183 Gly 166 Leu 158 Val 153 Lys 145 Thr 142 + (presumably) 25 terminators 2,196

29 Gix (= Glu or Gln), 26 Asx (= Asp or Asn) and 2 xxx (= unknown) have been excluded.

If these frequencies are plotted as a graph (to test whether, for example, a form of Zipf's law2 operates) two breaks, corresponding to the columns in which the data have been arranged here, are evident. The distribution is thus not to be represented by a simple function.

Using relative frequencies the data can be converted to a binary coding by Huffman's algorithm³. This algorithm allocates to each of the twenty-one categories a binary number such that the coding is optimum—that is, if information as to the sequences of a number of proteins, with the probabilities given here for the occurrences of the individual amino-acids, had to be sent over a telegraph circuit, this could be accomplished at the least cost. The coding procedure consists of the allocation of sequences of dichotomies using in turn pairs of categories which are nearly equi-probable. It has been shown that this procedure allocates binary digits so that the average number of bits in each category exceeds by less than one the optimum coding. In this communication, digram frequencies, transition probabilities, are neglected.

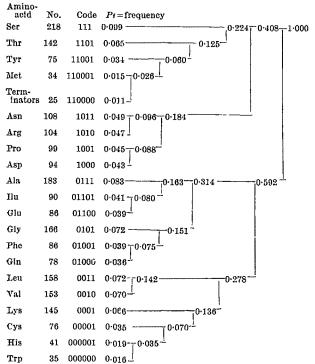
Table 1 shows the sequence of dichotomies and the resulting codings are given in the third column of Table 2. If all the codings were to be of the same length then they would have to contain five binary digits (and there would be 32-21=11 unused or nonsense codings). If the "negentropy" of the frequency distribution of amino-acids,

 $-\sum_{i=1}^{21} P_i \log_2 P_i$, is calculated, 3.97 bits in each category

is the absolute minimum required to code the twenty-one categories, neglecting interactions in higher order structure. In Table 2, column 4 gives the number of bits allocated to each category by the Huffman system (these numbers must be integers) and column 5, which gives the products of these numbers with the P_i , shows that the average number of bits in each category according to the Huffman algorithm is 4.257—only 0.29 higher than the theoretical minimum.

We now consider the way in which the twenty acids + terminator are coded by the natural genetic code of nucleotide triplets4. Each amino-acid is coded by a triplet of quaternary digits-U, C, A, G. There are thus (as described coincidentally in the early Chinese world system of the I Ching⁵) sixty-four different codons, each requiring six bits for its specification. Each possible codon but one has been allocated a provisional meaning. Serine, for example, is specified by each of six codons, so that the information content of a codon coding for serine is reduced by log₂ 6 bits from 6 to 3.4 bits. Similarly, if four codons code for alanine, the information content of one triplet coding for alanine is reduced to 4 bits. In

Table 1. RESULT OF APPLICATION OF HUFFMAN'S CODING ALGORITHM



Digit 1, upper branches in tree of dichotomies; digit 0, lower branches. Tree is read from right to left.

Table 2									
	Rel. prob. (Pi)	Huffman coding	No. of bits (N_t)	$N_t P_i$	No. of codons	No. of bits in codons N_0	N_0Pt		
1	2	3	4	5	в	7	8		
Ser Ala Gly Leu Val	0·099 0·083 0·076 0·072 0·070	111 0111 0101 0011 0010	3 4 4 4	0·297 0·332 0·304 0·288 0·280	6 4 6 4	3·4 4 4 3·4 4	0·337 0·332 0·304 0·245 0·280		
Lys Thr Asn Arg Pro	0.066 0.065 0.049 0.047 0.045	0001 1101 1011 1010 1001	4 4 4 4	0·264 0·260 0·196 0·188 0·180	2 4 2 6 4	5 4 5 3·4 4	0·330 0·260 0·245 0·180 0·180		
Asp Ilu Glu Phe Gln	0·043 0·041 0·039 0·039 0·036	1000 01101 01100 01001 01000	4 5 5 5 5	0·172 0·205 0·195 0·195 0·180	2 3 2 2 2	5 4·4 5 5 5	0·215 0·181 0·195 0·195 0·180		
Cys Tyr His Trp Met Term-	0·035 0·034 0·109 0·016 0·015	00001 11001 000001 000000 110001	5 5 6 6	0·175 0·170 0·114 0·096 0·090	2 2 2 1 1	5 5 6 6	0·175 0·170 0·170 0·096 0·090		
inator	0.011	110000	6	0-066	2 (ochre amber) 1 (?)	5	0.055		
21	1.000			4.247	64		4.320		

Table 2, column 6 shows the number of bits used to code each category. Multiplying these numbers by P_t gives the average number of bits in each category in the naturally developed code as 4.320. This number exceeds the "negentropy" by only 0.35 bits. If the twenty-one categories were equi-probable and coded optimally, $\log_2 21 =$ 4.4 bits would be required.

The genetic code is degenerate, but the degeneracy is not evenly distributed. There is a correlation between the frequency of occurrence of an amino-acid and the number of triplets coding for it, precisely as in a commercial code book alternatives are provided for frequent words, so that these cannot be identified from the frequencies of their coded representations.

The natural genetic code, given the base triplet mechanism, thus approaches remarkably closely to the optimum coding. This observation seems of significance when considering the evolution of the code by natural selection.

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Protective Immunity produced by the Injection of X-irradiated Sporozoites of Plasmodium berghei

STUDIES with avian malaria have shown that killed sporozoites as well as sporozoites inactivated with ultraviolet light can produce a partial immunity after injection into birds^{1,2}. On the other hand, attempts to use the erythrocytic stages of the parasite as the source of antigen have met with only limited success with avian3, rodent4 and monkey malaria^{5,6}. Previous attempts to use killed sporozoites of the rodent malarial parasite, Plasmodium berghei, to immunize rodents have been unsuccessful. We therefore sought to determine whether protective immunity to this parasite could be achieved by partial inactivation of the injected sporozoites as opposed to injection of dead parasites. X-irradiation was chosen as the inactivating agent, because of the partial immunity

obtained by vaccination with irradiated blood forms of malaria parasites⁷⁻⁹. This communication reports preliminary results on the production of protective immunity in mice by vaccination with X-irradiated sporozoites of P. berghei.

Sporozoites were obtained by dissection of the salivary glands of laboratory bred A. stephensi 14 days after feeding on a hamster infected with P. berghei. After homogenization of the salivary glands in 50 per cent human plasma diluted in saline, the suspension of sporozoites was irradiated in the 280 kVp X-ray beam of a Picker Vanguard teletherapy unit. The dose of radiation ranged from 2 to 15 krads. Irradiated parasites were injected intravenously into A/J mice, each mouse receiving between 5,000 and 75,000 sporozoites in an injection of 0.2 ml. In each experiment, a control group of A/J mice of the same age and sex was injected with non-irradiated sporozoites of the same pool to permit comparison with the infections produced by the irradiated parasites.

The amount of irradiation necessary to inactivate sporozoites sufficiently so that patent infection was prevented varied from 6 krads to more than 10 krads. The more viable the batch of sporozoites, the higher the dose of X-irradiation needed to produce this effect. A more detailed account of these experiments and additional data

will be published elsewhere.

Whether protective immunity resulted from the inoculation of irradiated sporozoites was determined by challenging those animals in which the injection of 5,000 irradiated parasites had failed to produce a patent malarial infection. These mice were challenged by intravenous injection with viable sporozoites (1,000/mouse) about 2 weeks after injection of the irradiated sporozoites.

The results indicate the presence of a certain degree of protection, for on challenge significantly less infection was observed in some groups of "vaccinated" animals, as compared with their controls. The best protection was conferred by sporozoites irradiated with 6, 8 or 10 krads. The degree of protection varied somewhat from experiment to experiment, and further data are needed to determine the relation, if any, between the dose of radiation and the degree of protection obtained.

More significant protection was observed when the immunizing dose, that is the number of irradiated sporozoites, was increased to 75,000/animal. This inoculum (75,000 sporozoites irradiated with 8 or 10 krads) was injected into forty-six mice, out of which only one developed a patent infection. All the remaining, apparently negative, animals were challenged with 1,000 viable sporozoites. The results are summarized in Table 1.

Table 1. VACCINATION WITH X-IRRADIATED SPOROZOITES OF P. berghei (75,000 SPOROZOITES/MOUSE)

Experi- ment No.	Cont No. : percent animals i	and age of	Day of challenge	Vaccinated a No. a percenta animals in	nd ge of	Protec- tion (per cent)
2* 3 4 5 6 Overall	9/10 7/10 10/10 12/12 12/12 38/42	90 70 100 100 100 90	$15 ext{th} \\ 19 ext{th} \\ 19 ext{th} \\ 15 ext{th} \\ 12 ext{th} \\ 12 ext{th} -19 ext{th}$	2/4 2/11† 1/5 1/7 8/11 14/38	50 18 20 14 73 37	44 74 80 86 27 59

All control and vaccinated animals were challenged with 1,000 sporozoites

All control and vaccinated animals were challenged with 1,000 sporozoites injected intravenously.

Percentage protection was calculated as 100 percentage minus per cent of vaccinated animals which became infected, correcting this value for an assumed 100 per cent infection of controls.

* The sporozoites in this experiment were irradiated with 10 krads; 8 krads were used in all other experiments.

Experiments 5 and 6 were challenged simultaneously so that they had their control group in common.

† Most of these animals remained protected against a second challenge, 36 days after this vaccination (67 per cent protection).

Although the degree of protection varied from experiment to experiment, the overall protection rate may be expressed by the fact that only 37 per cent of the vaccinated animals became positive after challenge, as compared with 90 per cent of the controls.

In vaccinated animals which became positive after being challenged, the prepatent period as well as the survival time was usually longer than in the nonvaccinated controls, but all animals ultimately died from their malarial infection.

These results differ somewhat from the attenuated P. berghei infections obtained in rats and in rats and mice vaccinated with X-irradiated blood forms and challenged with the same stage of this parasite.

As for the protected animals, at least in one instance, they remained significantly resistant to a second challenge, 36 days after the initial inoculation. Further work to determine the duration of this protection and its possible enhancement by the administration of repeated doses of irradiated sporozoites will be reported elsewhere.

It has previously been observed that the natural nonspecific resistance of the host to sporozoite induced infection can be greatly enhanced by various means, such as the administration of Freund's adjuvant, or of dead Corynebacterium parvum10. In view of this, it was necessary to ascertain the possible non-specific effect resulting from the injection of relatively large amounts of mosquito salivary gland tissue.

Homogenized salivary glands from non-infected mosquitoes were irradiated and injected intravenously into The amount of gland material (salivary glands mice. from nineteen mosquitoes into each mouse) was equivalent to that injected during a vaccination with 75,000 sporo-These mice together with controls were latered with sporozoites. The mice injected with zoites. challenged with sporozoites. normal mosquito tissue not only were not protected but had an even more virulent infection than the controls, with a shorter prepatent period and a shorter survival One possible explanation for this accelerated time. infection would be the considerable reticulocytosis observed in these animals, which would tend to favour the P. berghei infection.

Animals vaccinated with 75.000 irradiated sporozoites showed no protection even against a relatively small inoculum of parasitized red blood cells (1,000). Also in this case, and possibly for the same reason indicated in the previous paragraph, there was a certain enhancement of the infection in the vaccinated animals as compared with their controls.

This protection thus seems to be stage specific to the extent that it does not alter the course of blood induced infection of the same strain of P. berghei, its effect being limited to the sporozoite stage. A similar stage specific protection has been reported in vaccination with sporozoites of avian malaria2.

We can summarize the results of our experiments as follows. The X-irradiation of sporozoites of P. berghei inactivated these parasites, in certain conditions, so that they no longer produced patent malaria infections detectable by blood smears in A/J mice. It was further shown that a large percentage of these vaccinated animals were protected against the injection of viable sporozoites. Thus, if a sufficiently large number of irradiated sporozoites was inoculated (75,000), only 37 per cent of these vaccinated animals became infected on challenge as compared with 90 per cent of their controls. In the rest of the vaccinated animals there was a certain delay of the infection, but its invariably lethal outcome was unaltered.

The protection obtained by injection of irradiated sporozoites was apparently limited to challenge with this stage of the life cycle of P. berghei, being without effect against the injection of blood stages of the same parasite. Likewise, the injection of comparable amounts of normal mosquito salivary glands failed to protect against sporozoite induced P. berghei infection.

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Micropipetting Cytoplasm from the Mouse Egg

MICROMANIPULATION of living cells presents many intriguing problems because cells are so small and cellular substances are usually viscous. Placing a micropipette into a cell can be accomplished fairly easily by means of a precision micromanipulator, but removing a quantity of cytoplasm from a cell is difficult; to overcome this we have devised a vacuum micropipetting system.

The vacuum micropipetting system consists of a small vacuum pump, a pressure regulator and a plug-valve changer (Fig. 1). Negative pressure can be regulated by a 'Gomco' surgical suction tube, one end having a finger tip control and connexion to the distribution valve, the other end being connected to a vacuum bottle and then to the vacuum pump. Suction is produced by placing the thumb tightly against the finger tip control. Fluctuation of suction pressure within a narrow range can be maintained by the on and off action of the thumb on the finger tip opening of the pressure regulator, so that too fast a flow of cellular materials into the micropipette can be avoided.

The plug-valve changer has two interconnected valves both of which have two outlets. The outlets of one valve are connected by polyethylene tubing to the microinjector and the vacuum pump, while the outlets of the other valve connect with two micropipettes. Thus the function of either pipette can be quickly switched from suction to injection or vice versa.

The vacuum micropipetting system can be used to micromanipulate large and small living cells. In Fig. 2 living newly fertilized mouse eggs of about 70µ in diameter are shown. These were at the pronuclear stage and maintained in a drop of phosphate buffer saline plus bovine plasma albumin (10 mg/ml.) covered with mineral oil

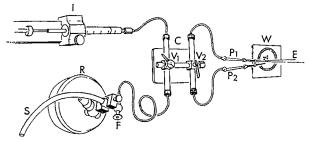


Fig. 1. Diagram of the vacuum micropipetting system. C, Plug-valve changer: V1, outlet valve 1, to microinjector or pressure regulator; V2, outlet valve 2, to micropipette P1 or P2; I, microinjector unit; E, egg-holder pipette; R, pressure regulator; F, finger tip control; S, suction tube to vacuum pump; W, 'Vasellne' well. Details are given in the text.

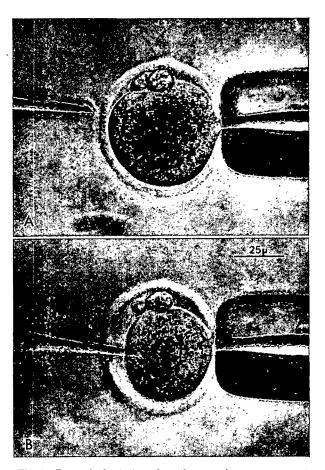


Fig. 2. Removal of cytoplasm from the pronuclear mouse egg. A, Holding of egg before suction; B, holding of same egg during suction. Compare the size of egg vitellus before and after suction.

in a 'Vaseline' well on a microscope slide. Using a Leitz micromanipulator, the egg was held by an "egg holder" which is a fire-polished blunt tipped pipette for holding the egg1, and a microsuctioning pipette with an oblique tip was operated from the opposite side as shown in Fig. 2. Both the egg holder and the suction pipette were maintained in position by a Leitz micromanipulator1.

Suction of the newly fertilized denuded egg can be started when the vacuum pump pressure reaches 125-250 mm of mercury, and the flow of cytoplasmic materials from the egg into the micropipette can be controlled at 505-635 mm of mercury. Negative pressure of 710 mm of mercury can be produced at the tip of the micropipette, but the danger of cells being destroyed is increased.

A pipette with an orifice about 3µ in diameter was used

because: (a) a high negative pressure may be required if too narrow; (b) particulate matter in the egg may block the pipette; and (c) the vitellar substance of the newly fertilized egg of the mouse is very viscous. Fig. 2 illustrates the removal of cytoplasm from the pronuclear mouse egg. Before suction was applied (A) the volume of the egg was estimated to be about 175,820 μ^{3} and the volume of its vitellus about 96,970 μ³. After suction had been applied (B), the size of the egg vitellus decreased, and was calculated to be about 54,365 μ^3 . Nearly half of the cytoplasm was removed from this egg after which the egg was still intact although the vitelline membrane had shrunk.

Reverberi² showed that the fertilized but still undivided egg of Ciona could develop into an embryo even after a large part of its substance had been removed (see also ref. 3). The quantity of cytoplasm removed from a mouse egg may determine its survival; the shrinkage of the vitellus does not cause death. Our previous work4

has shown that some of the shrunken eggs can survive without adverse effect.

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Melanin in an Insect. Lucilia cuprina (Wied.)

MELANINS, although somewhat ill defined, are dark pigments which occur in animals and plants and they are usually bound to protein. Thomson¹ in a review has pointed out that there are no satisfactory histochemical tests for the identification of melanin. Alkali solubility and reversible reduction merely indicate acidic and quinonoid properties-properties common to many different types of pigments. Mammalian and cephalopodan melanins in particular have been extensively studied and some have been isolated and examined chemically. Melanins are formed by the action of a phenolase on a phenolic substrate, but their structure is not known. Those derived from tyrosine, however, are considered to be built up from indolyl units. Nicolaus et al.2 classified melanins as "indole" or "catechol" types, depending on the degradation products formed on alkali fusion and permanganate oxidation. All melanins so far examined from animal sources are of the indole type, as are the melanins prepared by the oxidation of tyrosine and dopa.

In insects, except for one investigation, identification of melanin has relied on inadequate histochemical tests. Sometimes a search has been made for an accompanying phenolase system, but this is usually of little value because of the phenolase activated process of sclerotization which occurs in insects. Nevertheless, sclerotization and blackening (said to be melanin) have been shown to be independent processes in a few insects (for a review, see Hackman4).

I have a laboratory strain of the blowfly Lucilia cuprina (Wied.) in culture which is homozygous for three recessive mutants carrying yellow eyes, rusty body and a black puparium (the puparium of the normal wild strain is brown). Electron microscopy shows a layer of fine black granules at the inner surface of the epicuticle of the puparium. When empty puparia, from which pupal cuticles, other residues and lipids have been removed, are hydrolysed (6 normal hydrochloric acid) thin insoluble membranes remain which are "ghosts" of the original puparia. In the normal wild strain these membranes are colourless, but in the mutant they are an intense black and thicker. When radioactive tyrosine⁵ was injected into fully grown last instar larvae of the mutant, 79 per cent of the radioactivity was recovered from the puparia and 49 per cent of this was in the black membrane remaining after hydrolysis. With the normal wild strain these percentages were 59 and 16, respectively.

The black pigment was extracted (10 per cent aqueous sodium hydroxide) from the insoluble membranes remaining after acidic hydrolysis of puparia of the mutant and recovered by acidifying the extract and yielded 4.6 per cent on weight of puparia taken. The pigment was again subjected to acidic hydrolysis (6 normal hydrochloric acid) for 24 h. In alkaline solution the pigment showed only general absorption in the ultraviolet and visible regions of the spectrum. The pigment was subjected to alkali fusion² and 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid were identified among the degradation products. Permanganate oxidation6 of the pigment gave pyrrole-2,3,5-tricarboxylic acid as the principal product together with smaller amounts of pyrrole-2,3-dicarboxylic acid and pyrrole-2,3,4,5-tetracarboxylic acid. degradation products are the same as those obtained by Nicolaus and his collaborators^{2,3} from invertebrate melanins (from squid, cuttlefish and octopus inks and from Drosophila melanogaster tumours and Tropinota glabra elytra) and from melanin prepared by oxidation of dopa.

The black pigment in the puparia of the L. cuprina mutant has the physical properties of a melanin and gives degradation products characteristic of melanins of animal origin. This pigment is therefore classified as an indole melanin. Experiments with radioactive tyrosine show it to be formed from tyrosine, which is confirmed by the nature of the degradation products.

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Retraction of the Claim that Host Pregnancy affects Pupal Production by the Tsetse Fly

DURING the first two seasons of an investigation into the effect of host pregnancy on female Glossina austeni we claimed that the performance of the flies fed on pregnant goats was significantly better than that of those fed on non-pregnant goats¹⁻³. We must now retract this claim. The combined results for season 2 are given in Table 1, followed by the figures for the first experiment in season 3 in which there was a complete reversal.

Table 1. SURVIVAL AND PUPAL PRODUCTION BY FEMALE G. austeni WHEN FED ON PREGNANT AND NON-PREGNANT GOATS

37	A C Ct	No. of s	survivors	No. of pupae deposited		
No. of experiment	Age of flies (weeks)	Pregnant goats	Control (male and female)	Pregnant goats	Control (male and female)	
Season 2						
(all experiments	0	1,048	1,044	0	0	
combined)	9	639	406	2,865	1,852	
	20	87	36	4,841	2,665	
Season 3						
Expt. 1	0	600	600	0	0	
	9	163	367	1,032	1,766	
		Female goats		Female goats		
		(non-pregnant)	Male goats	(non-pregnant)	Male goats	
Expt. 2A	0	300	300	0	0	
	9	211	210	1,067	1,053	
		(Pregnant)		(Pregnant)		
Expt. 2B	0	600	600	0	0	
(same goats as in	9	424	441	2,038	2,104	
2A)	20	129	145	4,078	4,048	

Before this investigation began, attempts had been made to test goats for individual variation in attractiveness to fertilized female tsetse, but they failed as a result of concealment of the blood meal by the larva. After checking that the blood picture was normal for the three pregnant goats used in experiment I, we devised a test for the suitability of the host using unfed 2 day old male flies as the test organism. The proportion of such flies which have fed after application for 15 min is referred to as the "host suitability index"; indices were obtained for all goats. There is no evidence that certain goats are basically unattractive, but a few become exceedingly unsuitable because of skin thickening caused by excessive feeding by the flies or by susceptibility to tsetse saliva. Of forty goats tested, four had very low indices. By chance two of these four were in the team of three pregnant goats used in experiment 1; furthermore, all four had been included in the control herds used in seasons 1 and 2, where their presence would have lowered the control values and given rise to apparently better results from the pregnant goats.

Recently two teams of goats with equally high suitability indices were used. The performance of female tsetse flies was remarkably similar when fed on a team of non-pregnant female goats or on a team of castrated male goats (experiment 2A). Using the same goats, the experiment was repeated when the females were pregnant; again, fly performance was similar with the two types of host (experiment 2B). It should be noted that the superiority of these results over those of season 2 resulted from the introduction of a new type of cage2.

The error in the interpretation of our earlier results is extremely unfortunate. On the other hand, by culling any unsuitable animals, excellent yields of pupae can be obtained without having to use pregnant goats which are only seasonally available.

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Algebraic Model of the **Lactation Curve in Cattle**

Many factors may influence the total milk yield of a single lactation, but the general shape of the curve, defined by the locus of weekly yield, remains substantially unchanged. Economically, the configuration of the curve is important, for the animal which produces milk at a moderate level steadily throughout her lactation is to be preferred to one which produces a great deal of milk at her peak but little thereafter (see Cersovsky¹ for a review of the literature).

Some attention has been paid to the problem and many workers, chiefly in Europe, have noted changes in the shape of the curve as a result of season of calving2, age3 and fertility4, although the phenomenon appears to remain relatively unaffected by changes in management. British Milk Marketing Board⁵ has presented diagrams showing the effect of season of calving and parity on average lactation curves, and Makela^{6,7} has studied in detail the position and duration of peak yield, and has pointed out its importance to the milk yield of the lactation as a whole.

Little new has in fact emerged since Johanssen⁸ reviewed the subject in 1961, when he described Gaines formula $y = ae^{-Kt}$ (y is the yield in week t, e is the base of natural

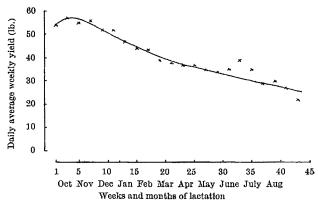


Fig. 1. Regression curve $y = 56.62 \ n^{0.03996} \exp(-0.00942 \ n)$ fitted to a single Friesian lactation.

logarithms, a and K are constants) which, however, does not give a very good fit. More recently, Nelder9 described an inverse polynomial which might furnish a rather better description, but at the same time our own studies have developed along slightly different lines.

The curve of lactation increases rapidly from calving to peak a few weeks later, followed by a more or less gradual decline until the animal goes dry in about 10 months. This is essentially a gamma-type curve, and may be expressed generally by the function

$$y_n = a \ n^b \exp(-cn) \tag{1}$$

where y_n is the average daily yield in the nth week and a, b and c are constants.

The total yield is then

$$y = \frac{a}{c^{b+1}} \vdash (b+1) \tag{2}$$

and yield to week t is given by

$$y_t = a \int_0^t t^b \exp(-ct) dt$$
 (3)

which may be evaluated, for example, by using tables of the incomplete gamma function.

Peak yield occurs therefore where n=b/c and is itself $y_{\max} = a(b/c)b e^{-b}.$

Other properties of the model may be deduced immediately.

The exponent, b, must be less than unity, otherwise we are faced with an ever increasing weekly yield. (b+1), is therefore close to unity, and total yield

$$y \leqslant \frac{a}{c^{b+1}}$$
 (from equation 2)

Hence $c^{b+1} < 1.0$, and so c < 1.0.

But the constant a is a general scaling factor associated with the average daily yield at the start of the lacta-Hence, for tion, because $y_n = a$ when $n^b \exp(-cn) = 1$. lactations starting at the same level, total yield, y, is a function of $c^{-(b+1)}$, which is therefore a measure of "persistency" or the extent to which peak yield is maintained. Putting $S = c^{-(b+1)}$, the relationship between persistency, total yield and level of production is given by

$$\ln(y) = \ln(a) + \ln(S) + \ln(\lceil (b+1))$$

For given a or S therefore variations in y depend almost entirely on variations in S or a, respectively.

The constants may be determined uniquely for any lactation by least squares methods. Equation (1) converts

$$\ln(y_n) = \ln(a) + b \ln(n) - cn \tag{4}$$

a straightforward linear regression model.

Evaluation of a single Friesian lactation chosen at random gave the following values, calculated from the 24 h production of one day in each week for the first 44 weeks of lactation: a = 56.62; b = 0.03996; c = 0.00942; from which $y_{\text{max}} = 57.6$ lb./day, $n_{\text{max}} = 4.25$ weeks.

The observed yield over 305 days was 12,215 lb., which

compares with 12,726 lb. calculated from equation (3), and the persistency factor $c^{-(b+1)}$ is 128.0. The curve is plotted with its expectation in Fig. 1.

If the errors of estimation are normally and independently distributed, a test of significance may be applied to the regression function (4) as in Table 1.

Table 1. REGRESSION ANALYSIS: LOG DAILY YIELD SINGLE LACTATION CURVE Source of variation df. Mean square Lactation curve Residual 0·170200* 0·001315 3 40 * P < 0.001.

The function accounts for 89.7 per cent of total variation in log daily yield, and the standard error of estimate is ± 0.036 , equivalent to ± 8.6 per cent of the expected daily yield. Hence 95 per cent of all errors of estimation will be less than 18.0 per cent of the expected yield.

The configuration of the curve may therefore be defined by three parameters which lend themselves to easy manipulation. If, however, the solution is to have any practical value, it must be possible to discriminate between economically distinguishable groupings and for this a random sample of 524 Friesian heifer lactations classified by sire and herd was taken from the National Milk Records register of calvings during the autumn and winter of 1960-61. Milk yield was recorded on one and the same day (p.m./a.m.) in each week of lactation, and curves were fitted individually.

The sample means of parameters are given in Table 2. Over all lactations, the combined standard error of estimate was ± 0.070 , equivalent to ± 17.5 per cent of expected yield, with 95 per cent limits of ± 38.4 per cent.

Table 2. PARAMETER MEANS

	J.	Tean .		
Constant	Log.	Natural	S.E.	S.D.
а	3.4201	30-57	±0.0163	+0.378
b	_	0.1889	± 0.0101	± 0.232
c	_	0.0306	±0.00098	±0.0219
2000				

The standard error and standard deviation given for the constant a are

The hierarchical analysis of variance is given in Table 3 together with the analysis of ln(S) = -(b+1) ln(c), the persistency factor.

Table 3. ANALYSIS OF VARIANCE

		Mean square						
Source of variation	df.	ln(a)	b	$(\times 10^{c7})$	ln(S)			
Between bulls	178	0.0440*	0.0845†	1,388†	0.1345			
Between herds within bulls	199	0.0144	0.0404	829*	0.1599			
Within herds	145	0.0232	0.0348	486	0.1014			
* $P < 0.01$; † $P < 0.00$	01.							

It is evidently possible in this sample to distinguish between progeny groups in respect of the three parameters and between herds in respect of c, but differences in persistency are not sufficiently marked as other workers have also found using other measures.

In Table 4 the principal characteristics of lactation are given for all bull progeny groups of ten or more daughters, and these are plotted in Fig. 2. The relationship between S and a is well demonstrated in Table 4, for example, by bulls 393 and 431. For these two animals the scaling

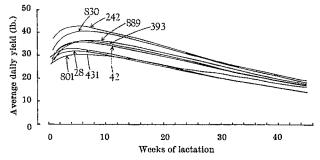


Fig. 2. Lactation curves of eight bull progeny groups.

Table 4. MEAN CHARACTERS OF PROGENY GROUPS

Bull	No. of daugh- ters	а	b	c	(equa- tion (3)) (lb.)	Peak (week)		tency factor (S)	Log (S)
28	11	29.72	0.1295	0.0267	7,798	4.9	$32 \cdot 1$	60	4.0945
42	14	30.98	0.1661	0.0265	9,380	6.3	35.6	69	4.2340
242	10	39.02	0.1419	0.0275	11,669	5.2	42.8	61	4.1109
393	10	31.33	0.1747	0.0276	9,219	6.3	36.3	68	4.2195
431	īi	31.36	0.1193	0.0271	8,582	4.4	33.2	56	4.0254
801	11	27.88	0.1203	0.0213	8.967	5.6	30.4	75	4-3176
830	19	34.07	0.1978	0.0298	11.578	6.6	40.6	67	4.2048
889	23	29.05	0.2314	0.0311	10,295	7.4	36.6	72	4.2766

factor, a, is almost identical but bull 393 with higher S has the higher yield.

The effect of age, season, disease or breed on the shape of the curve cannot be determined with these data, because all the animals were Friesian autumn-calving heifers, and no other information was recorded. There is, however, some evidence that the shape of the curve is modified particularly by season of calving, in that, superimposed on the basic curve, there is a seasonal fluctuation reflecting the May peak and winter trough noted else-

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Effect of Oestradiol and Testosterone on the Sexual Receptivity and Attractiveness of the Female **Rhesus Monkey**

THE ovarian hormones of the female rhesus monkey considerably influence sexual behaviour both in free-living groups¹ and in experimental situations in the laboratory^{2,3}. There are at least two properties in the female which are concerned in her interaction with the male: first, her sexual attractiveness, and second, her sexual receptivitythe willingness of the female to engage in copulation. These two components have now been found to respond differently to alterations in the female's hormonal state.

Experiments were carried out in the laboratory on oppositely sexed pairs of adult rhesus monkeys (Macaca mulatta). The males were intact; the females bilaterally ovariectomized. A series of quantitative observations were made on each pair from behind a one-way mirror after transferring the female to the male's home cage (size $50 \text{ in.} \times 30 \text{ in.} \times 35 \text{ in.}$) for periods of 30 min. All animals were housed singly between observations. Each female was paired successively on the same day, and in the same order, with two males; each series consisted of ten observation periods. Data from different series were compared by analysis of variance and t tests. This report is confined to experiments in which the female was untreated, or receiving either 50 µg/day of oestradiol benzoate or 1 mg/day of testosterone propionate by subcutaneous injection.

The male rhesus monkey copulates by making a series of mounts, each accompanied by intromission and a number of pelvic thrusts, before ejaculating. One way of expressing the level of a male's sexual activity is by means of the "sexual performance index", which is the product of the mean rate at which successive mounts occur and the mean number of thrusts delivered at each mount. This index is correlated with the time the male

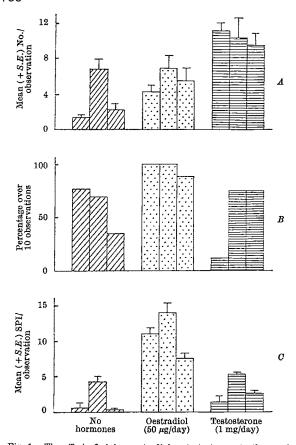


Fig. 1. The effect of giving oestradiol or testosterone to the ovariectomized rhesus monkey on her behaviour with the male. Values given are: means/observation period (A and C) and percentages over a series of ten observation periods (B). Oestradiol increased the female's attractiveness to the male (B) and thus stimulated his sexual activity (C). Testosterone increased the females' presenting behaviour (A), but her attractiveness and hence the male's sexual activity remained low. Examples are shown from three pairs of animals; see text for further details.

takes to ejaculate4, although this relationship is not at issue in this communication. The effect of giving oestradiol to the females of twenty pairs, made up from three males and ten females, was to increase the male's mounting activity (sexual performance index) and number of ejaculations (significantly, fifteen pairs). Females invite the male to mount by "presenting" in a number of ways, but there was no consistent change in the number of times they did so while receiving oestrogen. The mounting activity of the males was stimulated as much in pairs in which the presentations of the female decreased or were unchanged by oestrogen (twelve pairs) as in those in which an increase occurred (seven pairs). The proportion of presentations accepted by the male before ejaculation -that is, resulting in his attempting to mount—was higher when the females were treated with oestrogen (fourteen pairs), and a greater proportion of the male's attempts to mount occurred without being preceded by a recognizable presentation by the female. The females a recognizable presentation by the female. accepted the male's attempts to mount with equal frequency whether they were treated with oestrogen or not.

These results suggest that the administration of oestrogen to the ovariectomized female increases her sexual attractiveness to the male, but that this does not depend on changes in her "presenting" behaviour. If there is any effect on her receptivity, it is much much less than the effect on her attractiveness—a finding in contrast to that on many sub-primate females. The administration of progesterone to a female treated with oestrogen results in a behaviour pattern resembling that with the ovariectomized, untreated female³.

Testosterone was given to the females of eight pairs (three males and four females) and a very different pattern of behaviour resulted. The mean number of presentations offered to the male by the females increased to levels much higher than those in either untreated or oestrogen-treated females (six pairs). But there was only minimal stimulation of the male's sexual activity, and the proportion of the female's presentations accepted by the males remained similar to that in untreated females. Females accepted the male's attempts to mount equally while receiving this dose of testosterone as with oestradiol, and there was no increase in aggression between the animals. Fig. 1 shows data from three of these pairs.

These results indicate that testosterone stimulates the receptivity of the females but has little effect on their attractiveness to the male. The vaginal smears from these animals remained uncornified, although their sexual skins were reddened. This and the data on the effect of oestradiol support the earlier proposition derived from other experiments that intravaginal mechanisms which are sensitive to oestrogen are concerned in the transmission to the male of the state of sexual attractiveness of the female, and indicate that these mechanisms are not stimulated by testosterone. The effect of testosterone on the behaviour of the female monkey has an obvious parallel to clinical reports that androgen, rather than oestrogen, has been found to increase the libido of some women to whom it was administered8,9.

It seems that the sexual receptivity and attractiveness of the female rhesus monkey are affected differentially by oestradiol and testosterone, although further experiments are necessary to decide whether the behavioural role of oestrogen can be attributed principally to an action on the female genitalia, whereas androgen has its effect principally on the central nervous system.

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Antagonism between Cytokinins and Germination Inhibitors

The inhibition of lettuce seed germination by several naturally occurring growth inhibitors is reversed by kinetin in red light. Kinetin in red light also breaks the dormancy in Xanthium seed, presumably by counteracting the inhibitor present in the seed^{2,3}. A similar antagonism between kinetin and coumarin is shown in the germination of Brassica seed⁴. Actinomycin D inhibits the release from dormancy which is induced by kinetin in Xanthium seed2, which suggests that DNA-dependent RNA synthesis is involved in the dormancy release. There is, however, no evidence as yet to suggest whether the endogenous inhibitors act at the same or at different sites. evidence suggests an interplay of cytokinins, growth inhibitors, phytochrome and perhaps other factors in the control of seed germination and dormancy. This communication reports on the interactions in the germination of lettuce seed between zeatin^{5,6} (a naturally occurring cytokinin) and dormin^{7,8} (abscisin II), kinetin and dormin, zeatin and coumarin, and kinetin and coumarin.

Table 1. Effect of Red (R) and far red (FR) light on cytokinin (Kinetin or Zeatin) reversal of dormin inhibition of 'grand rapids' Lettuce seed germination (per cent)

Treatment	Water	Dormin	Dormin + kinetin	Dormin + zeatin
Dark	26	4	8	7
Light	100	6	42	55
R	98	7	35	49
R– FR	20	0	5	7
R– FR – R	98	2	32	40

10 min exposures at 20° C. Germination recorded after 3 days.

Lettuce seeds (Lactuca sativa, var. 'Grand Rapids', 1965 harvest) were germinated in 5 cm Petri plates lined with two disks of filter paper soaked in 2 ml. of test solution. Each experiment had three replicates of fifty seeds each. Emergence of the radicle from the seed coat was taken as the criterion of seed germination. Red light of approximately 800 $\mu W/cm^2$ was obtained by two 15 W, white cool fluorescent tubes covered with two layers of Dupont red 'Cellophane'. Far red light of approximately 800 $\mu W/cm^2$ from a 200 W incandescent bulb was filtered through a running water bath 2.54 cm thick and a Corning Filter No. 2600. Seeds were imbibed for 8 h in dark before exposure to red light. Where both red and far red light treatments were given, one followed the other immediately. Seeds were returned to darkness after light treatments, but in other experiments they were exposed to continuous light of 10 ft.-candles from a white cool fluorescent source.

The antagonism between zeatin and dormin and between kinetin and dormin is shown in Fig. 1. It is clear that dormin is a powerful inhibitor of lettuce seed germination at 25 mg/l. and 50 mg/l. Zeatin and kinetin at 50 mg/l. relieve this inhibition to a great extent. Zeatin and kinetin cause nearly complete reversal of inhibition by 25 mg/l. dormin in 96 h. These results strongly suggest that kinetin and zeatin act in a similar manner, although kinetin appears to be less effective in reversing the dormin inhibition of germination. This is consistent with other reports of the comparative effectiveness of these

Antagonism between kinetin and coumarin in lettuce seed germination has been reported earlier, and a critical examination shows that this antagonism is best brought out at 20° C rather than at lower temperatures. This may be due to the fact that while coumarin is less inhibitory to germination at low temperatures¹⁰, kinetin has little effect on germination between 20° and 25° C. Fig. 2 shows an interaction between zeatin and coumarin and between kinetin and coumarin in their effect on lettuce seed

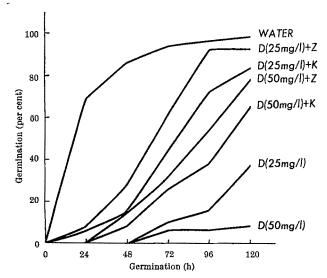


Fig. 1. Interaction between dormin (D) and zeatin (Z) and dormin and kinetin (K) in the germination of Grand Rapids' lettuce seed in continuous light at 20° C. Concentration of zeatin and kinetin: 50 mg/l.

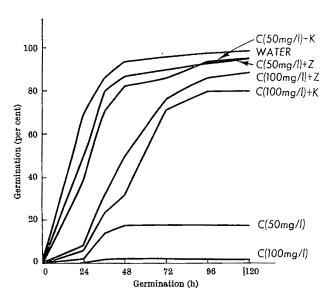


Fig. 2. Interaction between coumarin (C) and zeatin (Z) and coumarin and kinetin (K) in the germination of 'Grand Rapids' lettuce seed in continuous light at 25° C. Concentration of zeatin and kinetin: 50 mg/l.

germination at 25° C. Coumarin is nearly completely inhibitory at 100 mg/l., but this effect is nearly completely reversed by 50 mg/l. zeatin or kinetin. As before (see Fig. 1), zeatin is the more active of the two cytokinins.

An experiment was designed to test whether dormin blocks the red, far red photoreaction and whether this is prevented by cytokinin as shown earlier1. The results in Table 1 show that dormin in fact blocks the photoreversible system and that this is prevented by cytokinins, zeatin and kinetin.

The results reported here, together with other evidence1,2,4, strongly suggest that there is an interplay between the phytochrome system, natural inhibitors and cytokinins which function so as to regulate germination and growth. The mode of action of cytokinins and germination inhibitors is not known. Cytokinins have been shown to induce RNA synthesis 11 as well as to form part of certain transfer RNAs12. More recent work of Chrispeels and Varner13 shows that dormin inhibits the synthesis of gibberellin-induced hydrolases in barley aleurone layers in a manner similar to 6-methylpurine and 8-azaguanine, which suggests that cytokinins and biogenous inhibitors may participate in the regulation of nucleic acid metabolism during seed germination.

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Response to Photoperiod of Reported Long-day and Intermediate Varieties of Rice

Although Oryza sativa L. is generally considered to be a short-day plant, a delay in flowering by short-day treatment has been reported1-7. Varieties with such a response would have a potential use in studies of physiology and breeding, and would be most interesting ecologically. The data reported on such varieties do not present a clear picture of their reaction, however, and are insufficient to warrant their classification as intermediate, much less long-day, types. To clarify this point, we have carried out detailed tests on several varieties of rice previously reported to be long-day or intermediate. BPI-76', a variety known to be sensitive to photoperiod, was included as a control.

The plants were grown in the greenhouse during the day and transferred to different photoperiods in the evening. Table 1 shows the response of the varieties to photoperiod. All showed responses similar to short-day varieties. None could be regarded as a long-day or intermediate plant.

Table 1. NUMBER OF DAYS FROM SOWING TO FLOWERING OF THIRTEEN VARIETIES OF RICE GROWN AT DIFFERENT PHOTOPERIODS

	Photoperiod (h)					
Variety	8	10	12	13	14	16
'T-3 W356'	88	82	90	98	127	137
'B 76'	76	69	72	79	86	91
'Baok'	136	126	122	125	147	163
'GEB 24'	73	63	90	*	+	+
'CH-10'	78	76	78	78	80	85
'T.21'	91	77	82	88	103	98
T.136'	72	66	69	76	88	92
'Heenati 8963'	81	75	77	77	91	112
'Heenati 8976'	83	73	86	112	165	Ť
'Heenati 8965'	67	51	63	77	90	125
'Karang Serang'	127	115	111	111	114	121
'Karang Serang Sel.'	147	139	123	129	132	153
'BPI-76' (sensitive)	64	65	100	145	Ť	t

* Panicle formation but no emergence after 200 days of growth.

† No panicle initiation after 200 days of growth.

The seeds of 'T-3 W356', 'B 76', 'Baok', 'GEB 24', 'GH-10', 'T.21' and 'T.136' were obtained from the Central Rice Research Institute, Cuttack, India.

The conclusion of previous workers that these varieties were long-day plants, or that short-day treatment delayed flowering, was probably reached because only two photoperiods were tested, one of which was natural day length. For example, if 'Baok' is compared at photoperiods of 8 and 12 h, the 8 h treatment delays flowering by 14 days which leads to the conclusion that it is a long-day plant. The extra photoperiods of 10, 13, 14 and 16 h used in our tests, however, show definitely that it is a quantitative short-day plant. The delay in flowering with an 8 h photoperiod, which is sub-optimum, is a common phenomenon with the short-day rice varieties8,8. Other factors such as temperature may have greatly modified the response of these varieties in previous tests. Varieties like 'BPI-76', 'GEB 24' and 'Heenati 8976' are qualitative, whereas the other varieties are quantitative, short-day

More than a hundred varieties have been critically tested at the International Rice Research Institute and not one, so far, has shown a long-day response.

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Relationships among Functional Properties of Californian Grassland

Ecologists have become increasingly interested in general properties of communities and the relationships among species diversity, biomass, productivity, dominance and stability. Both verbal¹ and mathematical² models have been proposed to relate these properties. These models are important as initial generalizations in a developing predictive theory of ecology. The available data, however, are largely from planktonic systems and these data are contradictory. For example, Margalef³ has concluded from his extensive studies of marine systems that productivity and dominance are inversely related to stability and diversity. Increasing species diversity in the community leads to decreased dominance and productivity but increased stability. Patten4, however, has concluded that "high productive capacity is associated with high diversity" in summer plankton communities of Virginia's York River. Studies of grasslands on California's San Francisco peninsula, reported here, provide the first rigorous development of the relationships among diversity, dominance, productivity and stability of terrestrial communities. This report extends Margalef's general model to terrestrial systems and provides strong support for it

as a valid description of general properties of communities. From January 1 to July 1, 1966, standing crop was harvested in three 0.5 m² rectangular quadrats arranged linearly perpendicular to the slope and randomly placed at monthly intervals on north-east, north-west, southeast and south-west slopes on sandstone and serpentine soil types on Stanford University's Jasper Ridge Biological Experimental Area. The area studied had been free of grazing and fire for more than 5 years previously, undoubtedly long enough for the vegetation to come to equilibrium considering the rapidity with which this vegetation type responds after disturbance. Oven dried material from monthly samplings provided data on biomass and productivity of the grasslands during the principal productive period. During May, the month of peak standing crop, diversity data were collected by canopy interceptions along two 5 m transects on each sampling site with sampling points fixed at intervals according to fifty random numbers between 0 and 500 One transect was perpendicular to the slope and one was parallel to the slope. Dominance was assessed as a "dominance index" equal to the percentage of the total standing crop contributed by the two most important species. I am concerned here with the general properties of the grasslands as a model system and a more detailed treatment of their ecology will appear

Dominance in the grasslands was related to diversity according to Y=138-6.6~X, where Y is the percentage of the peak standing crop contributed by the two most

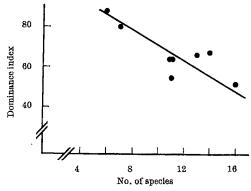


Fig. 1. Relationship between dominance (as percentage of total standing crop contributed by two most important species) and species diversity of grasslands on Stanford University's Jasper Ridge.

important species and X is the number of species recorded from 100 random points on the two transects (Fig. 1). The inverse correlation between these two properties is very significant (r = -0.842; 0.01 > P > 0.01). These data indicate that the tendency for biomass to become concentrated in a limited number of species decreases as the community becomes floristically richer.

The productivity of the Jasper Ridge grasslands was directly related to dominance according to Y = -0.79 +0.0188 X, where Y is productivity in g/m²/day and X is dominance as previously defined (Fig. 2). Productivity and dominance were significantly correlated (r = 0.852; 0.01 > P > 0.001), indicating that the efficiency of the grasslands as an energy-trapping system increases with increasing concentration of biomass in a limited number of species. The least diverse site was more than 2.5 times productive than the most diverse site.

Stability of communities can be estimated from the turnover time, the ratio of biomass to productivity. The greater the biomass in relation to productivity, the less dependent the community is on conditions favourable to productivity to maintain a relatively stable biomass. An elegant theoretical justification of this argument has been developed by Leigh2. In the California grasslands, stability declined with productivity according to Y = 3.95 -3.52~X, where Y is turnover time in years and X is productivity as previously defined (Fig. 3). The signoductivity nificant inverse correlation (r = -0.829; 0.02 > P)0.01) suggests that the community becomes increasingly susceptible to alteration in functional properties through environmental oscillation as productivity increases. rather severe price in stability seems to be paid for the increased productivity generated by greater dominance.

Although general, non-quantitative discussion of the ideas developed here is widespread among ecologists 10, this is the first time data from terrestrial systems have been rigorously related to the concepts in Margalef's model. I conclude from these data that dominance is directly related to productivity and inversely related to diversity and stability and infer the following general properties of communities: (a) diversity is principally a mechanism which generates community stability; (b) dominance is principally a mechanism which generates community productivity; and (c) increasing the number of species in a stand, rather than enhancing efficiency through more efficient exploitation of site resources, decreases efficiency, perhaps through competition. The similarity between the California grasslands and Margalef's planktonic communities emphasizes the general application of Margalef's conclusions to ecological systems and suggests that these conclusions represent extremely significant ecological generalizations.

The data presented here were collected during the tenure of a US Public Health Service postdoctoral traineeship

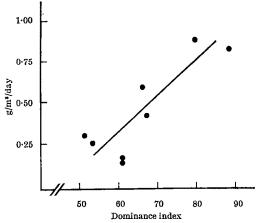


Fig. 2. Relationship between productivity and dominance in the grass-

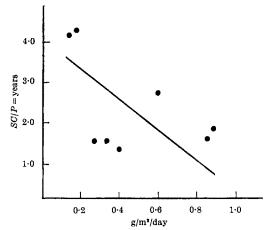


Fig. 3. Relationship between turnover time (in yr) and productivity of the grasslands.

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CYTOLOGY

Mitogenetic Radiation

Between 1920 and 1935 a great deal was published on the subject of mitogenetic radiation—a radiation usually considered to be ultraviolet, which is emitted by dividing cells and which stimulates other cells to divide. studies were originated by Gurwitsch1 and are still carried on in the Soviet Union, but almost ceased in Britain and the United States in the 1930s after much careful but negative work²⁻⁴. Nobody there was able to stimulate cell division with weak ultraviolet light or to detect radiation from rapidly dividing cells with photoelectric or biological detectors.

The sensitivity of photon counters has increased more than 200 times since the 1930s and modern equipment has been claimed to detect radiation from yeast and tissue cultures⁵⁻¹⁰. This communication reports an attempt to check these measurements.

According to a communication from Gurwitsch's laboratory quoted by Troitskiis, two drops of 6 per cent oxalic acid added to 3 ml. of 0.2 per cent potassium permanganate emit radiation of intensity similar to mitogenetic radiation. We have constructed a photon counter which readily measures light in the range 2000-6000 Å from this reaction.

The photon counter was equipped with an EMI 6255SAphotomultiplier which has a large 4 cm diameter photocathode. An air gap (1 mm) and a 3 cm silica light guide separate the photocathode from a silica cuvette of 25 ml. capacity, all of 4 cm diameter and coated externally with magnesium oxide which increases the light reaching the photocathode about tenfold. If the cell or light-guide

Table 1. LIGHT EMITTED IN THE RANGE 2000-6000 Å BY SUSPENSIONS OF YEAST OR BACTERIA

	Opa	icity								
Content	Measured with Maker's calibra- Welcome tion as millions		Light plus background		Background Before After		er	Light intensity (only if $> 2\sigma$)		
	opacity tubes	of $E. coli/ml$.	C.p.s.	σ	C.p.s.	σ	C.p.s.	σ	C.p.s.	σ
Escherichia coli	6.5	2460	24.3	0.3	24.5	0.3	25.0	0.3	0	0.6
	6.5	2460	24.6	0.3	24.8	0.3	24.3	0.3	0	0.6
Schizosaccharomyces pombe	4.0	1515	24.5	0.2	24.0	0.3	24.5	0.3	0	0.5
G 111 111	4.0	1515	24.7	0.2	24.5	0.2	24.2	0.3	0	0.5
Candida albicans	3.5	1330	24-0	0.3	24.5	0.8	24.0	0.3	0	0.6
Canalanamana	3.5	1330	24.2	0.3	24.0	0.3	24.7	0.2	o O	0.6
Saccharomyces cerevisiae	2.5	950	26.0	0.4	25.0	0.3	25.5	0.2	Ŏ	0.5
	2·5 2·5	950 950	$26 \cdot 2 \\ 24 \cdot 2$	0·1 0·1	25.5	$\begin{array}{c} 0.2 \\ 0.2 \end{array}$	27.0	0.3	Ŏ	0·5 0·3
Ditto (new sample)	6·0	2270	17.5	0.2	$\frac{24.5}{17.8}$	0.2	24·0 18·0	$\begin{array}{c} 0.1 \\ 0.2 \end{array}$	V	0.3
Divio (new sample)	6.0	2270	18.0	0.3	18.0	0.2	19.0	0.2	Ň	0.5
	6.0	2270	18.0	0.2	19.0	0.2	18.5	0.3	ő	0.5
Ditto (new sample)	4.0	1515	31.0	0.2	31.5	0.2	32.0	0.2	ŏ	0.4
Light from a reference solution of	f 0.01 males WMn	malam 10.0 ban	35.5	0.3	28.2	0-3	28.5	0.3	7.0	0.6
oxalic acid in water. (Passed	through a filter t	ronemitting 2000.	35-0	0.3	28.0	0.3	26.5	0.3	6.5	0.6
4000 Å)	mirough a misci s	imaminum 2000-	31.5	0.3	24.5	0.2	24.8	0.2	7.0	0.5
			30.0	0.3	24.0	0.2	24.3	0.2	6.0	0.5

touch the photocathode, spurious pulses are observed when liquids enter or leave the cell

Previous workers⁵⁻¹⁰ have specialized in ultra-low background counts which are increased slightly when the detector is exposed to dividing cells. This procedure is open to the objection that very low count rates are easily falsified by external interference of a temporary and unpredictable nature. We have preferred to accept the higher background counts from a large photocathode which can view a much larger volume of cell culture.

Suspensions of yeast or bacteria in their rapidly dividing logarithmic phase of growth (as shown by opacity measurements) were introduced into the cell by a syringe through a long, black plastic tube. The yeast and bacteria were suspended in a nutrient medium of soy trypticase broth which emitted detectable phosphorescence for up to 5 min after it entered the cell. After this no light could be detected from any of the yeast or bacterial suspensions listed in Table 1, although the light from the reference solution of Troitskii⁵ was easily measured with the same equipment.

We conclude that any radiation from these growing cells must be less than one-fifth of that from the reference solution quoted as a source of light comparable with mitogenetic radiation.

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Genetic Loads affecting Longevity in Natural Populations of Drosophila pseudoobscura

GENETIC loads consist of deleterious genes or gene complexes mostly recessive and concealed in the heterozygous state. The heterozygous individuals found in natural populations are usually superior in fitness to inbred progenies, which are made in part homozygous. This superiority is manifested in two ways: (a) the heterozygous combinations have a higher average in most components of the Darwinian fitness; (b) their phenotypic variability tends to be lower, because of the better buffering ability against environmental fluctuations1.

The components of genetic loads which reduce the viability of their carriers have been extensively studied, but considerably less information is available about the behaviour of other fitness components, particularly longevity. Many workers, however, have repeatedly observed the depressing effect of inbreeding on the mean life span of Drosophila2-5. Vetukhiv⁶ discovered a generalized heterosis for what concerns longevity, in crossing the strains of *D. pseudoobscura* from different localities. Spiess and Schuellein, on the other hand, working with different homo- and hetero-karyotypes of D. persimilis, found an average superiority of the heterokaryotypes in the rate of development, egg to adult survival and in fecundity, but not in longevity. This is why we decided to carry out a simultaneous analysis of the longevities of females, homozygous and heterozygous for the second chromosomes from natural populations of *Drosophila* pseudoobscura. An analysis of two other components of fitness-viability and fecundity-was also performed on the same material.

The samples of *D. pseudoobscura* were collected in November 1966 near Tucson (Arizona) by Dr W. Heed and at Borrego Valley (California) by Mr D. Richmond. To obtain the homo- and hetero-zygotes for wild second chromosomes, we used a standard technique⁸⁻¹⁰. longevity of the females was observed in sixty-seven homozygotes and in thirty-four heterozygous combinations, using three to five replications. Each replicate culture contained a group of ten flies which were kept in 9.5×2.5 cm vials with a synthetic medium and a constant amount of yeast11. The flies were placed in the vials after a mild etherization less than 24 h after emergence, and kept in an incubator at 25° C, with a humidity of 65-70 per cent. The numbers of dead flies were recorded on alternate days, and the medium was changed every fourth day. Fecundity and viability were studied in the same sixty-seven homozygotes and thirty-four heterozygous combinations, as described by Marinkovic¹⁰.

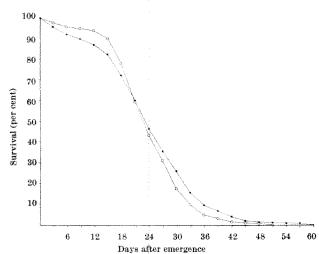
The mean longevities, viabilities and fecundities of the homozygotes and the random heterozygous combinations of wild second chromosomes are presented in Table 1. As was found earlier⁸⁻¹⁰, the viability and the fecundity of the homozygotes are significantly lower compared with the heterozygous controls (P < 0.001). The mean lon-

Table 1. Mean longevity (days), viability (per cent of wild type flies in the cultures) and fedundity (number of eggs laid in six days) of the females homozygous and heterozygous for wild second CHROMOSOMES

	Homozy $(N=0)$	gotes 87)	Heterozygotes $(N=34)$		
	x SE	S ²	x SE		
Longevity Viability	23.55 ± 0.77 28.88 ± 0.69	39·31 31·21	23.62 ± 0.63 34.12 ± 0.56		
Fecundity	151·3 ±7·3	3553.4	204·4 ± 8·0	2169-7	

gevities, however, are the same: the average longevity of the homozygotes is 23.6 ± 0.8 days and among the heterozygotes 23.6 ± 0.6 days. The variances in all three analysed characters are greater among the homozygotes (the variance ratios for the longevity and viability are significant at less than 0.02 and for the fecundity at P < 0.1, using a two-tailed test).

The correlation coefficients were calculated between the longevity and viability or fecundity of the homo- and hetero-zygotes. Among the homozygotes, a significant positive correlation was found between longevity and viability (r = 0.320; P < 0.02). Longevity and fecundity were also positively correlated, but the value is not significant (r = 0.183). Among the heterozygotes a high correlation was obtained between longevity and fecundity (r = 0.474) and a lack of correlation between the longevity and viability was found (r = 0.012). The calculated coefficients of correlation between longevity and viability or fecundity suggest a physiological relationship between them. A larger number of chromosomes, especially of heterozygotes, however, must be studied to ascertain the existence or non-existence of correlation of these characters.



-

and hetero-

Fig. 1 contains the survival curves of the flies which are homozygous (N = 2,206 individuals) and heterozygous (N = 1,184) for wild second chromosomes. The length of life among the homozygotes ranges from 1 to 60 days, while among the heterozygotes it ranges from I to 51 days. A probit test shows that a sigmoid function can be applied to both curves. Although the two curves look very much alike, the variance of the survival of the homozygous flies is greater than that of their heterozygous relatives ($F_{60^{\circ}48}=1.62$; P<0.1), giving a slightly higher level of probability than when the variances among the chromosomes and their combinations were compared.

These results suggest that (a) there is no difference in mean longevity between the homo- and hetero-zygotes, and (b) there is a statistical difference in the variances of these two groups.

Maynard-Smith12 pointed out a special importance of genes with sex limited effects on the longevity of D. subobscura, and Moriwaki and Tobari13 found that the Y chromosome is important in determining the longevity of the males in *D. melanogaster*. The lack of the difference in our experiment between the mean longevities of the homo- and hetero-zygotes for wild second chromosomes provides further information about the unequal role of factors located on different chromosomes in the determination of this character

Another property of the heterozygotes, namely, their smaller phenotypic variability, appears clearly in our results in all three analysed characters. It should be emphasized, however, that this variability is at the same time genotypic and environmental. The absence of difference in average longevity, associated with a significant difference in the variances of the homo- and hetero-zygotes, gives some indication of independence between these two parameters. On this basis, we would not agree with Rasmuson¹⁴ that an increase in environmental variance among the homozygotes is produced We would rather agree by their lower adaptedness. with Donald15 that a decrease in a component of the fitness and reduction of the buffering ability of the homozygotes are both concomitant consequences of inbreeding, and in the case of the factors studied, which are determining longevity, they seem to be independent.

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Transformation of Primary Rat Embryo Cells by a Weakly Oncogenic Adenovirus—Type 3

Although human adenovirus types 3 (ref. 1), 7 (ref. 2), 12 (ref. 3), 18 (ref. 4), 14, 16, 21 (unpublished work of M. Pina and M. Green) and 31 (ref. 5) have been shown to be oncogenic in hamsters and, in some cases, mice and rats as well, in vitro transformation has been reported only for "highly oncogenic" types 12 (refs. 6-9) and 18 (unpublished results of Rafajko and Huebner). primary rat embryo cells and a selective medium containing 0.1 mmolar calcium, a quantitative transformation system has been reported for adenovirus type 12 (ref. 9). It seemed that a similar system for the "weakly oncogenie" adenoviruses would be useful for quantitative studies of transformation by these two groups of adenoviruses, and this communication reports the transformation of rat cells by adenovirus type 3.

Primary cultures of cells derived from decapitated, near-term, inbred, Fisher rat embryos were planted in culture tubes with an inoculum of 105 cells/ml. in Hagle's basal medium¹⁰ supplemented with 10 per cent foetal bovine serum and 0.2 mmolar non-essential amino-acids. The cultures were fed on the third day and ineculated when confluent, usually on the fifth day after seeding. Adenovirus type 3, strain 15-520 (ref. 1) (titre 10° ID_{se}/ml. as determined by titration in primary human embryonic kidney (HEK) cultures), was inoculated into drained cultures, 0.1 ml. of virus stock in each tube. The purity of the adenovirus 3 pool was tested by breakthrough neutralization tests against type specific adenovirus type 3 rabbit antiserum in rhesus monkey kidney cultures¹ These tests were observed for 21 days, at which time 0.2 ml. of the culture fluid was blind passed into each of three HEK cultures. No cytopathic effect occurred in the HEK cultures during a 3 week observation period, indicating that the seed virus had been neutralized by adenovirus type 3 antiserum. Further, by means of complement fixation tests stock virus used in these experiments was found to be free of adenovirus associated viruses (AAV) types 1-4 (ref. 14). The cultures were incubated at 37° C for a 2 h adsorption period and the tubes were rotated at 15-20 min intervals. Low calcium medium (1 ml.)15,16 (Eagle's minimum essential medium without calcium, supplemented with 0.1 mmolar calcium chloride, 5 per cent dialysed calf serum, 2 per cent foetal bovine serum and 0.2 mmolar non-essential amino-acids) was added to each tube. The cultures were re-fed at 2-3 day intervals and were maintained in a stationary position in atmosphere containing 5 per cent carbon dioxide in air. Cultures were observed for at least 60 days.

No cytopathogenic effects were observed in the inocuted cultures. Thirty-five days after inoculation, there lated cultures. appeared, against a fibroblastic background, morphologically altered areas consisting of small epithelioid cells forming distinct domed foci (Fig. 1) similar to those previously described in primary rat embryo cultures transformed by adenovirus type 12 (ref. 9). Although the number of tubes with foci continued to increase up to 50 days after inoculation, the number of foci in each individual tube remained quite constant. At the end of 60 days some tubes contained no foci, some contained a single focus, sometimes 6-8 mm in diameter and some tubes contained two or three foci. At this low frequency of foci in a tube, any foci caused by re-seeding could not have altered the rate of transformation significantly. In each of two experiments, twenty-seven tubes/dilution were inoculated with virus doses ranging from 106 to 108 HEK ID₅₀/0·1 ml. Based on the original virus inoculum, the rate of transformation was linear, with approximately

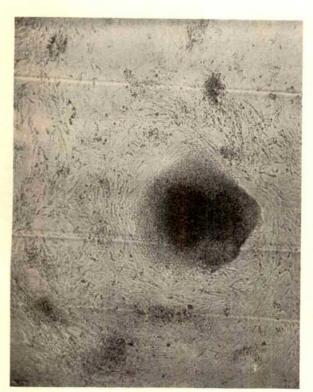


Fig. 1. Focus of rat embryo cells transformed by adenovirus type 3, 60 days after inoculation. (x c. 33.)

Table 1. RELATIONSHIP BETWEEN VIRUS DOSE AND THE NUMBER OF TRANS-FORMED FOCI

Virus dose	Transformed foci		No. of tubes counted*		Foci tube	
$(ID_{50}/{ m tube})$	Experi- ment 1	Experi- ment 2	Experi- ment 1	Experi- ment 2		Experi- ment 2
10* 10 ⁷ 10 ⁴	26 4 0	23 3 0	18 20 17	24 27 27	1·4 0·2 0	1·0 0·1

* In each experiment, a certain number of tubes were lost because they were not properly sealed.

Table 2. specific complement fixing antigens in rat embryo cells transformed by adenovirus 3

Antigen				
20 per cent extract line 8426	From hamsters Adeno 7	bearing tumo Adeno 12	urs induced by SV40	Convalescent adenovirus heterotypic human serum
Pa	4†	< 4	< 4	The second residence of the
Po	4	< 4	< 4	-
P 13	32	< 4	< 4	< 4
P_{18}	16	< 4	< 4	<4
P 29	32	< 4	< 4	< 4

1-2 transformation events occurring for each 108 infectious virus doses (Table 1). Based on the number of rat cells in each tube at the time of inoculation, approximately 0.0003 per cent of the cells were transformed by the inoculum containing the highest virus dose. Previously, it has been reported that approximately 106 infectious doses of adenovirus type 12 were required for one transformation event in rat cells and that 0.01 per cent of the cells were transformed9.

Some of the cultures bearing transformed foci were subdivided and six cell lines were derived from the two experiments. During the first ten to fifteen passage levels these lines contained low but significant complement fixation titres¹⁷ of tumour (T) antigen when tested against a pool of serum obtained from animals bearing transplanted tumours originally induced with adenovirus type This serum pool was used because it was difficult to obtain high titre sera from hamsters bearing adenovirus type 3 tumours1; adenovirus types 3 and 7 tumour antigens are known to cross react1. At a higher passage level, one of the cell lines transformed by type 3, No. 8426, produced tumour antigen with high complement fixation activity (Table 2). The results of a complement fixation test shown in Table 2 indicated that the rat cells had been transformed specifically by an adenovirus of the type 3-7 sub-group, because there was no evidence of T antigen induced by adenovirus type 12 or SV40. There was no serological evidence of infectious adenovirus in the transformed cells. The specificity of the transformation was substantiated further by the following tests. At the twenty-second passage level, the cells of line 8426 were stained directly with a hamster tumour (adenovirus type 7 induced) serum which had been conjugated with fluorescein isothiocyanate. Almost all of the cells contained the nuclear "fleck" antigen which has been described in adenovirus tumour or transformed cells6. Using SV40 hamster tumour sera, however, indirect fluorescent antibody studies were negative. Thus it was unlikely that an adenovirus 3-SV40 hybrid virus was responsible for the cell transformation because virtually all cells transformed by this hybrid contained the SV40 tumour antigen in the nucleus as determined by fluorescent antibody tests18.

Fujinaga and Green have reported that cell lines, derived from hamster tumours induced by adenovirus type 3, yield messenger mRNA which hybridized with the DNA of adenovirus types 3 and 7, but not with that of adenovirus types 12 or 18 (ref. 19). One of the rat embryo cell lines transformed by adenovirus type 3 was studied by these investigators and was also found to contain mRNA which hybridized with DNA from adenovirus type 3 but not with DNA from adenovirus type 12 (personal communication from M. Green and K. Fujinaga).

Not tested.
 4-8 units of serum was used in each test.
 Reciprocal of complement fixation titre.

To substantiate the results of complement fixation which suggested that cell line 8426 was free of infectious virus, a 20 per cent frozen and thawed extract of each cell line was inoculated and sub-passaged in ten primary human embryonic kidney cultures. Both sets of culture were observed for 21 days without evidence of infectious

Between the fifteenth and eighteenth passage levels 106 cells of each of three transformed cell lines were inoculated subcutaneously into newborn Fisher rats, and sixteen out of fifty-eight developed tumours 50-60 days after inoculation. Histological examination showed that these tumours contained uniform, small, "undifferentiated sarcoma" cells which were typical of those described in adenovirus induced tumours. A cell line was derived from one of the tumours and, at passage 3, a 20 per cent extract had an adenovirus 3 tumour antigen complement fixation titre of 1:4. Sera from animals bearing tumours 3-7 cm in diameter did not contain demonstrable levels of T antibody. When these tumours were transplanted into weanling rats, however, T antibody was found which, diluted 1:40, reacted with adenovirus 3 or adenovirus 7 tumour antigen. There were no tumours in eleven newborn rats inoculated subcutaneously with the slower growing control rat embryo cells which were at the sixth passage level.

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Fixation of Ejaculated Spermatozoa for Electron Microscopy

EJACULATED spermatozoa cannot be preserved satisfactorily by conventional fixation procedures for electron microscopy. Osmium tetroxide (OsO4) fixation of crude ejaculate consistently produces a variety of artefacts such as separation of the plasma membrane from the

acrosome, widening of nuclear vacuoles, erosion of the acrosome, and swelling of mitochondria1-3. These alterations could be the consequence of the rapid destruction of the fixative by the proteins of the seminal plasma. Similar results are obtained whenever the semen is washed either in salt solutions3,4 or in other media5-7 before osmium tetroxide fixation. In these cases, the structural alterations of the sperm are probably caused by osmotie imbalances. Fixation with glutaraldehyde is also unsuecessful in that it is associated with the typical artefacts of this fixative (our unpublished work).

Previous fixation experiments with a buffered pierie acid-formaldehyde (PAF) solution had shown that this fixative penetrates faster than conventional electron microscopy fixatives, and it is affected only a little by tissue fluids and plasma proteins8. Spermatozoa were very well preserved when the PAF fixative was tested on

human ejaculates.

The fixative, a modification of Bouin fluid, is prepared as follows: 20 g of para-formaldehyde is added to 150 ml. of a double-filtered, saturated aqueous solution of pierie acid. Dissociation of the para-formaldehyde into formaldehyde in the strong pieric acid solution is obtained by heat (about 60° C) and on alkalinization of the mixture with drops of a 2.52 per cent sodium hydroxide in water. Thus a clear solution is obtained, which is filtered in a cylinder, allowed to cool, and then made up to 1,000 ml. with a phosphate buffer used by Karlsson and Schultz for perfusion fixation of the central nervous system. The buffer is prepared by dissolving 3.31 g of NaH2PO4.H2O and 33.77 g of Na₂HPO₄.7H₂O in 1 l. of water. The PAF fixative has an osmolarity of 900 mOsm and a pH of 7.3. The fixative can be prepared in large quantities because it is very stable and withstands exposure to light at room temperature for 12 months without deteriorating.

The first two drops of the ejaculate are collected directly into 20 ml. of PAF at room temperature. The seminal plasma coagulates immediately into clumps of different sizes. These consist mostly of proteinaceous components of the seminal plasma precipitated in a granular form, and very few spermatozoa. These clumps, including those at the limit of visibility, are removed to obtain a nearly pure cellular suspension which is spun for 10 min at 1,000 r.p.m. The supernatant is discarded (fixation time is about 25 min), and the pellet of highly concentrated spermatozoa (Fig. 2d) is washed for 15 min in several changes of phosphate buffer. It is then post-fixed for 15 min in 10 ml. of 1 per cent osmium tetroxide¹⁰, and rapidly dehydrated. The pellet is finally reduced into small fragments and embedded in 'Epon 812'.

With this fixation procedure the spermatozoa show intact plasma membranes (Fig. 1 and Fig. 2a), well preserved acrosomes (Fig. 1b), nuclear vacuoles of a

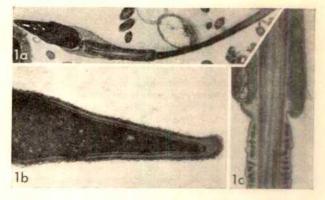


Fig. 1. (a) Longitudinal section of a spermatozoon in the human semen (×4,500). (b) Anterior portion of a sperm head. Note the integrity of acrosome and plasma membrane, and the close relationship between these two structures. The nuclear vacuoles are uniformly small (×16,500). (c) Transitional area between middle and principal piece of sperm flagellum (×22,000). All are stained with lead hydroxide.

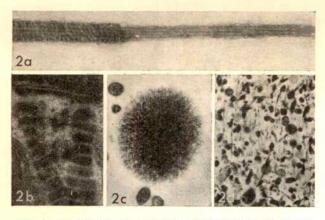


Fig. 2. (a) Principal and end pieces of the flagellum of a human ejaculated spermatozoon. The plasma membrane is continuous throughout and closely adherent to the underlying structures (\times 16,000), (b) Cross-section through the proximal centriole (\times 52,500), (c) Semian plasma components precipitated in a crystalline form (\times 8,750). (d) Light microscopic picture showing the high concentration of spermatozoa in the pellet. (Section about $\ln c$ in thickness, \times 675.) (a, b and c stained with lead hydroxide and d with toluidine blue.)

consistently small diameter (Fig. 1b), well preserved flagella (Fig. 1a and c, and 2a and b), and mitochondria of uniform density (Fig. 1a and c). Areas of separation between plasma membrane and acrosome are not observed (Fig. 1b). There are numerous crystals of a varying size among the spermatozoa (Fig. 2c). presence of these crystals, which obviously consist of inorganic components of the seminal plasma precipitated by the fixative, makes the blocks difficult to section. This inconvenience is eliminated by using very soft 'Epon' mixtures (7 parts of 'Epon A' and 3 parts of 'Epon B').

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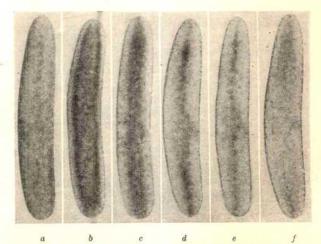
Vital Staining of Insect Eggs by Incorporation of Trypan Blue

MORPHOGENETIC movements which occur during the early stages of animal embryogenesis are difficult to study unless some kind of marking technique is used. In vertebrates vital staining of small cell patches1 or application of charcoal powder² has permitted the construction of maps showing extensive morphogenetic movements during early embryogenesis. Similar techniques have proved useful for the study of morphogenesis in other animal phyla. There are, however, no reports on successful intra vitam staining of insect eggs. This may be because the protective covers of most insect eggs do not permit dyes to enter locally, while eggs deprived of their covers cease to develop. Morphogenetic movements taking place during the plasmodial stages³ of insect Morphogenetic movements embryogenesis therefore had to be reconstructed from a series of fixed material4-6 or were analysed by using local defects as markers7,8 and by time lapse studies7,9,10. Defect marking more than other techniques involves the danger of misinterpretation. Time lapse studies and investigations on fixed material share the disadvantage that only a limited range of structures located in the egg plasmodium can be traced satisfactorily, for example, nuclei or special inclusions of the cytoplasm; furthermore, work with fixed material is technically difficult and cinematography fails to reveal movements within bulky eggs sufficiently clearly. Renewed attempts to overcome these handicaps have produced a technique which, although far from being as versatile as vital staining in amphibians, serves to reveal the type and extent of movements within the whole egg plasmodium of the house cricket (Acheta domesticus L.) during germ band formation and anatrepsis.

The technique is based on the observation 11 that trypan blue injected into females of Panorpa is incorporated into growing oocytes in a way similar to the incorporation of proteins from haemolymph into yolk bodies. A short pulse of trypan blue offered to growing oocytes of different sizes should therefore lead to incorporation of the dye into different concentric yolk layers. In the house cricket, this result is obtained by injecting 0.03 ml. of 0.7 per cent trypan blue dissolved in insect Ringer solution into virgin These are mated the following day and are allowed to oviposit 1 day later, when a certain percentage of the eggs will be stained in different layers of the yolk system (Fig. 1). The females survive and can be used for

another injection some days later.

In most eggs studied the stain was confined to the yolk bodies and remained so for at least the first half of embryogenesis; however, in very rare instances the trypan blue appeared to be attached to material which soon was taken up by the cytoplasmic islands of certain vitellophages. The percentage of stained eggs failing to develop varies somewhat but does not notably exceed the percentage observed in eggs of untreated females. The remaining eggs develop quite normally although trypan blue is highly teratogenic in vertebrates12; the development may merely proceed more slowly. Owing to the rather uniform distribution of the stain within the coloured layer the technique at present does not permit the construction of fate maps showing the positions of prospective organs. The marking of different egg layers, however, is very instructive so far as translocation of materials within the egg plasmodium is concerned. Eggs stained superficially corroborate on the whole the results of earlier studies¹³. Streaming movements of surface material collect many preblastoderm nuclei at two regions



Eggs of cricket with trypan blue incorporated into different yers of the yolk system. Unstained control at the right. Fig. 1. Eggs of cricket states layers of the yolk system.

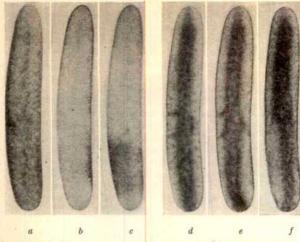


Fig. 2. Distribution of stained material in three corresponding stages of a superficially stained egg (a-e) and of an egg stained around its central core (d-f) as seen from the left-hand side.

situated dorsolaterally on each side of the posterior half of the egg. From there they are moved ventrally to form the germ anlage while more material is added from the anterior surface of the egg. Photographs taken at different stages of development reflect these movements by the varying distribution of stained material (Fig. 2a-c). Staining of deeper egg layers reveals that the movements shaping the superficial germ anlage are not limited to the surface. The surface streams are matched by extensive shifting of material deep inside the egg plasmodium. When the surface material moves dorsolaterally, the central mass of the posterior egg half is found displaced ventrally (Fig. 2b and e), while postero-ventral movements on the surface are compensated by dorsal and forward movements of central material (Fig. 2c and f). The whole yolk system is involved also in the initial stages of the movement of germ anlage around the posterior egg pole (anatrepsis)¹⁴. During this movement the plasmodial system is agitated by a series of slow and predominantly internal movements best described as internal peristalsis, and thereby becomes divided into separate yolk cells.

Incorporation of trypan blue into growing oocytes has been achieved in a number of other insect species as well. In these, however, differential staining of egg layers may not be equally good because it is influenced by varying parameters such as rate of protein incorporation into the ovary, speed of oocyte growth, and uptake of the stain by other organs of the mother. Nevertheless, the technique after suitable modification should prove useful for further research in insect embryology.

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Effect of Adenosine Diphosphate on Adhesiveness of Platelets in vitro and in vivo

Adenosine diphosphate (ADP) is known to cause reversible aggregation of human and animal platelets in vitro1-4. Hellem et al.5 used a column of glass beads6 to determine an increase, within certain limits, in the adhesiveness of platelets in response to ADP. Born and Cross and Chins have also shown that intravascular injection of ADP produces a rapid and transient decrease in the number of circulating platelets, which is probably caused by the adhesion of platelets to the endothelium and the aggregation of platelets leading to intravascular trapping?. and Stehbens, have used ear chambers to demonstrate the intravascular aggregation of platelets in rabbits caused by intravenous infusion of ADP

We have added ADP (Sigma Chemical Co.) to the citrated whole blood of healthy subjects in vitro to study the changes in platelet adhesiveness with the glass filter method10. As Fig. 1 shows, the adhesiveness began to increase 10 min after the addition of ADP, and had reached its maximum and returned to the initial value within 50 min. The decrease in the platelet count of filtered blood5,10 might thus be caused by the capture in the filter of the mass of aggregated platelets as well as the adhesion of platelets to the surface of the filter glass. Our experiments also confirm that the increase in the adhesiveness of platelets induced by ADP is reversible.

We have also studied the influence of intravascularly injected ADP on circulating platelets in rabbits. Fig. 2 shows the number of circulating platelets and the fluctuation of adhesive and non-adhesive platelets, measured by the glass filter method, after the injection of 10 mg of ADP in physiological saline into the marginal vein of the ear lobe of four healthy rabbits. After the intravenous injection of ADP, the circulating platelets started to decrease, reaching a minimum 20 min later, and then returned towards the initial concentration except in one rabbit. Together with the marked decrease in the circulating platelets there was a pronounced fall of the adhesive platelets, although non-adhesive platelets showed scarcely any change. No such decreases were seen when physiological saline alone was injected. Thus the decrease reported7,8 in circulating platelets in response to the injection of ADP was confirmed. The decrease in adhesive platelets which was seen at the same time can be explained by the intravascular reversible adhesion or aggregation of platelets in response to ADP which causes them to become trapped in the endothelium. On the other hand, the absence of changes in non-adhesive platelets is difficult

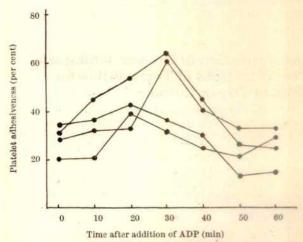


Fig. 1. Changes in adhesiveness of platelets after addition of ADP as a solution in physiological saline, in a final concentration of 0-1 μ g/ml., of citrated whole blood collected from four healthy donors. ADP added at zero time.

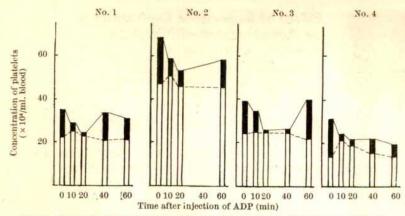


Fig. 2. Changes in circulating platelet count and the number of adhesive and non-adhesive platelets after intravenous injection of 10 mg of ADP into four rabbits, ADP injected at zero time. Columns represent circulating platelet count: _____, adhesive platelets; ______, non-adhesive platelets.

to explain, except by the difference in the dose of ADP used. The effect on the increase in the platelet adhesiveness of the concentration of the ADP added has certain limitations in vitro11. The mechanism of adhesion of platelets to a glass surface or to each other through the action of ADP is not yet clear, however, and a different reaction mechanism seems to be involved between platelet adhesion and aggregation. Further investigation is also necessary before it can be decided whether there are two different kinds of platelets—one which reacts with ADP and one which does not.

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Determination of Growth Inhibitory Concentrations of Tetracycline for Bone in Organ Culture

Long bones of premature children given tetracycline in therapeutically high dosages have shown up to 40 per cent growth inhibition during 2-4 weeks of treatment1. This effect was reversible, and approximately 2 weeks after the last administration of tetracycline the growth rate returned to normal. It is well known that large doses of tetracycline depress overall growth of chick embryos and mice2, but in other studies when therapeutic doses of tetracycline were given to rats and humans, growth inhibition was not observed^{3,4}. It seemed probable that growth inhibition was dose dependent and that perhaps growth inhibiting concentrations of tetracycline could be reached in premature human infants, and so we decided to determine whether tetracycline would inhibit

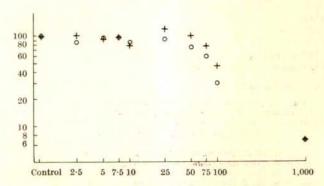
long bone growth in an organ culture system and, if so, at what concentration of tetracycline in the medium the inhibition The method developed by occurred. Proffit and Ackerman⁵, which evaluates growth of bone by measuring uptake of tritiated thymidine and of 14C proline from the culture medium, was used. Separate experiments indicated that, although there was some uptake of calcium-45 into bone spicules, there was no calcification of cartilage in this system. culture method is similar to that used by Saxén⁶⁻⁸, who observed effects of tetracycline on calcification of immature bone in vitro but did not describe an effect on growth.

Five day old rats were killed and four bones from each forepaw (first, second and third metacarpal and third phalanx) were placed on small sections of 'Millipore'

filter in a plasma-thrombin clot. The bones were then placed in culture tubes containing standard 199 medium, supplemented with penicillin-streptomycin and 10 per cent horse serum. Labelled proline and thymidine were added to monitor the synthesis of structural protein and DNA, respectively. Tetracycline was included in and DNA, respectively. varying concentrations from 0 to 1,000 µg/ml. The pH of the medium was found to be independent of the amount of tetracycline except at a concentration of 1,000 µg/ml. when a lowering of 0-15 U was observed. Bones were cultured in a roller drum apparatus at 37° C. After 6 days in culture, isotope uptake was evaluated by liquid scintillation counting of the residue after the dried bones had been burned. In addition, other bones were cultured in control and tetracycline containing media for histological examination.

Fig. 1 shows graphically the uptake of tritiated thymi-dine and ¹⁴C-proline. The decrease in uptake of tritiated thymidine is statistically significant (P < 0.01) at 50 The effects of tetracycline on the uptake of ug/ml. ¹⁴C-proline are significant at 75 μ g/ml. (P < 0.005).

Ultraviolet microscopic examination of bones cultured in medium containing tetracycline revealed the golden yellow fluorescence characteristic of tetracycline in the cartilage lacunae, in the fibrous capsule to a lesser extent, and in the calcified parts of the bones. Fig. 2 shows a control and an experimental section of the cartilage. Brightly fluorescing areas in the cartilage lacunae can be clearly seen. These fluorescent areas are probably not the nuclei, they are more likely to be in the cytoplasm of the cell. Loose binding of tetracycline to cytoplasmic elements has been noted with several other tissues9-11, but the particular affinity for maturing cartilage cells in this system is interesting.



Concentration of tetracycline in medium (µg/ml.)

Fig. 1. Uptake of tritiated thymidine and ¹⁴C-proline as a function of tetracycline concentration in the culture medium (control considered as 100).

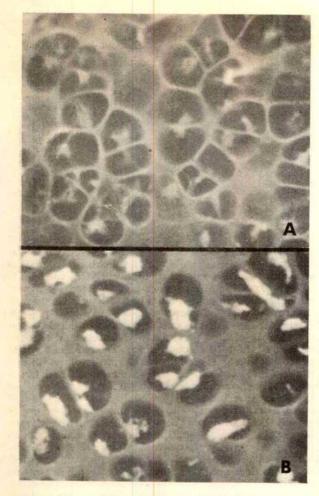


Fig. 2. Fluorescence photomicrographs of proliferating cartilage from rat metacarpal. A, Control, cultured in medium without tetracycline; B, experimental, cultured with tetracycline in medium (40 μg/ml.), Fluorescent areas, representing tetracycline, can be seen in the cartilage lacunae.

Finland¹² reported that in adult human males given therapeutic doses of tetracycline, the amount of drug circulating in the serum was about 4 µg/ml. From our data, which indicate that about 50 µg/ml. of tetracycline in the medium depresses long bone growth, it does seem possible that serum concentrations in premature infants given tetracycline could become large enough to depress long bone growth. The mechanism of direct incorporation of tetracycline into cartilage probably explains the inhibitory effect, for growth of long bones in vivo and of the metacarpals used in these in vitro experiments depends largely on proliferation of cartilage cells. It is probable that the tetracycline is much less tightly bound to cartilage than to calcifying bone, and that growth of the bones for a short time in a medium not containing tetracycline would remove the drug from the cartilage cells.

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MICROBIOLOGY

Influence of Pulmonary Dust Load on the Development of Experimental Infection by Mycobacterium kansasii

For a long time1 it has been known that the experimental infection of guinea-pigs could be aggravated by exposure to silica particles. Many surveys have tried to specify the interactions: "dust-infection" into lungs. We have determined the pathogenic role of Mycobacterium kansasii in guinea-pigs exposed to the inhalation of mineral dust particles²⁻⁷ and have established the following facts. (a) Dust particles aggravate experimental infection by Mycobacterium kansasii, and dusting is liable to make pathogenic a certain dose of bacteria which, without dust, would not induce lesions. (b) There is increased severity of the pulmonary lesions when there is dusting before, but not after the inoculation of an infecting dose of about 5 x 105 micro-organisms. (c) The previously established absence 8-11 of increased virulence of the strains after passage and extensive multiplication in dusted animals is confirmed and so is (d) the exacerbation and precociousness of tuberculin-allergy after the double

aggression of dust and mycobacterial infection.

We have assessed the importance of the quantity of dust particles contained in the lungs12,13 and have used a precise qualitative and quantitative means of dusting. Mycobacterium kansasii, the part of which in human pulmonary pathology is recognized, was given intravenously to avoid the over-traumatizing intratracheal route. Three series of experiments were performed with guinea-pigs weighing approximately 500 g each, and with three different kinds of dust: group 1, 98 per cent coal and 2 per cent quartz (final pulmonary concentration in quartz was 5 per cent); groups 2 and 3, 85 per cent coal and 15 per cent quartz (final concentration in quartz was 30 per cent). Coal was estimated by the method of King and Gilchrist*, and quartz by King's method¹4. Dusting was effected in automatically regulated cages, during 3 and 6 days, and 2, 3, 6 and 8 weeks, in order to obtain increasing amounts of dust particles in the lungs. The members of each experimental group were injected intravenously, on the same day, with Mycobacterium kansasii, variety luciflavum strain WR. Group 1 received 800,000 bacilli, group 2 770,000 bacilli and group 3 650,000 bacilli. Groups 1 and 3 were killed after 6 weeks and group 2 after 12 weeks.

The weight of the guinea-pigs was checked, weekly systematic pulmonary radiography was carried out before infection and on days 30 and 45 after inoculation of bacteria, and, on the same days, tuberculin allergy was investigated. At the end of the experiments, the lesion index was recorded for each animal according to a preestablished scale; the right lungs were removed for bacteriological examination, and the viable mycobacterial units were counted, while the left lungs and mediastinal lymph nodes were kept for histopathological study. The extent of the specific lesions, with respect to the total pulmonary parenchyma, was estimated in the left lungs by cutting off and weighing the projections of the histological lesions on 'Kodatrace' paper.

The changes in weight, radiological opacities and tuberculin reactions are of little significance, and the intensity of the lesions in each experimental group was estimated from the number of bacilli cultivated from the

Experi- mental group	Dusting	Pulmonary Total dust	dust content	Infection (<i>Mycobacterium kansasii</i>) Infecting Time of		Mean lesion Mean lesion and and histological		No. of bacilli	
	Nature of the dust particles	Duration (days)	particles (mg)	Quartz (per cent)	dose	observation	anatomical indexes	indexes (left lung)	(right lung)
1	Coal (98 per cent) + quartz (2 per cent)	3 7 14 21 42	13·4 26·6 34·5 62·2 83·5	3·25 5·1 5·75 5·0 6·1	$8 \times 10^{\circ}$ bacilli	6 weeks	1·87 4·31 3·51 5·03 6·64	1·40 6·90 13·19 12·84 24·83	$\begin{array}{c} 50 \times 10^3 \\ 33 \times 10^2 \\ 424 \times 10^2 \\ 462 \times 10^2 \\ 676 \times 10^3 \end{array}$
2	Coal (85 per cent) + quartz (15 per cent)	3 7 14 21 42	9·5 13·7 27·7 37·8 68·0	24·0 32·5 36·9 35·9 41·4	7.7×10^{5} bacilli	12 weeks	1·87 1·86 3·38 2·98 5·69	0·02 1·03 0·71 2·69 10·62	$\begin{array}{c} 8\times 10^{8} \\ 15\times 10^{9} \\ 117\times 10^{2} \\ 596\times 10^{2} \\ 6,730\times 10^{2} \end{array}$
3	Coal (85 per cent) + quartz (15 per cent)	3 7 14 21 42 56	$\begin{array}{c} 6 \cdot 2 \\ 11 \cdot 5 \\ 12 \cdot 8 \\ 15 \cdot 7 \\ 64 \cdot 5 \\ 65 \cdot 3 \end{array}$	38·7 23·8 33·0 44·1 34·3 32·5	6·5 × 10° bacilli	6 weeks	0·74 0·86 2·24 2·52 5·46 6·64	2·19 0·89 5·24 4·17 14·49 18·78	$\begin{array}{c} 45 \times 10^2 \\ 86 \times 10^2 \\ 1,295 \times 10^2 \\ 1,425 \times 10^2 \\ 3,765 \times 10^2 \\ 3,296 \times 10^2 \end{array}$

right lungs, the anatomical lesion index and the histological lesion index.

There is a statistical correlation between the duration of dusting and the amount of dust detained in the lungs on the one hand, and the intensity of the tuberculous lesions on the other hand. In each group of guinea-pigs the coniotic content of the lungs increased in proportion with the duration of dusting (Table 1). There is a very close correlation between these two parameters. nodular lesions enumerated on the surface of the pulmonary lobes were all the more numerous and voluminous as dusting increased and the dust content became large. The statistical correlation between these two parameters is remarkably significant.

The extent of the tuberculous lesions, assessed by graphical analysis of the histological sections, is proportional to the quantity of dust particles inhaled, whatever may be the initial content of quartz particles (2 or 15 per cent) in the mixture of coal and quartz employed. The importance of the final pulmonary concentration in quartz (comparison of groups 1 and 3) has not promoted the spread of the tuberculous lesions, probably because of the protective power of coal with respect to quartz, a

property which we have recently detected 15

The histological aspect of the lesions remained identical after 6 weeks, in all the animals of groups 1 and 3. The lesions consisted of tuberculous nodules frequently with necrotic centres containing dust. In contrast, the cellular reaction area constituting the tuberculous granuloma was rather poor in mineral particles. It was surrounded by a peripheral area devoid of real fibrous capsule, but in which the alveolar septa seemed to be thickened and infiltrated. The alveoli in process of collapse are coated with cuboidal cells and enclose pulmonary macrophages heavily loaded with mineral particles.

After 3 months' infection (group 2), the lesions were more fibrous and less extensive than in the animals killed 1.5 months after mycobacterial inoculation. The absence of fundamental distinctive histological characteristics between the lesions observed in these animals and those exposed to the inhalation of a mixture poorer in quartz seems to be worth recording. The spontaneously regressive characteristics of the tuberculous histological lesions progressively lessened while the amount of pulmonary dust particles increased. The comparison between the bacteriological and anatomo-pathological results shows that, beyond a certain limit in dust load, the tuberculous lesions become spontaneously irreversible (during our periods of observation).

In each of the experiments, the number of the viable mycobacterial units isolated from the right lungs was proportional to the dust content of the lungs. In this respect, the coefficients of statistical correlation are very significant. For the same dusting (a mixture of 85 per cent coal and 15 per cent quartz), extended to periods of 3-20 days, fewer bacilli were isolated from the right lungs after 3 months infection than after 1.5 months. This bacteriological aspect of the regressive characteristics of the tuberculous lesions is no longer observed when the dust content exceeds a certain threshold. Beyond this threshold, tuberculosis was spontaneously irreversible.

These experiments on guinea-pigs infected by Mycobacterium kansasii, after a more or less long inhalation of a mixture of coal and quartz particles in variable proportions, show the existence of a linear relationship between the quantity of dust particles in the lungs and the importance of the tuberculous lesions. The excellent statistical correlation observed between the means of the various criteria (dust content, indexes of anatomical and histological lesions, counting of the viable mycobacteria in the lungs) emphasizes the proportionality between the importance of the dust deposits in the lungs and the aggravation of the infection. For light dustings, involving a moderate charge, the pneumoconio-tuberculous lesions prove spontaneously regressive 3 months after myeobacterial inoculation. Nevertheless, this regressive aspect of the lesions does not appear any more after an important dusting involving a higher dust content. This fact, supported by bacteriological and anatomo-pathological arguments, involves the existence of a threshold in the pulmonary dust content beyond which specific lesions become spontaneously irreversible.

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effects of Anti-thymocyte Serum on 17-D Yellow Fever Infection in Adult Mice

Many neurotropic viral infections are accompanied by inflammatory cellular infiltrates of the central nervous system. With some of these viruses, notably lymphocytic choriomeningitis (LCM), the cellular response is believed to be responsible for the morbidity and mortality associated with infection1, and suppression of the lymphocytic response by treatment with anti-thymocyte serum prevents clinical signs of disease2,5. Yellow fever infection caused by the 17-D virus in mice is characterized by a diffuse encephalomyelitis consisting of mononuclear cell infiltration of leptomeninges and perivascular areas, as well as neuronal degeneration and glial proliferation4. We have used rabbit anti-mouse thymocyte (RAMT) serum to prevent cellular responsiveness to 17-D virus, in order to evaluate the role of delayed hypersensitivity in the pathogenesis of experimental yellow fever.

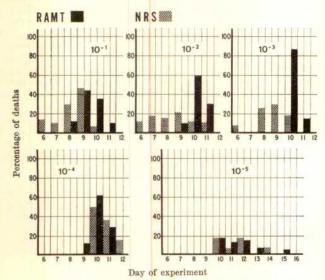


Fig. 1. Comparison of times of death in groups treated with normal rabbit serum (NRS) or rabbit anti-mouse thymocyte (RAMT) serum, at virus dilutions from 10⁻¹ to 10⁻².

Adult ICR mice of both sexes were used in all experiments. RAMT serum was prepared as described by Levey and Medawars, and assayed by its ability to (a) diminish peripheral blood lymphocyte counts by 50 per cent within 4 h, and (b) prevent clinical and histological evidence of lymphocytic choriomeningitis in ICR mice inoculated with LCM virus when 1 week old. All RAMT sera were shown to be free of anti-yellow fever activity by mouse neutralization assay. The virus used was a 20 per cent w/v suspension of suckling mouse brain of 17-D vaccine strain passed three times in adult mice and twice in suckling mice. It contained 104.4 LD 50/0.03 ml. when titrated by intracerebral inoculation of adult ICR mice. A hundred and seventy-two mice were divided into two equal groups, one which received RAMT serum, the other normal rabbit serum (NRS). The sera were intraperitoneally administered in 0.3 ml. volumes at 3 day intervals beginning 1 week before virus inoculation, and ending at the termination of the experiment. The virus was intracerebrally inoculated on day 0 in 0.03 ml. volumes, in dilutions of 10-1-10-6. The animals were observed daily for signs of clinical illness. Animals which died were autopsied, and sections of their organs were examined by light microscopy. Titrations of individual brains from both groups killed on the same day were performed by intracerebral inoculation of adult mice.

At low dilutions of virus (10⁻¹, 10⁻², 10⁻³), mice treated with NRS developed clinical illness 1-2 days earlier than



Fig. 2. Brain section of mouse treated with 10⁻⁴ dilution of 17-D virus and NRS, dying 12 days after infection. Meningeal and perivascular inflammatory infiltrates present. Stained with haematoxylin and eosin (× 525).

their counterparts treated with RAMT serum. As shown in Fig. 1, the times of death at these dilutions were also delayed by treatment with RAMT serum, although only at two dilutions $(10^{-2}, 10^{-3})$ were the differences highly significant $(P < 0.01, \text{ using the } \chi^2 \text{ approximation of the Kolmogorov–Smirnov non-parametric test). At higher dilutions <math>(10^{-4}, 10^{-5})$ no differences in morbidity or mortality were observed between the two groups. Other than the times of onset and death at low dilutions, the clinical features of the illness in both groups were identical. The animals first became hypokinetic, and shortly afterwards they developed progressive and ascending flaccid paralysis, ruffled fur, conjunctivitis and dehydration.

Histological examination revealed marked differences between dying animals of both groups at all dilutions. While animals treated with NRS showed characteristic leptomeningeal and perivascular infiltrates of brains, these findings were nearly absent in animals treated with RAMT serum (Figs. 2 and 3). Neurones in both groups showed moderate degenerative changes with focal decreases in Nissl substance, pyknosis of nuclei and cell liquefaction. Virus titres in brains of animals killed on the same days were equivalent in both groups at all dilutions, despite the differences in histological findings.

These studies suggest that the inflammatory reaction to 17-D virus in adult mice plays a slight but significant potentiating part in the development of morbidity and mortality after virus inoculation at low dilutions. That the antiserum is not acting against either the virus itself or the target cell of the virus is indicated by (a) the absence of 17-D neutralizing activity in RAMT serum and (b) the absence of differences in virus titre between the experimental and control groups. It is also clear, however, that the cellular response is not of primary importance in the pathogenesis of 17-D infection in adult mice, as it is in lymphocytic choriomeningitis virus infection*,3; neither is the cellular response apparently necessary for protection from the effects of the virus, as evidenced by equal LD_{50} titres in both groups. The reasons for the lack of protective effect of RAMT serum at higher virus dilutions are obscure. Perhaps with the smaller virus doses and subsequently longer incubation periods, other host mechanisms, such as humoral antibody formation

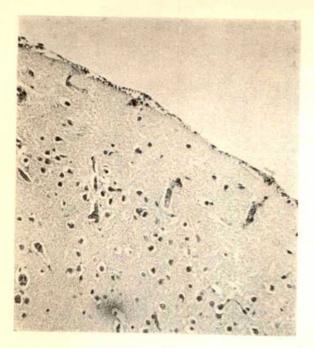


Fig. 3. Brain section of mouse treated with 10⁻⁴ dilution of 17-D virus and RAMT serum, dying 12 days after infection. Inflammatory infiltrate minimal. Stained with haematoxylin and cosin stain (×525).

or reticulo-endothelial activity, are more important defence mechanisms than delayed cellular immunity.

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IMMUNOLOGY

Inhibition of Complementary Lysis by Rheumatoid Sera

In suitable conditions, anti-γ-globulin inhibits lysis of sensitized red cells1. Rheumatoid factor is apparently an antibody against γ-globulin, and so it might be expected to do the same. This report describes the results of "inhibition of lysis" titrations on sera from normal subjects and patients with rheumatoid arthritis, and shows that the correlation with sensitized sheep cell agglutination tests and latex fixation tests is high.

Heimer et al.2 have reported that whole rheumatoid sera delay the lysis of sensitized sheep erythrocytes rather more when human heterophil antibody, as opposed to rabbit-produced amboceptor, is used to sensitize the cells. This inhibition of lysis they attributed chiefly to a material designated "inhibitor (Ag-Ab)" which was distinct from rheumatoid factor^{2,3}, although it may be simply the moiety of rheumatoid factor with primary specificity for human-γ-globulin4. The amount of "inhibitor (Ag-Ab)" correlates well with latex fixation titre⁵.

Most of Heimer's work^{2,3,5} is related to the ability of "inhibitor (Ag-Ab)" to inhibit complement fixation by a

specific precipitate (human thyroglobulin and rabbitproduced antibody against it) and is less relevant to the present work, although the mechanism involved may be the same. Schmid and Roitt⁶ have also briefly reported inhibition of complementary lysis using rheumatoid materials and have found good correlation with agglutination tests for rheumatoid factor.

In the present work, the sensitivity of the test for inhibition of lysis was increased in several ways, as follows. Romeyn and Onysko1 have shown that complement (C') and anti-γ-globulin compete for sites on sensitized cells. Furthermore, because the anti-y-globulin delays rather than inhibits lysis entirely, the equilibrium between sensitized cells and anti-y-globulin appears to be a dynamic one in which molecules of anti-y-globulin are released and later re-attach themselves so that gradually the C' can attach and accomplish lysis of most of the cells. Furthermore, any other material in the suspending fluid, notably free \gamma-globulin, can unite with anti-y-globulin and inhibit its attachment to sensitized sites. Thus three things are done to increase the sensitivity of the test: first, the sensitized cells are washed free of other rabbit serum components before adding to the test sera; second, the sera are allowed to react with the sensitized cells for 20 min before C' is added; and third, the final lytic incubation after C' is added is shortened to 20 min. In such tests it is also necessary to ensure that inhibition of lysis is not simply caused by destruction of C' and the method for doing this is described

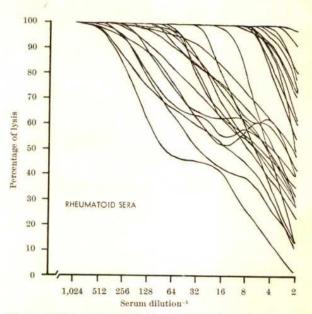


Fig. 1. Inhibition of haemolysis titration curves of sera from twenty-seven rheumatoid arthritis patients.

The technique was essentially that described by Romeyn and Onysko¹ except that group O Rh-negative human cells and amboceptor produced by rabbits against them were used instead of sheep erythrocytes, which would have been lysed by heterophil antibody in the test sera. Briefly, the test involves serially diluting the serum, adding erythrocytes sensitized with $3\,HD_{50}$ amboceptor (washed after sensitization and before use in the test) and incubating for 20 min at 37° C. Guinea-pig C' is then added, $4HD_{50}$, and the mixture incubated for a further 20 min at 37° C. After centrifugation in the cold, the supernatants are read spectrophotometrically against blanks in which untreated red cells are used instead of sensitized cells. Percentage lysis is calculated, using as 100 per cent lysis the reading obtained in a tube in which the serum is replaced by saline.

Any reduction in lysis to less than 100 per cent might be caused by destruction of complement or protective union of rheumatoid factor with the sensitized cells. The test to rule out C' destruction depends on a substantial though not absolute specificity of rheumatoid factor for rabbit as opposed to guinea-pig-γ-globulin. Therefore. to 3.0 ml. of the supernatants are added after reading 1.0 ml. amounts of rabbit erythrocytes sensitized with amboceptor produced by guinea-pigs and then washed. After incubation for 20 min at 37° C and centrifugation, spectrophotometric readings are again made using the same blanks as before. The resultant increase in optical density approaches (but does not quite reach) 100 per cent lysis of the rabbit erythrocytes, indicating no substantial destruction of complement in the original test. This test is carried out after all inhibition of lysis titrations.

It is worth remarking that, when complement fixation tests are performed on rheumatoid sera (for syphilis, for example), the sera are often reported to be anti-complementary. It seems probable from our experience that what is actually occurring in the serum control is not

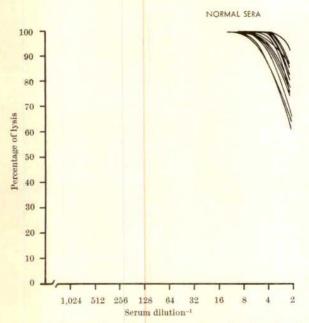


Fig. 2. Inhibition of haemolysis titration curves of sera from twenty normal humans.

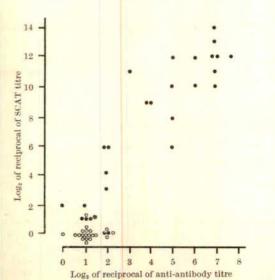


Fig. 3. Scatter diagrams of sensitized sheep cell agglutination titres (SCAT) and inhibition of haemolysis titres on twenty-seven rheumatoid and twenty normal sera. ●, Rheumatoid; ○, normal.

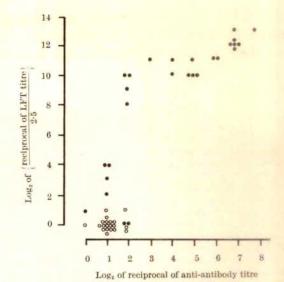


Fig. 4. Scatter diagrams of latex fixation titres (LFT) and inhibition of haemolysis titres on twenty-seven rheumatoid and twenty normal sera. •, Rheumatoid; O, normal.

complement destruction but inhibition of lysis through the protective mechanism operative in the presently reported tests.

Figs. 1 and 2 show the titration curves for twentyseven sera from rheumatoid arthritis patients (ages 25-78 yr, mean 51 yr, 78 per cent female) and twenty sera from normal subjects (ages 23-60 yr, mean 42 yr, 65 per cent female), respectively. It is clear that the rheumatoid sera differ markedly from the normal sera, although there is some inhibition of lysis produced by normal sera at high concentration (1:2 and 1:4).

Sheep cell agglutination titres (SCAT) and latex fixation titres (LFT) were also determined. For comparison with these, the "inhibition of lysis titre" was defined as the least concentration of serum required to produce at least 7.5 per cent inhibition of lysis, using the initial serum concentrations before the addition of cells and complement. Figs. 3 and 4 are scatter diagrams showing the relation of inhibition of lysis titres to SCAT and LFT. Log to a base 2 of the reciprocals of the SCAT titre and of

reciprocal of LFT titre is plotted on these figures (and

was also used in calculating the correlation coefficients). The figures show a high positive correlation between the tests. The correlation coefficient relating inhibition of lysis titre to SCAT was 0.89 (P=0.001), and relating inhibition of lysis titre to LFT was 0-81 (P = 0.001)

Although inhibition of lysis correlates well with SCAT and LFT, in this small series it was not more sensitive or more specific in distinguishing normals from patients.

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Immunological Cross-reactivity between Plasma Membrane and Endoplasmic Reticulum of Ehrlich Ascites Carcinoma

MICROSOMAL membranes of Ehrlich ascites carcinoma can be separated into membrane classes of high and low density, which on the basis of enzyme, physiochemical and immunological criteria 1-4 are considered to be derived from endoplasmic reticulum and plasma membrane, respectively. Because the membranes from the endoplasmic reticulum lack the antigens involved in the agglutination and/or immune lysis of intact Ehrlich ascites carcinoma by rabbit and horse antisera directed against microsomal membranes of Ehrlich ascites carcinoma^{1,2}, we wished to test, by means of antibody-binding, whether there are not some antigens which the two membrane classes have in common.

Plasma membrane, endoplasmic reticulum and immune horse immunoglobulin were prepared as in earlier work^{1,2}. The immunoglobulin was labelled with 14C-acetic anhydride5 without loss of agglutinating activity from the carcinoma. Antibody absorption by plasma membrane and endoplasmic reticulum was measured by equilibrating stated amounts of membrane and immunoglobulin in 1 ml. of 0·15 molar sodium chloride, 0·005 molar calcium chloride, 0.01 molar tris hydrochloric acid (pH 7.4) for 16 h at 0° C, and then for 0.5 h at 37° C. The samples were then made to 5.0 ml. with the buffer and centrifuged for 45 min at 50,000 r.p.m. and 4° C (Spinco L-2 ultracentrifuge, rotor SW 50). The supernatants were decanted, the tubes fully drained and the pellets resuspended in 5.0 ml. 0.15 molar sodium chloride and 0.005 molar calcium chloride. After another centrifugation at 50,000 r.p.m. and 4° C for 45 min. the supernatants were again decanted, the tubes drained and carefully blotted on the inside and the pellets suspended in 0.5 ml. 0.15 molar sodium chloride. Samples (0.05 ml.) of these suspensions were mixed with 1.0 ml. of hydroxide of hyamine 10x (Packard Instrument Co.) and 10 ml. of Bray's solution and counted to 5 per cent error in a Tricarb liquid scintillation counter. The supernatants and appropriate dilutions of unabsorbed immunoglobulin were counted in the same way. All counts were carried out in duplicate as was each absorption experiment. We used two different membrane preparations and two lots of labelled antibody prepared from the same antiserum. To measure antigens common to endoplasmic reticulum and plasma membrane the immunoglobulin was first absorbed with varying amounts of endoplasmic reticulum as described. The pellets of endoplasmic reticulum were then removed by centrifugation and samples of the supernatants equilibrated with a constant amount of plasma membrane for $16\,\mathrm{h}$ at $0^{\circ}\,\mathrm{C}$ and $30\,\mathrm{min}$ at $37^{\circ}\,\mathrm{C}$. The plasma membrane pellets were then sedimented, washed and counted. Preliminary studies showed negligible absorption of normal horse immunoglobulin and showed that the washing procedure used was adequate to reduce adventitious counts to insignificant levels. Labelled immunoglobulin was used as the internal standard and results are thus expressed as μg of immunoglobulin absorbed by plasma membrane or endoplasmic reticulum.

Absorption tests with plasma membrane show that, below an immunoglobulin: membrane weight ratio of 2, the amount of antibody absorbed is constant (about 0.04 mg/mg). Specific absorption declines with higher proportions of antigen and is about 0.02 mg/mg at a 1:1 With endoplasmic reticulum (Fig. $\bar{1}$, curve a) specific antibody absorption progressively diminishes as the proportion of antigen increases. Maximum antibody binding is attained at an immunoglobulin: endoplasmic reticulum ratio of 0.5. Further increases in the proportion of endoplasmic reticulum do not lead to additional absorption of antibody. When a given amount of antibody (0.7 mg) is first equilibrated with increasing amounts of endoplasmic reticulum (as in Fig. 1a), and, after removal

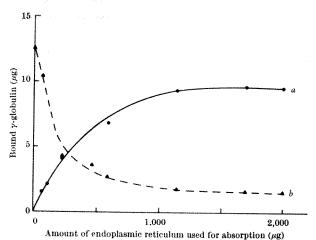


Fig. 1. Antibody-binding by plasma membrane and endoplasmic reticulum. (a) —— Antibody-absorption curve of endoplasmic reticulum. 0-7 mg of immunoglobulin was equilibrated with the amounts of endoplasmic reticulum shown, under the conditions given in the text. (b) A - - - A, Decrease in the antibody bound by 0-5 mg of plasma membrane after prior absorption of the immunoglobulin with increasing amounts of endoplasmic reticulum.

of the endoplasmic reticulum, is absorbed with a constant amount (0.5 mg) of plasma membrane, curve b in Fig. 1 results. This shows a reduction in the amount of antibody bound to plasma membrane from 0.025 mg/mg of membrane in the controls to 0.003-0.004 mg/mg after absorption with excess endoplasmic reticulum.

These results, which show that there are antigenic sites on plasma membrane which are either lacking or inaccessible in endoplasmic reticulum, are consistent with the previous demonstration1,2 of the lack of absorption by endoplasmic reticulum of horse anti-Ehrlich ascites careinoma agglutinating antibody. On the other hand, the data also imply that the two membrane classes, although differing in a number of critical features, have several structural components in common.

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Effects of Phytohaemagglutinin on Homograft Rejection

Although phytohaemagglutinin (PHA) has been widely investigated in vitro, relatively little is known about its effects in vivo1. It has been used in humans with aplastic anaemia, who subsequently showed evidence of increased bone marrow activity in some cases2. Gamble3 found that intravenous injection of PHA in mice resulted in increases in the cellularity, the proportion of immature cells, and the weight of the spleen, which were most marked 3 days after administration. Calne et al.4 used combined PHA and azathioprine ('Imuran') therapy in an attempt to prolong the survival times of renal homotransplants in dogs. Intravenous PHA potentiated the immunosuppressive action of azathioprine and was thought to have some immunosuppressive activity when used alone.

Because of the stimulating effect of PHA on lymphocytes in vitro, it is conceivable that there would be a profound effect on the immune system of the organism if adequate concentrations could be attained in vivo. If generalized de-differentiation occurred in the lymphoid system of the organism after administration of PHA, it is possible that the ability to respond to antigenic stimulus would be severely compromised. The work reported here was designed to determine whether PHA in vivo has any effect on the ability of a host to reject a skin homograft.

Mixed male and female closed colony White Swiss and inbred virgin C57BL mice, aged 2-3 months, and weighing between 27 and 44 g, obtained from Roswell Park, Buffalo, New York, were used. PHA was injected in doses of 0.5 ml. into the tail veins of mice with sterile tuberculin syringes and 27 gauge needles and the maximum dose tolerated was determined by injecting increasing amounts of PHA. Most of the animals survived a dose of 0.5 ml., whereas most animals died after injections of 1.0 ml. Phytohaemagglutinin M (General Biochemicals) used in this project is apparently not as toxic to mice as PHA-M (Difco), which has been used extensively by other investigators. Whereas Micklem⁵ found a mortality rate of 50 per cent using 0.4 ml. of PHA (Difco), most of the animals we used survived the standard dose of 0.5 ml. PHA (General Biochemicals). Several of the mice in group 1, however, showed gross hepatic necrosis on autopsy which was apparently similar to that found in 50 per cent of Micklem's animals.

The mice were divided into four groups (Table 1). Group 1 consisted of eight White Swiss male (WSM) and eight White Swiss female (WSF) mice. Four were killed immediately and the remaining twelve animals were given 0.5 ml. of PHA and killed on subsequent days in groups of two, beginning 2 days after injection. Body weights, spleen weights and peripheral white blood cell counts were recorded and spleen smears were made. Group 2 consisted of five WSM and five WSF mice which received 0.5 ml. of PHA and the skin homograft on the same day. Another dose of PHA was given 3 days later. The technique of skin grafting and observation is described in ref. 6. Group 3 consisted of five WSM and five WSF mice which received 0.5 ml. of PHA 3 days before the skin graft, on the day of the graft, and 3 days after it. Group 4 consisted of four animals treated like those in Group 3, except that they were injected with saline rather than PHA.

After necropsy of the animals in Group 1, the spleen was placed on a dry filter paper and immediately weighed

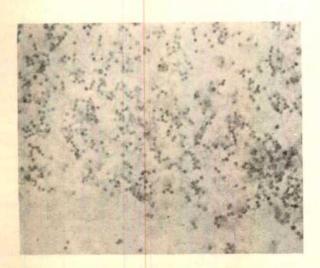


Fig 1. Low power magnification of a control spleen smear.



Fig. 2. Low power magnification of a spleen smear, 4 days after administration of PHA.

on a Mettler analytical balance to the nearest 0.0001 g. The spleen was macerated between two glass slides and the smear stained with Wright's stain.

Peripheral white blood cell counts on the animals in Group 1 revealed counts ranging between 6,100 cells/mm³ and 16,850 cells/mm³. No trend was established during the 6 days after administration of PHA. The peripheral white blood cell count was inconclusive as a criterion of the *in vivo* effect of PHA, perhaps because of many variables in production, release, and sequestration of leucocytes.

Gamble³ found earlier that PHA affected spleen weight and cellularity, and we confirmed this (Table 2). The average spleen weight of the control animals was 0·1144 g, and the average ratio of spleen weight to body weight was 0·00286. The values obtained from the animals which received PHA were moderately elevated with very little overlap into the control group, both in splenic weights and in the ratios of spleen weight to body weight.

Figs. 1-4 illustrate the differences observed in the spleen smears under high and low power magnification. Fig. 1

Table 1. SPLEEN WEIGHTS AND SPLEEN WEIGHT/BODY WEIGHT RATIOS AFTER INTRAVENOUS ADMINISTRATION OF PHA

Days after PHA	Sex	Spleen weight	Body weight	Ratio
2	m	0.1465	38	0.00386
7-	f	0.1453	39	0.00373
3	m	0.1685	33	0-00510
	f	0.1395	34	0.00410
4	m	0.1232	37	0-00334*
	f	0.1622	35	0.00464
5	m	0.1689	41	0.00412
	f	0.1786	34	0.00526
6	m	0.1265	35	0.00362
	f	0-2365	35	0.00678
9	m	0.1142	38	0.00301
	f	0.1325	31	0.00427
Controls	m	0-1225	44	0.00278
C CARCA SEE	f	0.1351	40	0-00338
	m	0.1207	39	0-00309
	1	0.0795	37	0.00214

The average spleen weight of mice, 2–9 days after administration of PHA, is 0-1563 g as compared with the control average of 0-1145 g. The average ratio of spleen weight/body weight in animals treated with PHA is 0-00433; average control animal ratio is 0-00286. t value on these ratios = 2-54. (P < 0.05, P > 0.01.)*A portion of this animal's spleen was lost during splenectomy.

	Table 2.	HOMOGRAFI	REJECTION	TIMES IN	GROCES	24.0	Wan a		
Gro	No. of up mice	Days betv admini -3	stration of 1	aft and PHA +3	Rejection date +8 +9 +10 +11				
2	7	PHA	PHA an		3	2	1	1	
3	9		PHA an	d PHA	2	3	4	-	
4	4	Saline	Saline ar	ograft d saline	-	2	2	-	

No significant difference in homograft rejection is apparent between animals treated with PHA and controls treated with saline.

is from a mouse which received no PHA, and Fig. 2 is from a mouse 4 days after intravenous administration of PHA. The absence of small, dark-staining lymphocytes and the presence of larger, more immature cells are quite evident in the second photomicrograph. High power magnifications of normal spleen cells as compared with those stimulated by PHA are shown, respectively, in Figs. 3 and 4.

The twenty-four White Swiss mice bearing skin homografts from C57BL mice all rejected their grafts without any significant deviation from the normal rejection time⁶. Neither the dosage of PHA nor the timing of its administration in relation to application of the skin homograft caused any prolongation or shortening of the rejection time. The length of time that a homograft will remain compatible was previously determined at this laboratory to be 9±1 days7. Rejection times in this project ranged from 8 to 11 days, with only one of the grafts becoming incompatible on day 11 (Table 2).

Intravenous administration of PHA in divided doses of 1.0 to 1.5 ml. failed to alter significantly first-set homograft rejection times. Animals receiving lesser doses of PHA showed evidence of lymphoid mitogenic activity in their spleens. The failure of PHA to render the animals immunologically tolerant may be caused by a variety of factors⁸⁻¹⁰. One possibility is that an inadequate concentration of PHA or an inadequate period of exposure to PHA was attained for its full biological effect to be

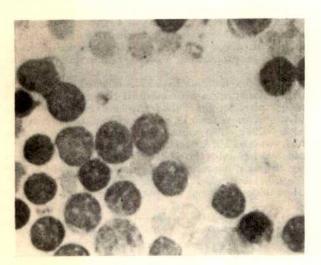


Fig. 3. High power magnification of a control spleen smear.

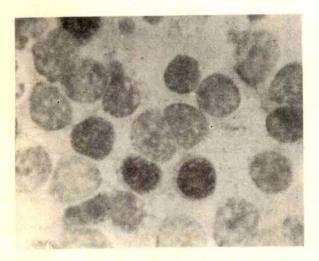


Fig. 4. High power magnification of a spleen smear 4 days after administration of PHA.

exhibited. Toxicity becomes a problem with higher dosesand is usually manifested by sudden death. Because PHA is a relatively crude extract, further biochemical study may, in the future, yield a pure substance of high mitogenic activity and lower toxicity.

In vitro studies reveal that PHA affects 70-95 per cent of the cells in lymphocyte cultures. The percentage of lymphoid cells that are affected in vivo is unknown. Even if PHA concentration was optimal, there might still exist in vivo enough functional, mature lymphocytes to maintain unaltered defences against foreign antigens 11,12.

A less likely possibility is that, in spite of a profound effect on the morphology and kinetics of the lymphocyte, stimulation with PHA may not alter its ability to react with foreign histocompatibility antigens.

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GENETICS

Serum Albumin Polymorphism in North American Indians

ALBUMIN variants, migrating anodally to the usual type in starch gel, cellulose acetate and paper electrophoretic systems, have been reported in Algonkian-speaking and neighbouring North American Indian populations1 and in several Indian and non-Indian pedigrees2-5. Variation in the electrophoretic mobility of the Indian and one non-Indian, fast migrating variant has been established1. An additional albumin variant with a slow electrophoretic mobility occurs, with relatively high frequency, in Mexican Indian populations1, and has been observed in a Pima population of the south-western United States (our unpublished work).

We have examined sera from northern Minnesota Indians with varying degrees of Ojibwa-Anglo genetic admixture and a representative sample of Blackfoot Indians from Montana (our unpublished work). sera were diluted 1:7 with 0.15 molar sodium chloride before vertical starch gel electrophoresis was carried out for 8-10 h at 11 V/cm and 4° C. Starch gels, consisting of 15 g per cent hydrolysed starch, were prepared according to Bowman and Bearn⁶. In both populations an albumin variant with identical electrophoretic mobility on vertical starch and polyacrylamide gels has been encountered migrating anodally to the usual type. Characteristic vertical starch gel electrophoretic patterns of representative sera are shown in Fig. 1. Phenotype frequencies encountered within the two populations are shown in Table 1.

Immunoelectrophoretic analysis produced patterns concordant with the results of starch and polyacrylamide gels. Ouchterlony immunodiffusion of sera from indi-

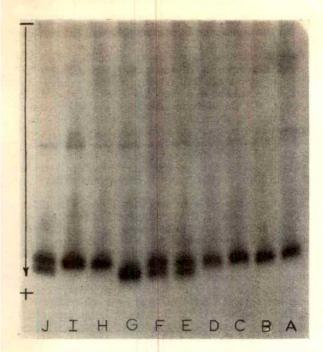


Fig. 1. Vertical starch gel representation of human serum albumin phenotypes. (A-D, H and I) individuals homozygous for allele AlA; (E, F and J) heterozygotes AlA!/AlA; and (G) a homozygote for the Algonkian variant, AlA!/AlA!.

viduals homozygous and heterozygous for the variant albumin and homozygotes for the common type developed precipitin arcs characteristic of immunological identity when tested against rabbit, goat and horse anti-human albumin antisera.

The presence of individuals homozygous and heterozygous for a fast-migrating albumin variant within the same populations (as in this case and ref. 1) and evidence from pedigree analysis^{1,2} indicate hereditary transmission through an autosomal, co-dominant, equally expressive allele to that in control of synthesis of the usual type of human serum albumin, AlA. Family and linkage analysis, now in progress, on the Ojibwa material should provide additional relevant information on the inheritance pattern of the variant albumin.

Table 1. DISTRIBUTION OF ALBUMIN TYPES IN POPULATIONS OF NORTH AMERICAN INDIAN

Population	N	AlA	IJALAI	Phenotypes Al Al /Al A		Al A/Al A		Source
Ojibwa Blackfoot Naskapi Montagnais Sioux Athabaskan Tlingit	250 97 151 112 160 230 100	0 1 2 0	% 0·4 0·6 1·8 0·4	No. 11 4 37 14 2 11 1	% 4·4 4·1 24·5 12·4 1·3 4·8 1·0	No. 238 93 113 96 158 218 99	% 95·2 95·9 74·8 85·7 98·7 94·8 99·0	Present study Present study Ref. 1 Ref. 1 Ref. 1 Ref. 1 Ref. 1

Assuming that the electrophoretically identical variants encountered among the Naskapi–Montagnais¹ and the Ojibwa–Blackfoot populations, as well as the Cree variant³, constitute the same molecular species of serum albumin, we have provisionally designated the controlling allele as AlAlgonkian (AlAl). Such a designation utilizes the linguistic affinity uniting these populations¹ and has the merit of emphasizing probable genetic relationship between the constituent populations. The sporadic occurrence of the Algonkian variant among Siouan, Athabaskan and Tlingit-speaking peoples¹ is best attributed to gene flow at the periphery of the extensive territory inhabited by Algonkian-speaking peoples in Canada and the northern United States.

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PATHOLOGY

Neural Mechanism in Induction of Dioestrus and Tumour in the Androgen Sterile Rat

THERE is accumulating evidence of a connexion between hormone imbalance and tumorigenesis. A gonadotropin imbalance in the endocrine environment has been related to the development of granulosa cell tumours of the ovary¹, and to the induction of mammary gland tumour in mice in which prolactin release is a feature of the

experimental hormonal environment2.

When chemical carcinogens were administered for prolonged periods to induce mammary gland tumours in mice, Jull described their effects on target tissues as mimetic of oestrogen or progesterone³. Studies of more immediate effects of chemical carcinogens have shown decreased prolactin content of pituitaries of lactating rats after the administration of 3-methylcholanthrene (3MC) (ref. 4), which was found, together with 9,10-dimethyl-1,2-benzanthracene (DMBA) and 3,4-benzpyrene (BP), to initiate mammary secretion and maintain lobulo-alveolar structure in oestrogen-primed female rats. The ability of these three carcinogens to elicit mammary cancer may depend partially on stimulating prolactin secretion⁵.

A direct correlation between carcinogen-associated endocrine effects and the development of a tumour has not been reported. In this communication, we give evidence of a possible association between changes in the endocrine environment and the development of tumours. Our data are based on investigations of tumorigenesis in rats, aspects of which we have reported previously (ref. 6 and paper at symposium on Biorhythms in Experimental and Clinical Endocrinology, Florence, 1966, to be pub-

lished).

In the basic experiment, 50 day old androgen sterile and control Sprague-Dawley female rats were given a single intragastric dose of either DMBA administered according to the regimen of Huggins et al.7 or the solvent vehicle. Androgen sterility as defined by Barraclough* was induced by a single subcutaneous injection of 1.25 mg of testosterone propionate at 5 days of age. Body weight and the state of the vaginal cycle were monitored on a regular basis. For each animal vaginal smears were taken daily from the day the vagina opened until necropsy. Individual animal weights were noted weekly from 21 days until necropsy; more frequent observations were made during treatment. Necropsies were conducted either 3 or 100 days after carcinogen administration, and particular attention was given to the mammary glands, adrenals, pituitary, ovaries, uterus and vagina. Animals necropsied 3 days after carcinogen had been given established adrenal necrosis as a possible response to carcinogen. and so a finding at necropsy 100 days after carcinogen.

of adrenal cortical calcification or fibrosis with little or no regeneration of the reticularis, was interpreted as an indication of adrenal necrosis after administration of the carcinogen*. An additional reflexion of the endocrine environment was the functioning of the mammary glands, shown by the presence of milk or the histological observation of lobulo-alveolar development and secretion from necropsy preparations 100 days after carcinogen. The vaginal response of dioestrus was considered to have been seen if the smears indicated dioestrus within 4 days of treatment and if the smears indicated oestrus for each of the 3 days before treatment; if smears did not indicate oestrus for each of these 3 days, the response was considered to be indeterminate. The vaginal response noted in the daily vaginal smears is confirmed by histological examination of the vaginal wall 3 days after the carcinogen. There is absence of keratinization, and neutrophiles are found in the vaginal mucosa and lumen. The endometrium shows a related response in that the lining epithelium is markedly reduced in height and there is depletion of stromal mucopolysaccharide.

In considering the associations between the endocrine environment and tumorigenesis, comparisons among the treatment groups do not provide for distinguishing responses which result from the carcinogen independent of tumour development from those that are more directly associated with tumours. To consider associations within treatment groups may be more instructive. The number of tumour-free animals may be so small, however, that the contrast of endocrine responses on the basis of tumour development is uninformative. In our basic experiment, for example, only two of the twenty-five control animals treated with carcinogen failed to develop either mammary gland or ovarian tumours before necropsy at 100 days after treatment with carcinogen; it is not informative that no association with tumour development was seen with respect to these data. The androgen sterile animals treated with carcinogen are more informative in that seven out of twenty-three failed to develop tumours. Although the number of tumour-free animals is small, it is still worth while examining the data for possible associations.

Results are given in Table 1. Changes which may indicate an immediate endocrine response to the administration of carcinogen are considered with reference to tumour development. The changes considered are vaginal dioestrus in previously constant oestrus animals, adrenal damage and change in body weight during the 3 days after carcinogen administration. The development of milk secretion by 150 days is included.

A hundred days after treatment, sixteen of the twentythree treated androgen-sterile rats (TD) had developed tumours and eleven of these had developed mammary gland tumours while five had granulosa cell tumours of

Table 1. TUMOUR DEVELOPMENT AND CARCINOGEN-INDUCED RESPONSES OF ANDROGEN STERILE RATS NECROPSIED AT 150 DAYS (100 days after carcinogen)

	Tumours MGT	Treated present GCT	Tumours absent	Control Tumours absent
No. of rats	11	5	7	24
Vaginal response*	**	Ü	•	
Yes	7	5	1	4
No	2 2	Ō	5	19
Indeterminate	2	Ô	ì	ĩ
Milk present				**
Ŷes	11	5	7	17
No	0	Ö	Ò	7
Adrenal damage				
Yes	8	4	4	0
No	3	1	3	24
% Increase in body weight age 50-53 days				
Mean	-0.50	-3.1	-0.1	5.3
Standard deviation	2.5	5.5	3.7	2.0

MGT, Mammary gland tumour; GCT, ovarian granulosa cell tumour.

* Vaginal response in constant oestrus rats indicates arrest of keratinization and neutrophil migration, or mucification, within 96 h of administration of DMBA at age 50 days, as shown by daily vaginal smears. The vaginal response is considered to be indeterminate in animals not in vaginal oestrus for the 3 days before administration of carcinogen.

the ovary. In no case were both mammary gland and ovarian tumours found in one rat. None of the untreated androgen sterile rats (TV) developed tumours.

Vaginal response is positively associated with subsequent development of tumours ($\chi^2 = 6.03$, P = 0.007, TD necropsy at age 150 days). All five animals with ovarian granulosa cell tumours showed the vaginal response. Of the eleven rats that developed mammary gland tumours, seven showed the response, two gave no response, and response was indeterminate on the other two. Of the seven tumour-free animals, one showed the response. An alternative expression of the association is that twelve of the thirteen responders developed tumours but only two of the seven non-responders developed

No association with tumour development is demonstrated in the case of the development of lactation or in the immediate response to the carcinogen as reflected in the change in body weight and adrenal damage. These results do not necessarily indicate a lack of association, but they do indicate the lack of a strong association with tumour development. Huggins and Morii did not consider adrenal damage a prerequisite to mammary cancers. There are several effects of treatment with carcinogen, and the results reported here may be interpreted as showing that the effects are not strongly associated among themselves and that the tumour-free rats were not completely immune to the effects of the carcinogen.

It would be interesting to correlate tumour development with changes which could only be determined by killing the animals immediately after giving the carcinogen. If vaginal response is considered to be a predictor of tumour development, in that there is a positive association of this response with the subsequent development of tumours, it is possible to associate indirectly observations made at necropsy 3 days after giving carcinogen with eventual tumour development. We can examine their distribution in animals which did or did not show the vaginal response. Although such correlation is tenuous, it is worth considering. In our experiment, four of the twenty-two androgen sterile rats (TD) that were necropsied 3 days after carcinogen had been given gave no vaginal response. Results of other responses for animals necropsied at 53 days, grouped according to vaginal response, are given in Table 2. It is of interest that the organ weights for the non-responding group match corresponding weights of untreated androgen sterile animals necropsied at the same age.

Table 2. CARCINOGEN-INDUCED CHANGES ACCORDING TO VAGINAL RESPONSE
IN ANDROGEN STERILE RATS NECROPSIED AT 53 DAYS
(3 days post-carcinogen)

	Vaginal	response	Statistical significance	
	No.	Yes	\tilde{P}	
No. of rats*	4	17		
Pituitary weight (mg)	$12.5 \pm 2.5 \dagger$	10.1 ± 2.1	0.058	
Adrenals weight (mg)	58.7 ± 8.3	86.6 ± 23.8	0.028	
Ovaries weight (mg)	36.7 ± 4.6	30·0 ± 6·1	0.066	
Uterine weight (mg)	331.6 ± 34.0	261.3 ± 45.0	0.008	
Percentage change in boo	ly			
weight days 50-53	1.15 + 1.41	-1.49 + 6.37	0.14	

Statistical significance was evaluated from t ratio using pooled variance estimate; comparisons of organ weights were made from logarithms of the observed weights.

* Response indeterminate on one animal.

† Mean \pm standard deviation.

In TD animals necropsied 3 days after the carcinogen was given, the vaginal response is related to a decrease in weight of pituitary, ovaries and uterus. It is not clear whether the results can be accounted for as unrelated, separate target organ responses or whether they are related through the initiation of a cycle. The association of losses in organ weight with vaginal dioestrus suggests that the vaginal response is not an isolated peripheral effect of the chemical carcinogen but that it may be related to release of hormones by the pituitary and ovaries. If this is so, it is plausible that the endocrine effects are to a large extent the result of central action of the DMBA; action on the hypothalamus, in particular,

is suggested.

A possible central effect is supported by additional con-The finding of augmented lactogenesis in androgen sterile animals 100 days after treatment with carcinogen⁶ implies a sustained release of prolactin by the pituitary and is consistent with a neural action of DMBA. Second, the interruption of vaginal keratinization followed by dioestrus and proestrus (androgen sterile rats) after administration of DMBA resembles the vaginal response to progesterone in persistent oestrus rats¹⁰ and in androgen sterile rats¹¹. The nervous system may be involved in this response¹². Barraclough has proposed that treatment with progesterone lowers thresholds of excitability within the hypothalamus of sterile rats13. By analogy, the carcinogen DMBA may affect the hypothalamus. An additional support is the release of pituitary prolactin within 16 h of administration of the carcinogen 3MC. If the effect of DMBA is similar in time, our finding that the vaginal response is delayed for approximately 48 h is at least consistent with the possibility that endocrine effects are largely secondary to a central effect.

The endocrine effect of vaginal response was found to be associated with tumorigenesis, and we have used this association in considering the possibility that other endocrine effects are similarly associated with tumour development, and the results are suggestive of such associations, but are not conclusive. If the immediate endocrine effects, and the vaginal response in particular, arise chiefly in response to a central neural effect of the carcinogen, then a plausible inference from the association between vaginal response and tumour development is that the suggested neural effect of the carcinogen is a significant factor in

tumorigenesis. This work was supported by an American Cancer Society institutional research grant and the California Institute for Cancer Research. Statistical services were supplied by the University of California, Los Angeles, Health Sciences Computing Facility, supported by a grant from the National Institutes of Health, US Public Health Service.

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Increased Incidence of Carcinoma induced by DMBA in the Hamster Cheek Pouch in response to Vitamin A

VITAMIN A, when administered topically or systemically in high doses, retards the growth and inhibits the induction of benign and malignant tumours1-3. When it is applied locally, vitamin A inhibits the development of tumours of the cervix and vagina of hamsters induced by 9,10-dimethyl-1,2-benzanthracene (DMBA), but has

no protective effect on the perineal skin1. Topical application of excessive vitamin A, however, is known to cause epithelial hyperplasia and mild atypicality, disorientation of the basal layer4-6, rapid epithelial proliferation7 and mucoid metaplasia, with the formation of glandlike structures8,9; and an increased incidence of avian leukosis has recently been reported in response to excess vitamin A (ref. 10). We describe here the effect of topical hypervitaminosis A on the induction by DMBA of careinoma in the hamster cheek pouch.

The right cheek pouches of thirty-five Syrian golden hamsters aged 2 months were painted three times a week with one of the following substances dissolved in liquid paraffin: 0.5 per cent DMBA (fifteen animals, DMBAgroup); 0.5 per cent DMBA combined with 10 per cent vitamin A palmitate (E. Merck AG, Darmstadt; 1 g= 1.7×10^6 IU) (fifteen animals, DMBA-A-group); or 10 per cent vitamin A palmitate (five animals, A-group). Half the animals from each group were killed after 9 weeks, and the remainder after 11 weeks.

After 9 weeks the pouches of all animals in the DMBAgroup showed areas of irregularity of the mucosa and a few small papillomatous tumours. Histologically, lesions included epithelial hyperplasia with very prominent hyperkeratosis, benign papillomata, intra-epithelial carcinoma, and occasional foci of early infiltrating squamous cell carcinoma.

The pouches of all animals of the DMBA-A-group revealed diffuse irregularity of the mucosa and many tumours which were larger, more numerous and more irregular than those in the DMBA-group. Histologically, the most outstanding feature was the presence of many feet of intra-epithelial carcinoma and infiltrating squamous cell carcinoma. There was mucoid metaplasia of some tumour cells with the appearance of gland-like structures in the infiltrating tumours. Non-tumorous areas showed generalized epithelial hyperplasia, but the marked hyperkeratosis found in the DMBA-group was absent, and the epithelium was covered by a thin layer of pale staining keratin.

The pouches of all animals in the A-group revealed areas with marked acanthosis, hyperplasia and mild pleomorphism. In most areas there was only a thin layer of pale staining keratin. In some areas, however, the keratin was absent.

The animals killed after 11 weeks of painting revealed similar lesions. There was, however, a striking increase in the number and size of tumours in the DMBA-A-group as compared with the DMBA-group. The pouches of the A-group showed marked acanthosis and focal epithelial atypicality. No foci of mucoid metaplasia, however. were present. Table I summarizes our results.

ble 1. RESULTS OF LOCAL TREATMENT OF HAMSTER CHEEK POUCHES WITH DMBA, VITAMIN A, AND A COMBINATION OF BOTH SUBSTANCES Table 1. 11 weeks

9 weeks Group DMBA

Few small papillary tumours, histologically ranging from benign papillomas to intraepithelial carcinoma, and only occasional small foci of infiltrating carcinoma.

DMBA-A Numerous, irregular tumours. Histologically, many foci of intra-epithelial and invading carcinoma.

A Histologically, foci of mild epithelial hyperplasia and pleo-

thelial hyperplasia and pleo-morphism.

Scattered slightly irregular tumours. Histologically as at 9 weeks, but more foct of infiltrating carcinoma.

Many large, irregular tuneours.
Histologically, many careinomas with extensive infiltration.
Histologically, foel of marked acanthosis and pleomorphism.

Squamous cell carcinomas induced in hamster cheek pouches by DMBA are known to appear 10-11 weeks after the initial painting, and pre-malignant lesions after 7-8 weeks11. In our experiments, tumours appeared after 9 weeks of painting with DMBA only, but we found many more and larger tumours at the identical stage in the These differences were even more DMBA-A-group. obvious after 11 weeks.

The mechanism underlying the earlier development of malignant tumours in the DMBA-A-group may be related

to an increased mitotic index, rapid epithelial proliferation and altered differentiation of epithelial cells as reported after the topical application of moderate quantities of vitamin A (refs. 4-9). This may cause the malignancy induced by DMBA to appear earlier and more extensively in epithelium altered by a moderate excess (10 per cent solution) of vitamin A. Other factors which may be involved include altered permeability of cellular and subcellular membranes 12,13 and the release of lysosomal proteolytic enzymes induced by vitamin A (refs. 14, 15).

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Non-self-maintaining Kinetics of Proliferating Blasts in Human Acute Leukaemia

THERE is still uncertainty about the cytological characteristics of stem cells, their production and kinetics. As far as pathology is concerned the acute leukaemias are characterized by the presence and multiplication of the youngest recognizable cells of each haemopoietic line; these are the blasts. The identity of the blasts and their histogenetic connexions with stem cells are not known, and the possibility that acute leukaemia blasts are stem cells has recently been raised 1-3. These blasts are often similar to the normal parent cells of the various haemopoietic lines and can therefore be identified as myeloblasts, lymphoblasts or monoblasts. Sometimes, however, their features are even more undifferentiated and/or atypical, and thus the definitions stem cell leukaemia, haemocytoblastic acute leukaemia, acute histioleukaemia.

Taking the leukaemic blast population as a whole, allowance must be made for the fact that only some of the blasts are able to proliferate as shown before4. Clearly the problem can be posed more precisely by considering only those of the blasts which multiply. If an acute leukaemia blast population is divided into classes on the basis of cell diameter, it is possible to isolate a class of large blasts, the labelling index of which is similar to that of normal bone marrow blasts4. The class of large and normally proliferating blasts constitutes less than 10 per cent of the whole population when this population is in a relatively steady state, but this can rise to 15-20 per cent when the population is expanding rapidly. Can this class of proliferating blasts be taken as a class of stem cells? Cronkite recently pointed out that "one cannot be too certain on this issue and much further work is needed to answer the question"3.

We believe that one way of tackling this problem is to assume that if the normally proliferating blasts are stem cells for the entire acute leukaemia population, they should show the kinetic features of a self-maintaining cell population. We have therefore studied four patients with untreated acute myeloblastic leukaemia by means of in vivo pulse labelling with tritiated thymidine (0.2 µc./g of body weight, specific activity 2 c./mmole). In all four cases, the blast population constituted more than 90 per cent of nucleated cells in both bone marrow and peripheral blood. In the days before and during the experiment, clinical and haematological conditions were constant. The bone marrow and peripheral blood were sampled 1 h after injecting the tracer and thereafter at different times. The autoradiographic preparations were made with a technique described before \$\frac{4}{3},6-8\$.

Blast cells always varied considerably in size, and cell diameters ranged in case 1 from 8 to 21µ, in case 2 from 7 to 16.5μ , in case 3 from 8.5 to 19.5μ , and in case 4 from 8 to 19.5\mu, that is there was at least a doubling in each case. This variation is much greater than the differences in diameter—shown to be 20 per cent—seen in the same types of cell in S and G_1 (ref. 9). We were thus dealing with real cell heterogeneity which was only slightly dependent on the different phases of the cell cycle. The blast population was divided into three equal classes according to diameter. The percentage distribution of the cells in the three classes was on average: large blasts 9 per cent of the whole population; medium blasts 51 per cent; and small blasts 40 per cent. In all classes, cell distribution was constant throughout the experiment.

Table 1. Labelling indices and mean grain count after injection of tritiated thymidine

			4.	*** * * * * * * * * * * * * * * * * * *	× 111 7 141	*******				
	77		belling	indices ((%)		Mean grain count			
Cases	Hours after injec- tion	Total blast popu- lation	Large blasts	Med- ium blasts	Small blasts	Total blast popu- lation	Large blasts	Med- ium blasts	Small blasts	
P.L.,	1	9.7	30.4	$7 \cdot 2$	9	60.7	79.0	52.0	0	
female	10	11.3	17.4	11.8	$7 \cdot 9$	33.4	55.3	30.6	22.0	
M.L.,	1	5.7	$52 \cdot 2$	1.1	0	65.0	61.1	45.0	0	
female	15	8.6	21.6	18-6	4.6	34.5	48.7	34.6	30.1	
	48	4.8	6.9	6.7	3-8	27-3	38.6	28.8	20.4	
	120	5.1	9.9	7.1	$3 \cdot 2$	19.7	16.7	29.3	18.0	
M.B.,	1	$5 \cdot 2$	46.0	3.0	0	59.7	61.6	54.0	0	
male	17	6.7	13.3	9.6	4.6	29.8	34.0	29.6	29.2	
	70	10.4	7.6	11.4	8.5	28.1	25.0	30.3	29.6	
R.A.,	1	$3 \cdot 1$	23.8	1.1	0	51.0	52.6	47.7	0	
female	13	5.3	13.8	5.8	2.0	25.0	41.0	22.1	18-1	
	24	8.2	11.2	6.6	10.4	22.7	35.0	22.5	21.1	
	70	9.3	9.0	9.1	9.5	23.2	31.0	23.6	21.2	

Labelling indices were determined by counting from a minimum of 300 cells, for the large blasts (representing the smallest part of the population) to a maximum of 2,000 cells. The mean grain count was estimated by counting the grains on a number of labelled cells varying from at least seventy (large blasts) to more than 200 cells

As shown in Table 1, 1 h after injecting the tracer we observed a high labelling index in the bone marrow in all large blasts (values about 40 per cent, that is similar to those of blasts in normal bone marrow); a very low labelling index in the medium sized blasts and no evidence of labelling at all in the small blasts. Between 10 and 17 h the mean number of grains/cell halved in all cases. This means that during this period the cells which were in S at the time of pulse labelling completed this phase, passed the G2 phase and underwent mitosis. After this first division, there was a sharp fall in the labelling index in the class of large blasts (on average from 40 per cent to 16 per cent) and this was accompanied by an increase of the labelling index of the medium sized blasts and by the appearance of labelled small blasts. On subsequent days the labelling index of the large blasts remained almost stationary at low values or decreased slightly more. This means that many more than 50 per cent of the daughter cells become small after division and come out of the class of large blasts for good, or at least for a period of time longer than the generation time. The class of large blasts does not therefore behave as a pool of self-maintaining cells but rather as a normal multiplicationmaturation compartment.

Table 2. CELL FLUXES IN AN AGUTE LEUKAEMIA BLAST POPULATION (MEAN VALUES FROM FOUR CASES)

	Large blasts	Medium blasts	Small blasts
Relative number (N)	88	506	406
Labelling index $\left(\frac{Ns}{N}\right)$	42.3%	1.6%	0%
Proliferation rate $\left(\frac{Ns}{Ts}\right)$	2-32	0.52	0
Influx (\overline{Ki}) Sum of the proliferation		2.94 = 2.84	1.92
Total influy in non-prol	iferating compartme	ant = 4.86	

Ns, number of cells in DNA synthesis; Ts, DNA synthetic period = 16 h; Ki, number of new labelled blasts appearing/h in medium and small blasts.

We were also able to quantify the kinetics of proliferating blasts as follows. In all four cases we evaluated fluxes between the various classes of blast in the first 10-17 h after the precursor was injected; we used a modification of the method already used for normal bone marrow10. We considered: (a) the labelling indices of the various classes at 1 h and after the first division; (b) the rate of appearance of new (medium and small) labelled blasts in the bone marrow in each hour during the period considered; (c) cells discharged into the peripheral blood in the same

period. This evaluation was based on the number of grains lost from the bone marrow/1,000 cells, divided by the mean number of grains/labelled cell which appeared in the peripheral blood; and (d) the duration of the S phase of proliferating blasts this was evaluated in one case only, in which it was possible to take repeated bone marrow samples. On the basis of the progression of the percentage of labelled mitoses, the duration of S was found to be 16 h, which agrees with previous evaluations for acute leukaemia2,11; we adopted this value for all cases. Table 2 shows the values which we obtained.

The rates of proliferation, calculated on the basis of the labelling indices and the duration of S, are much lower than the total influx into the non-proliferating part of the population as calculated from the number of cells which appeared in the non-proliferating part of bone marrow and peripheral blood. This result also suggests that the pool of proliferating blasts does not behave as a self-maintaining system.

There is thus some evidence that even if a comparatively small section of the blasts with the same proliferative activity as normal marrow blasts is isolated from the whole blast population in myeloblastic acute leukaemia, its kinetic behaviour is not what would be expected from stem cells. It is rather the behaviour of a system which has to be continually replenished in cells from another part of the population.

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Substrate Limiting Melanogenic Inhibitor in Malignant Melanomas

The presence of a heat stable, tyrosinase-inhibiting factor in various malignant melanomas has recently been reported¹⁻⁴. The reported inhibitor(s) has been partially purified and its mode of action has been determined to be competitive. Although it was not determined whether competition was with enzyme or substrate, the presence of a tyrosine residue within this factor seemed to suggest competition with substrate. We report here the presence of a separate, heat labile inhibitory substance within malignant melanoma tissue which is competitive with tyrosinase, that is, it competes with tyrosinase for the substrates tyrosine and 3,4 dihydroxyphenylalanine The independence of this inhibitory activity (dopa). from either added pyridoxal phosphate or α-methyldopa, from ATP, and furthermore, its absence in normal hamster liver, provides evidence that it is not due to either tyrosine or dopa decarboxylase or transaminase, or in vitro protein synthesis.

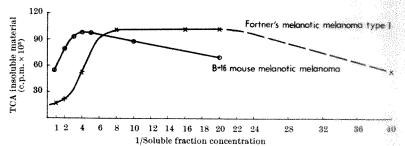


Fig. 1. Tyrosinase radio-assay of Fortner's melanoma soluble fraction at various concentrations.

We first obtained evidence for the presence of this substance while studying the total amount of melanin formed under low substrate conditions using varying concentrations of the soluble fraction, obtained after centrifuging at 105,000g for 1 h, of the homogenate of Fortner's melanotic melanoma type No. 1 (1 g tumour to 5 ml. 0.25 molar sucrose) or of B-16 mouse melanoma (1 g tumour to 10 ml. 0.25 molar sucrose). When various dilutions of the soluble fraction were incubated at 30° C for 16 h in 0·1 molar phosphate buffer, pH 6.8, with 0.27 $\mu c.$, 2.8×10^{-8} molar DL-tyrosine-2-14C, along with 92 μg/ml. chloramphenicol^{6,7}, the presence of an internal inhibitor became apparent (Fig. 1). Only with more dilute preparations is the total amount of melanin formed (TCA insoluble material) approximately proportional to the concentration of the enzyme present in the soluble fraction (Fig. 1); as the enzyme preparation becomes highly concentrated, the amount of melanin synthesized becomes inversely proportional to preparation concentration. Furthermore, as shown in Table 1, the 1:1 undiluted soluble fraction converts less tyrosine to melanin than a 1:20 dilution. The addition, to the 1:1 incubation, of an original amount of soluble fraction after the completion of 16 h and further incubation for 4 h fails to increase the amount of melanin formed, while the addition of an original amount of tyrosine-14C increases the amount of melanin formed. Thus all available tyrosine has already been converted to TCA insoluble material, and it is apparent that some of the original tyrosine is now unavailable for melanin synthesis, although active tyrosinase is still present. Chromatography of the TCA supernatant from the 1:1 incubation, using either paper or Gelman silicate thin-layer

Table 1. TYROSINASE RADIO-ASSAY OF FORTNER'S MELANOMA SOLUBLE FRACTION OF 1:1 AND 1:20 DILUTIONS

Incubation 1:201:1+S - 1:20+S - 1:1+E - 1:20+ECPM of TCA precipitate (duplicates) $14,600 \\ 15,400$

S, Substrate added; E, soluble fraction added.

chromatography media and water saturated phenol, yields two radioactive peaks. One of these corresponds to a standard tyrosine solution (probably representing D-tyrosine-14C); the other has an R_F 0.3 under the described conditions and represents a reaction of this inhibitor with tyrosine-14C. Chromatography of the TCA supernatant from the 1:20 incubation yields a tyrosine peak, but only a trace peak at R_F 0.3. Preliminary studies using gel chromatography indicate this inhibitor has a molecular weight under 10,000. While the presence of a low molecular weight enzyme which utilizes tyrosine or dopa as substrate cannot be excluded at this time, any substance which serves to lessen melanin formation through substrate limitation belongs in the category of substrate limiting melanogenic inhibitor. The enzyme exhaustion observed after incubation for 16 h with 1:20 dilution remains to be clarified in relation to enzyme stability in dilute solution, the recently described enzyme directed inhibitor, and reaction inactivation.

The present finding of this substrate binding inhibitor should be considered in the future investigation not only of tyrosinase activity but also of disturbed melanogenesis, particularly because albino melanocytes⁸ and amelanotic melanoma cells, have been shown to contain tyrosinase and to undergo melanization of their premelanosomes if sufficient free L-tyrosine is made available in vitro7. In addition, the recent report of Demopoulos10 on the selective inhibition and regression of malignant melanomas by tyrosine deprivation must be further evaluated together with these findings.

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Identification of 3,4-Dimethoxyphen-ethylamine from Schizophrenic Urine by Mass Spectrometry

THE occurrence of the "pink spot" in the urine of schizophrenies was first reported by Friedhoff and van Winkle¹, who tentatively identified the substance as 3,4-dimethoxy. phenethylamine (3,4-DMPEA). Subsequent interest in this amine is linked to the possibility that its formation may result from an aberration in the metabolism of the endogenous amine, dopamine, as either a resultant or aetiological factor in mental disorders². Reports of both confirmatory3-7 and conflicting8-14 nature have appeared with respect to the identity and origin of the "pink spot" 15,16. The methods which have been used to isolate and identify the "pink spot", solvent extraction, ionexchange chromatography, paper and thin-layer chromatography, counter-current distribution and various colour reactions, have not provided unequivocal identification. Much of the controversy originates from the non-specificity of chromatographic separation and the complexity of amines present in human urine. Indeed, several "pink spots" occur in urine extracts which are isographic in one or more of the solvent systems used to isolate the 'pink spot''

In the work reported here we have applied a new technique for both the isolation and identification of the "pink spot"17. The initial extraction and chromatography was performed as follows: urine was obtained from two female schizophrenics daily for 5 days; the collections were made in 1 normal hydrochloric acid/toluene to a final pH of 1 and stored frozen; the urines were pooled

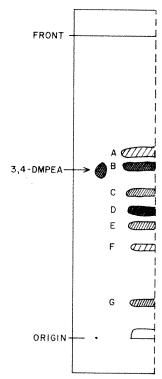


Fig. 1. Thin-layer chromatogram of "pink spot" area. Solvent system: nitroethane, acetic acid, water (90:24:12). Plate, Analtech preparative silica gel, 750 mm. Visualization, ninhydrin/Erhlich¹. Area B taken for dansylation.

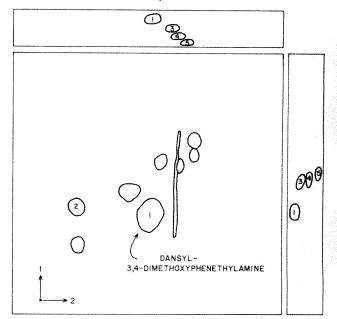


Fig. 2. Thin-layer chromatogram of dansyl products formed from the "pink spot". Solvent systems: 1, ethylacetate, cyclohexane (3:4); 2, benzene, triethylamine (8:1). Plate, Analtech silica gel, 250 mm. Dansylamine standards: 1, 3,4-DMPEA; 3, 2,3-DMPEA; 4, 2,5-DMPEA; 5, p-methoxy PEA. Spot 2, dansylamide. Spot 1, "dansyl-3,4-DMPEA". Fluorescence visualization: "Mineralight UVS 11'.

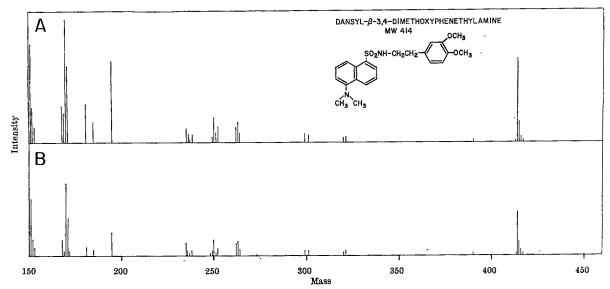


Fig. 3. Mass spectra of A, dansyl derivative of β -3,4-dimethoxyphenethylamine; B, dansyl derivative of "pink spot". (AEI MS9 mass spectrometer, direct inlet, 300° C, 70 eV.)

(volume 8 l.), the pH adjusted to 10 with 5 normal sodium hydroxide, extracted with 12 l. of methylene chloride, the organic solvent removed in vacuo, the residue suspended in 10 ml. of methanol and clarified by centrifugation. A portion (5 ml.) of the extract was streaked on Whatman No. 3 paper and developed in butanol, acetic acid and water (4:1:1) by descending chromatography. A band between R_F 0.52 and 0.66 which corresponded to 3,4-dimethoxyphenethylamine (R_F 0.58), and which gave on a guide strip a pink colour with ninhydrin/Ehrlich, was cut out and eluted by descending chromatography with 0.05 normal hydrochloric acid. The extract from the paper was chromatographed on silica gel plates in several solvent systems revealing at least seven pink spots. The optimum resolution was obtained with nitroethane, acetic acid and water (90:24:12) as shown in Fig. 1.

The area corresponding to 3,4-DMPEA (Fig. 1B) was extracted with acetone-water (5:1), centrifuged and the supernatant was reacted with 5-dimethylaminonaphthalenesulphonylchloride in the presence of excess sodium bicarbonate to form the "dansylamine" derivative. After 4 h, an excess of water was added and the dansyl derivative was extracted into ethyl acetate. The solubility in organic solvents and the intense yellow to blue fluorescence on ultraviolet irradiation make the "dansylamines" ideal The two-dimensional for thin-layer chromatography. chromatogram of the dansylated products formed from the components in "pink spot B" (Fig. 1) is shown in Fig. 2. The principal spot, which exhibited a bright yellow fluorescence, corresponded closely with the migration of authentic dansyl-3,4-DMPEA and was clearly separated from the dansyl derivatives of 2,3- and 2,5-DMPEA and The "dansyl-DMPEA" p-methoxytyramine. was extracted into ethyl acetate and the concentration estimated fluorometrically (Aminco-Bowman, uncorr.; activation 340 mµ; emission, 510 mµ). A minimum value which is uncorrected for losses during isolation of 4 $\mu g/g$ creatine was obtained.

The dansyl derivative was then analysed by mass spectroscopy (Fig. 3). The chief component had a mass of 414 which corresponded exactly with that obtained with authentic dansyl-3,4-DMPEA. The remaining pattern of fragmentation of both the unknown and authentic dansyl derivatives was identical. Although other structures can be devised for a mass of 414 they are, in light of the other properties of the unknown, quite untenable. Thus the isolated material is certainly a DMPEA. 2,3-, 2,4-, 2,5-, 2,6- and 3,5-isomers were eliminated on the basis of their chromatographic behaviour. It is most probable on the basis of colour reactions and chromatographic behaviour that the material is the 3,4-substituted amine.

This finding demonstrates that low concentrations of dimethoxyphenethylamine were present in the schizo-phrenic urine sample examined. The "pink spot" band, isolated by paper chromatography, however, consisted of at least seven different compounds as shown by subsequent thin-layer chromatography. DMPEA was thus only a minor constituent of "pink spot" in this urine sample, and its ultimate origin from either a dietary source, bacterial metabolism or endogeneous metabolism and any relationship between its presence in urine and mental disease remains to be investigated using a specific method such as described here. Kuehl and co-workers¹⁷ have recently carried out such a study on the occurrence of 3,4-dimethoxyphenylacetic acid in urine. They used a combination of gas chromatography and mass spectrometry and have reported 3,4-dimethoxyphenylacetic acid in both normal and schizophrenic urine.

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PHYSIOLOGY

Origin of Delayed Implantation in Marsupials

Much work has been done on delayed implantation in macropod marsupials and it is now known that the phenomenon is widespread through the family¹⁻⁷. Sharman has shown that the average length of pregnancy in the red kangaroo (Megaleia rufa) is 33.17 days and that post-partum oestrus occurs about 2 days after parturition and the entry of the neonatus into the pouch. Although copulation will now occur, the new foetus-the species is monovular-will remain in one of the two uteri in the blastocyst stage for as long as the pouch young is suckled. If the neonatus is removed from the pouch, lactation is arrested, the quiescent blastocyst starts to develop, and the next generation is born after about 31 Usually, the "joey" or pouch young remains in the marsupium for about 236 days and it is not unusual in some macropod species for a young-at-foot to be fed from one nipple, a naked neonatus from another while a third generation, in the form of a blastocyst, lies dormant yet viable in one of the uteri. This species inhabits arid and semi-arid country over a vast area of Australia.

Certainly such an extraordinary mechanism could not arise, nor would it be retained, unless it conferred great benefit on the species that possess it. Waring4 said that "the selective advantages of delayed implantation in the large kangaroos which traverse great distances can be easily imagined. The doe is known to discard her joey when harassed by predators and the same circumstances may separate her from her buck . . . the selective advantage . . . for the quokka [the small scrub-dwelling Setonix brachyurus] is not so obvious and of course we may well take the view that it was developed for other circumstances and is now just built into the gene structure". Sharman3, too, said the emergence of a second offspring without preliminary mating is "presumably of some adaptive significance if the animals, in times of sparse grazing, are dispersed over a wide area". Ealey^{5,8} and Sadleir, who worked with kangaroos in the arid northwest of Western Australia, showed that the post-partum mating and its resultant quiescent blastocyst probably benefited both Macropus robustus (personal communication from Ealey) and M. rufa by enabling repeated reproduction at appropriate times.

These observations, however valid in themselves for xerophilous animals, do not give us an explanation of the general utility of the post-partum oestrus and delayed implantation in the macropod marsupials as a whole. The history of aridity in much of Australia is relatively brief and, judged by its wide distribution in the Macropodidae, the history of the post-partum oestrus and delayed implantation is very long.

Several authors are careful to point out that in most species in which delayed implantation is apparently controlled by lactation, the gestation period is slightly shorter than one oestrous cycle and one has gone so far as to suggest that, while it is of benefit to the species, "post-partum ovulation may be merely an unavoidable by-product in marsupials that have a gestation period shorter than the oestrous cycle". More recently, too, it has been concluded that lactation-controlled delayed implantation may have evolved independently in a number of species in which the gestation period was extended and that it has "no special significance except that the mechanism prevents the occupation of the pouch by young of different ages" 10.

The length of the oestrous cycle is probably very adaptive. The important event is surely the post-partum oestrus that (as, for example, in the domestic mare) allows successful insemination to occur again soon after the emptying of the uterus. This means that the doe kangaroo is almost always pregnant but, because implan-

tation and development are delayed, little or probably no loss of mobility occurs.

From information already assembled¹¹ it can be seen that the post-partum oestrus and lactation-controlled implantation, or post-partum oestrus alone, is not restricted to marsupials inhabiting arid, periodically drought-stricken regions. Thus such animals include a small retkangaroo, the quokka which inhabits relatively lush, well-wooded areas; the tammar wallaby (Protemnodon eugenii), which lives in island scrubs; the red-necked wallaby (P. rufogrisea), which confines itself to heavy scrub or brush; the long-nosed rat-kangaroo or potoroo (Potorous tridactylus), an inhabitant of dense damp scrubs; the swamp wallaby (Protemnodon bicolor) which prefers damp valleys; the boodie (Bettongia lesueuri), a small nocturnal rat-kangaroo adapted to islands as well as deserts; and finally, the red kangaroo and the euro, both of which live in dry, periodically drought-stressed regions.

On the other hand, the grey kangaroo or forrester (Macropus cangaru), which inhabits well watered and sheltered country near the coasts, does not delay implantation. When the pouch young was removed during the season of increased breeding frequency (the warmer months September to March) the female usually returned to oestrus within 13 days and so quickly became pregnant again. If pouch life was not interrupted this lasted for about 312 days (compare 236 days for the red kangaroo in more hazardous circumstances). Also because of the relatively restricted peak breeding period each female forrester will usually produce only one joey each year¹².

These data show that lactation-controlled delayed implantation occurs in such phylogenetically different types as the giant red kangaroo and the small rat-kangaroos (for example, Bettongia) and in such ecologically diverse animals as the desert-dwelling euro, the coastal swamp wallaby, and the potoroo, which inhabits lush damp Tasmanian forests. Clearly, then, the phenomenon has greater significance than merely to replace pouch young lost by the bigger kangaroos in arid conditions. Rather, it can be said that the macropod stock was pre-adapted to deal with such losses because there seems little doubt that the mechanism is of greater antiquity than is aridity in most areas of Australia. True macropod kangaroos are known from the Miocene when members of the ferocious Thylacinidae (marsupial "wolves") were also present. The great diversification of macropods took place during the Pliocene during a "fluviatile regime"13. This was a period of widening pastures but, although rainfall was decreasing, Australia was not yet a place of widespread aridity.

The question, then, arises: what was the primary function of the mechanism? The most probable explanation is that macropod marsupials almost always produce only one offspring at a time, although in highly exceptional circumstances twins may be born. They are timid animals, invariably fleeing from danger and, with few exceptions, do not take refuge in hollow logs or burrows. The young-at-foot is always highly vulnerable, and the large pouch young is often lost, or deliberately unloaded by the doe in desperate flight. It seems likely, then, that predator pressure on timid, relatively defenceless, monovular animals has made for the evolution of the mechanism—perhaps more than once—in ancestral forms and its retention in a variety of modern macropods inhabiting a wide range of habitats. On this reckoning delayed implantation (but not the post-partum oestrus) has probably been lost in M. cangaru, and has arisen by convergence in the minute pigmy possum (Cercartetus concinnus).

That the herbivorous fauna of Australia was subject to widespread predation in, for example, the Pliocene is evident from an analysis by de Vis¹⁴, who found that about 5 per cent of some hundreds of bones were "pitted, scored, cracked, chopped and crushed" by teeth. In

historical times notable predators have been the wedgetailed eagle (Uroaetus audax), the dingo (Canis dingo), the Tasmanian wolf (Thylacinus cyanocephalus) and, in the case of the smaller wallabies and rat-kangaroos, the Tasmanian devil (Sarcophilus harrisii), the tiger-cat (Dasyurops maculatus) and no doubt others. Although a relatively rare sight today except in remote parts of the continent, the wedge-tailed eagle was one of the com-monest birds in the days of early European settlement (for example, Vancouver¹⁵); in fact, all these predators were formerly extremely plentiful. Furthermore, both the Tasmanian wolf and the devil inhabited the Australian mainland until relatively recent times.

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Mechanism for the Exchange of the Calcium in Bone Mineral

THE exchange of the calcium in bone mineral with radioactive calcium and other bivalent metals has been studied in living animals1 and in vitro2, and various physical processes which lead to exchange have been suggested3. Little attention has, however, been paid to the process of exchange itself—that is, the chemical reaction by which the calcium on the surface of bone mineral is replaced by radioactive calcium or other metals.

Even in pure metals, the dissimilarity in potentials between the inner and outer sides of a crystal surface may cause the structure of the surface to differ from that of the interior4,5. Bone mineral, which is considered to be chiefly hydroxyapatite3, is markedly impure and contains not only water of hydration, but carbonate, citrate, magnesium, strontium, and traces of other elements, many of them on the crystal surface. In addition, one side of a crystal may be bound to the organic matrix, while other sides correspond to sections through different planes of the unit cell, and therefore differ not only from the side bound to organic matter but from each other as well. Moreover, extensive work on heterogeneous catalysis has shown that the surface is more dynamic than the interior, and lateral migration of different atoms over it leads to a continual fluctuation of the chemical composition on a submicroscopic scale.

It is therefore extremely improbable that the surfaces of bone mineral crystals have the same structure as the interior and equally improbable that there is any sharp

distinction on the surface between screw axis or columnar calcium ions of bone mineral or hydroxyapatite. These surfaces are therefore best pictured schematically, as in Fig. 1a. No attempt has been made to draw to scale either atoms or their distances from the dashed line, which represents the "average" surface.

Many of the familiar reaction mechanisms in chemistry begin with the addition of one atom or molecule to another. I suggest that the similar addition of a calcium ion to the surface of bone mineral, rather than the expulsion of a previously bound calcium ion, is the first step in exchange. In bone mineral, a radioactive ion from surrounding fluid would have much more chance of forming a bond with an oxygen atom, which is always accessible, than of striking a vacant calcium site. Most of the bonds formed, as shown in Fig. 1b, are unstable and immediately break up. A small proportion, however, may remain until the neighbouring calcium ion is ejected (Fig. 1c). The new ion then slips down to occupy the more stable position now available to it (Fig. 1d).

This sequence of events is plausible and in accord with present knowledge of the behaviour of crystals, and it is supported by striking indirect evidence obtained with zinc-65.

When used without carrier, zinc-65 reacts more rapidly and to a greater extent with defatted bone powder and anorganic bone than do calcium-47, strontium-85 and barium-133. Even in the presence of 2.5 mmoles/l. of calcium, almost 100 per cent of zinc-65 tracer is taken up by anorganic bone in 5 min^{7,8}. This is in great contrast to the effect of calcium in reducing the uptakes of strontium-85 tracer² and barium-133⁸. More rapid uptake of zinc-65 than of calcium-47 would scarcely be expected if the ratedetermining step were the removal of a calcium ion; it would be expected if the rate-determining step were the addition of a zinc-65 ion as in Fig. 2. Because zinc is more electrophilic than calcium, has a smaller ionic radius, and forms a more covalent bond¹⁰, the neighbouring calcium ion is released more readily than in Fig. 1, and is rapidly replaced by zinc-65.

In the presence of 2.5 mmoles/l. of stable zinc, the reaction appears to go even further. Not only does zinc replace calcium, but additional zinc may add on, possibly in the form of ZnOH+, as in Fig. 2e.

It may be objected that an alternative explanation of the behaviour of the zinc is possible: that it reacts with the bone mineral to form a zinc phosphate. When zinc-65 is used without stable zinc carrier, however, it is obviously

Fig. 1. Exchange of calcium-47 with stable calcium on surface of bone mineral.

(e)
$$\frac{^{65}Z_{n}^{2n+}}{^{0}}$$
 $\frac{^{0}H}{^{0}}$ $\frac{^{0}C_{a}}{^{0}}$ $\frac{^{0}P}{^{0}}$

Fig. 2. Exchange of zinc-65 with stable calcium on surface of bone mineral.

at too low a concentration to form a precipitate of zinc phosphate, and either adds on to the bone mineral or exchanges with it. With 2.5 mmoles/l. of zinc carrier, the possibility of the formation of a precipitate of zinc phosphate has been ruled out by experiments on the removal of the radioisotope which has been taken up by exchange, and by mcre detailed studies of the uptake of stable and radioactive zinc with time (my unpublished

It is likely that the fluoride ion exchanges with the hydroxyl ion in hydroxyapatite and 32PO₄3- with surface PO₄3- by similar addition mechanisms.

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Fertilization and Embryonic Loss in Sheep after Insemination with Deep Frozen Semen

Although there are reports of satisfactory lambing after artificial insemination with frozen ram semen1-4, most investigators have obtained disappointing results (reviewed by Emmens and Robinson⁵ and Sadleir⁶). It has been claimed, that frozen ram spermatozoa penetrate to the cranial part of the cervix more slowly than those from fresh semen. In an experiment⁸ with a limited number of animals, cervical insemination yielded only unfertilized eggs (eight ewes), and although after tubal insemination fertilized eggs were recovered from all three ewes examined, only two out of seven ewes lambed. We have conducted two experiments with frozen semen—the first at the McCaughey Memorial Institute, Jerilderie (Table 1) and the second on a private property at Yass, NSW (Table 2).

Semen was collected from mature merino rams by means of an artificial vagina. Ejaculates of good initial motility were pooled and diluted 1:1 at 30° C with a hypertonic ($\Delta = -0.90^{\circ}$ C) egg yolk-citrate-raffinose diluent, containing 3 per cent glycerol. Semen pellets (0.03 ml.) were prepared on dry ice, and stored in liquid

Table 1. EXPERIMENT 1: FERTILIZATION RATES WITH FROZEN RAM SEMEN AFTER BITHER CERVICAL, UTERINE OR TUBAL INSEMINATION

	(A		(B) Eggs fertilized/ eggs recovered Number Per cent			
Method of insemination	Ewes with fer ewes from white recov Number	ch eggs were				
Cervical Uterine Tubal	5/16 15/17 13/23	31 88 57	5/20 15/17 13/24 (B)	25 88 54		
Cervical against uterine: Cervical against	$\chi_{(1)}^2 = 8.95; P < 0$	0.01	$\chi_{(1)}^{3} = 12.36; P < 0.001$			
tubal:	$\chi_{(1)}^2 = 1.51$; N.S.	•	$\chi_{(1)}^2 = 2.73$; 0.08	S < P < 0·10		
Uterine against tubal:	$\chi^2_{(1)} = 3.29$; 0.05	< P < 0·10	$\chi_{(1)}^2 = 3.83; P =$	0.05		

Table 2. EXPERIMENT 2: FERTILIZATION AND/OR NON-RETURN RATES WITH FROZEN RAM SEMEN AFTER EITHER CERVICAL, DEEP CERVICAL OR UTERINE INSEMINATION

Method of insemination	Ewes fertilize ewes from eggs recov	d eggs/ n which were		rtilized/ covered	Non-returns/ ewes inseminated					
	Number	Per cent	Number	Per cent	Number	Per cent				
Cervical					0/68	0				
Deep cervical			_	_	10/67	15				
Uterine	25/27	93	27/29	93	23/68	34				
Cervical against deep cervical: non-returns, $\chi_{(1)}^2 = 8.89$; $P < 0.01$										
Deep cervical against uterine: non-returns, $\chi_{(1)}^2 = 5.54$; $P < 0.02$										
Uterine: ewes fertilized against non-returns 20 = 24.40: P<0.001										

nitrogen for 4-12 weeks. The pelleted semen was thawed in 2.6 per cent sodium citrate (2:1) at 37° C and used for insemination within 10 min.

Four methods of insemination were used. For cervical inseminations 0.30 ml. of thawed semen $(120 \times 10^6 \text{ motile})$ spermatozoa) was deposited into the entrance of the cervix of ewes 10-25 h after the onset of oestrus. "Deep" cervical insemination was carried out by traction on the cervical papilla with forceps permitting semen deposition 1-3 cm inside the cervical canal. Uterine and tubal inseminations, following laparotomy, were performed on most of the ewes within 6 h (range 1-8) after ovulation. In experiment 2, an additional eight ewes were inseminated just before ovulation. For uterine insemination 0.1 ml. of thawed semen $(40\times10^6$ motile spermatozoa) was deposited into the cranial region of the appropriate uterine horn(s). Tubal inseminations were achieved by depositing 0.05 ml. of thawed semen $(20 \times 10^6 \text{ motile})$ spermatozoa) into the ampulla of the appropriate fallopian tube(s). Rates of fertilization were determined by egg recovery and examination for cell cleavage 48 h after either uterine or tubal inseminations and 60 h after Sperm transport through the cervical insemination. genital tract of the ewe was examined by flushing the fallopian tubes of three ewes at 3, 6, 12 and 24 h after cervical insemination 10.

In experiment 1 (Table 1), fertilization rates after cervical insemination with frozen semen were low (25 per cent of eggs or 31 per cent of ewes fertilized). In a concomitant experiment performed by T. D. Quinlivan (personal communication) using sheep from the same flock, 80 per cent of eggs were fertilized after cervical insemination with fresh undiluted semen $(100\times10^6$ spermatozoa). A mean of only twenty-four spermatozoa from each fallopian tube was recovered from twelve ewes after cervical insemination with frozen semen. number is very low compared with that observed after natural mating11 or artificial insemination with fresh semen¹⁰ when a population of several thousands is usually found. There were no differences between the four times of sperm recovery. The results indicate that an impaired pattern of sperm transport through the genital tract of the ewe was the principal cause of low fertilization rates after cervical insemination with frozen semen. Primary failure of sperm transport probably occurred in the cervix. This claim is supported by the results of experiment 2 (Table 2) where "deep" cervical insemination with frozen semen was markedly superior to the normal

Frozen ram semen can produce high rates of fertilization when the cervix is by-passed with the uterine insemination technique. This was first shown in experiment I and subsequently confirmed in experiment 2 with egg fertilization rates of 88 per cent and 93 per cent, Tubal inseminations were less successful. respectively. Despite the finding of a high proportion of ewes with fertilized eggs in experiment 2, only 34 per cent did not return to service within 22 days of insemination, which implies that normal development of the embryos failed in some 64 per cent of the ewes containing fertilized eggs.

Such a high rate of embryo mortality, however, may not be entirely the effect of freezing. It is possible that

some eggs were "aged" at the time of fertilization (review by Salisbury¹²), although in eight ewes inseminated just before ovulation the rate of non-return (38 per cent) was not significantly higher than that for the remaining sixty ewes (33 per cent). It is also possible that the technique of uterine insemination caused changes in the environment of the genital tract, so as to impair survival of the embryo. In these preliminary experiments, uterine insemination with fresh semen was not examined. Lopyrin et al.13, however, have obtained normal pregnancy after uterine (three out of four ewes) and tubal (six out of eight) insemination with fresh semen. Finally, it must be borne in mind that even after natural service under apparently ideal conditions early embryonic loss is usually within the range of 10-20 per cent^{14,15}.

Nevertheless the results indicate that early embryonic mortality is extremely high in the sheep after fertilization following uterine insemination with frozen semen. There may also be additional loss of the embryo at a later stage of development, as indicated by the difference between

non-return and lambing results^{6,16}.

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Calcification in vitro of Human Aortic Tissue

CALCIFICATION in vitro of excised rat and rabbit acrta has been described previously1-5, but no comparable studies have been reported for the in vitro calcification of human aortic tissue. This communication reports the in vitro calcification of human aortic tissue using a procedure which measures the simultaneous deposition of calcium-45 and phosphorus-32. The mineral formed in the tissue was identified by X-ray diffraction analysis as hydroxyapatite.

Aortic tissue specimens were obtained at the autopsies of a 26 yr old male who died of cancer, a 37 yr old male who died of stab wounds and a 56 yr old female who died of cancer, to be referred to as tissues I, II and III, respectively. Tissue sections free of atherosclerosis were taken from the thoracic aorta. Calcification of the

tissues was induced in the following way. Fresh tissue was washed by mechanical shaking in physiological saline for 6 h to remove traces of blood. The wash solution was changed once after 3 h. After rinsing the washed tissue in triple distilled water, pieces (about 0.1 g) were cut, except for tissue II where 0.4 g pieces were cut, and a wet weight was taken. To ensure uniform penetration of the solution during calcification, incisions about 2-3 mm apart were made at least half-way through the tissue with a scalpel. The calcifying solution used for these experiments has been described before except that 100 mg of sodium ampicillin/l. calcifying solution was used instead of thymol. The quantities of calcium and phosphorus in the solution were adjusted to concentrations of 11 mg per cent and 5 mg per cent, respectively. The solution contained 20 μc . of calcium-45 and 20 µc. of phosphorus-32 as inorganic phosphate/l. of solution, and was freshly prepared for each experiment. The pH was 7.3. Control tubes containing only calcifying solution and stored in conditions identical to the calcification experiment remained stable for several weeks. The washed aortic tissue was placed in 28.8 ml. of calcifying solution in a tightly stoppered acid-washed glass tube. Calcification was allowed to proceed at 37° C. At specific time intervals of 2-123 h for tissue II, and 2-200 h for the other two specimens, tissue samples were removed and rinsed thoroughly in distilled water. Tissues were then ashed in a muffle furnace and the ash was dissolved in 2 ml. of 0.2 normal hydrochloric acid. An aliquot of the dissolved ash was pipetted into a naphthalene-PPO-POPOP-dioxane scintillation solution and calcium-45 and phosphorus-32 were counted simultaneously in an ambient temperature liquid scintillation counter. This method has previously yielded highly sensitive and reproducible results when applied to known calcification systems in vitro.

To determine the extent of calcium uptake resulting from true calcification it was necessary to measure (a) the amount of calcium bound in the absence of phosphate as well as (b) the exchange, if any, of residual tissue calcium with calcium-45 in the solution. The uptake of calcium by these means will be referred to as non-specific incorporation. These determinations were carried out by exposing aortic tissue samples to a solution containing all of the ingredients of the calcifying solution except for inorganic phosphate. All other conditions remained the same. The incorporation of calcium-45 from the solution into the tissue was followed with time. Tissue samples were treated as described here for the calcification pro-The samples were assayed as described for calcium-45. Similarly, the binding of labelled phosphate in the absence of calcium, also referred to as non-specific incorporation, was investigated in the same way as that described for calcium alone, using a solution containing all the ingredients for calcification except calcium. At various time intervals each tissue was removed, washed,

ashed and assayed as described before.

It is evident that there is some uptake of calcium-45 in the absence of phosphate (Fig. 1). The data shown in Fig. 1 indicate that some of the calcium taken up during calcification could result from non-specific incorporation. Also, some of the phosphorus-32 uptake could take place by some means other than calcification (Fig. 1). might be the result of a net incorporation of calcium (or phosphorus) unrelated to calcification or an exchange of the unlabelled element retained by the washed tissue with the labelled element from the solution. It is also possible that some combination of both mechanisms is responsible for the non-specific incorporation of calcium and phosphorus during calcification. These reservations must be considered in evaluating the results.

The first experiment made use of tissue II. From the results (Fig. I) it was evident that additional calcification could be achieved by extending the experimental period. The subsequent experiments involving tissues I and III were therefore extended to 200 h. Also, tissue weights

for the longer experiments were reduced from 0.4 g to 0.1 g in an attempt to reduce the amount of calcium and phosphorus being withdrawn from the solution. results of these experiments using solutions containing both calcium-45 and phosphorus-32 revealed that simultaneous deposition of calcium and phosphorus into aortic tissue increases for at least 123 h in the case of tissue II and at least 200 h for the other two specimens (Fig. 1), whereas the incorporation of either calcium or phosphorus alone showed little increase after 24 h. Because the uptake of either calcium or phosphorus alone shows no significant increase after 24 h in any of the specimens (Fig. 1), it can therefore be assumed that subsequent calcium and phosphorus uptake is chiefly, if not entirely, caused by calcification. Considerably more calcification took place in the experiments involving tissues I and III (Fig. 1). This was probably because of the time and weight changes previously described.

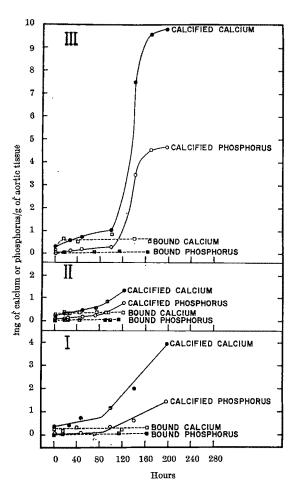


Fig. 1. The uptake of calcium () and phosphorus () during calcification is represented by the curves marked "calcified calcium" and "calcified phosphorus". The incorporation of calcium in the absence of phosphorus () and phosphorus in the absence of calcium () the represented by the curves marked "bound calcium" and "bound phosphorus". I, 26 yr old male; II, 37 yr old male; III, 56 yr old female.

A comparison of the rates of uptake of calcium-45 and phosphorus-32 during in vitro calcification of the three tissue samples as shown in Fig. 1 reveals some interesting similarities. The graphs for each tissue (Fig. 1) show a lag period followed by an inflexion point between 80 and 100 h and a subsequent increase in the slope of the curve. The patterns during the lag period are very similar and for tissues I and III are nearly superimposable up to the inflexion point. Apparently the weight difference between tissues I and II had no effect on the experiment until after the time of the inflexion point. The significance of the lag period described here is currently being investigated.

Aortic tissue samples from tissues I and III placed in calcifying solution for 200 h were subjected to X-ray diffraction procedures. The results showed a hydroxyapatite pattern, indicating that hydroxyapatite was formed during the calcification study. It is interesting that hydroxyapatite has also been found during calcification of aortic tissue in vivo? as well as in in vitro calcification studies with animal aortic tissues4. A Von Kossa stain done on the in vitro calcified aortic tissues was positive within the media as early as 72 h when cell nuclei were distinctly identifiable in haematoxylin and eosin.

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Alteration by Pretreatment with Iproniazid and an Inactive Mescaline Analogue of a Behaviour Change induced by Mescaline

THERE have been several conflicting reports on the interaction of mescaline with monoamine oxidase inhibitors. Friedhoff and Goldstein¹, who gave 100 mg/kg iproniazid to rats, 24 h and 3 h before mescaline, found that this pretreatment had no effect on the gross behavioural changes induced by mescaline at 10, 25 and 100 mg/kg. The iproniazid pretreatment itself had produced a slight behavioural excitation at the time of administration of mescaline, and urine estimations of labelled mescaline showed that levels of the unmetabolized amine were considerably increased. In vitro experiments suggest that monoamine oxidase does not affect the metabolism of mescaline. Diamine oxidase (histamine oxidase) and a specific mescaline oxidase (found in rabbit liver), however, are probably involved in the oxidation. Iproniazid is not a specific monoamine oxidase inhibitor and is capable of inhibiting both histamine and mescaline oxidase, which would explain the increase in unmetabolized mescaline in urine. Goldwurm and Gualandri (personal communica-

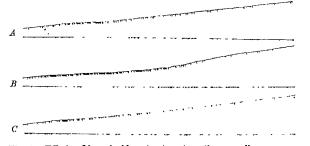


Fig. 1. Effects of ipronlazid pretreatment on the mescaline response. A and C, 17.5 mg/kg mescaline; B, 17.5 mg/kg mescaline pretreated with 50 mg/kg ipronlazid.

tion) found that pretreatment with iproniazid abolished the inhibitory action of mescaline on shuttle box avoidance in the rat. Steiner and Sulman² reported that iproniazid given before mescaline in the rabbit induced an anxiety reaction together with EEG changes.

We have examined the effect on rats of pretreatment with iproniazid. The subjects were three experimentally naive male hooded rats which were trained on a continuous discriminated avoidance schedule in a Skinner box until a stable level of performance was achieved. On this schedule an animal receives a shock of 0.5 see duration every 10 see unless it makes a bar press which postpones the shock for 30 sec. During the last 10 sec of this response-shock interval a discriminative stimulus light is turned on inside the experimental chamber. The stimulus light remains on until the animal makes a response which initiates a new cycle.

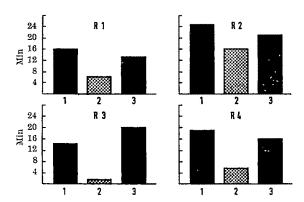


Fig. 2. Duration of inhibitory period for four experimental animals subjected to the three drug conditions. 1 and 3, 12·5 mg/kg mescaline; 2, 12·5 mg/kg mescaline pretreated with 25 mg/kg 2,4,5-trimethoxy-phenylethylamine.

Throughout the experiment, each subject was tested at the same time every day for 2.75 h. The experimental session can be sub-divided into a 15 min "warm-up" period, a 30 min pre-injection or control period and a 2 h test period. Each subject served as its own control and was injected with a control physiological saline solution on the days before and after drug administration. With the exception of the days before the treatment started, all drugs were given after the pre-injection period and the subject was immediately placed in the Skinner box for the 2 h test period. A pilot study revealed that 100 mg/kg of iproniazid had some behavioural effects on the avoidance schedule so the pretreatment dosage was halved to 50 mg/kg. At this dose there was no behavioural effect at any time after injection. Initially, the response of each animal to 17.5 mg/kg mescaline was determined (Fig. 1A). Previous experiments had shown that this dosage would have a noticeable, but not extreme, effect on behaviour. Fourteen days later each animal received 50 mg/kg of iproniazid (intraperitoneally) 3 h before the administration of 17.5 mg/kg of mescaline. As a further control 17.5 mg/kg of mescaline was given again after 4 weeks. Fig. 1B shows the influence of iproniazid pretreatment on the action of this dose of mescaline. There is a marked increase in the effect of the drug. The entire procedure was repeated for each animal and the increase in the effect of mescaline was verified.

This finding does not support the theory that mescaline acts through a metabolite but suggests that the free amine level is the critical factor. An alternative hypothesis is that the increased levels of other monoamines, after iproniazid pretreatment, in some way facilitate the hallucinogenic activity of mescaline. A number of experiments are suggested by the latter hypothesis. If endogenous amine levels are involved, then pretreatment with amine depleters such as reserpine or tetrabenazine

should decrease hallucinogenic activity and specific monoamine oxidase inhibitors (possibly N-octanol) should The reversal of the enhance hallucinogenic activity. mescaline effect by pretreatment with tetrabenazine would avoid any interpretation of the results as the summated effects of two independent processes. because both mescaline and tetrabenazine block the conditioned avoidance response, then any reversal of the mescaline effect would be in the opposite direction to both manipulations. Furthermore, because pharmacological agents are available which selectively interfere with the synthesis of particular amines, then it might be possible to delineate further the precise action of mescaline.

A second pretreatment experiment was undertaken in order to throw some light on the specificity of the mescaline molecule. Four experimentally naive male hooded rats were kept on a restricted 23·5 h water deprivation schedule. All subjects were trained to press a lever in a Skinner box for access for 1 sec to water reinforcement. After preliminary training, reinforcements were only delivered when a response occurred at least 15 sec after the previous one (DRL 15). When the animals had reached a stable level of performance, each subject was initially treated with 12·5 mg/kg mescaline. The effect of the drug is to cause a period of complete suppression of lever pressing. It has already been shown that the duration of the inhibitory period is a good index of drug dose.

Fourteen days later these animals were given 25 mg/kg of 2,4,5-trimethoxyphenylethylamine (2,4,5-TMPE) followed after 15 min by 12·5 mg/kg of 3,4,5-trimethoxyphenylethylamine (mescaline). The pretreatment compound is inactive at this dosage³. After a further 14 days, 12·5 mg/kg of mescaline was again administered. The duration of the inhibitory period was measured for all three conditions (Fig. 2). It is clear that the effect of pretreatment was to decrease the mescaline response.

The antagonism between these two closely related compounds would suggest that 2,4,5-TMPE is capable of occupying the mescaline receptor site or blocking related transport mechanisms, though itself having no central effect. In this case a systematic study of the other fifteen inactive ring methoxylated phenylethylamines in this situation may yield data as to properties of the receptor site involved.

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Weissenberg Effect as an End-point in Coagulation Studies

During a recent investigation of the viscosity changes which occur when thrombin is added continuously to fibrinogen, the substrate solution suddenly and rapidly climbed the shaft of a motor driven paddle at about the conventional coagulation point. This phenomenon is familiar to investigators of artificial high polymers as the Weissenberg effect¹⁻³, which is usually observed where the macromolecules or other structures form a network of temporary entanglements or permanent cross-linkages.

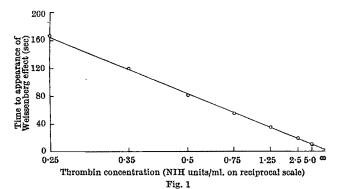
To our knowledge, the effect has not been noted in the case of fibrin. It occurred to us to investigate whether the appearance of the effect might provide a useful end-point in coagulation studies.

We used a commercial standardized concentrate of bovine thrombin (Parke, Davis and Co., thrombin "Topical") diluted with normal saline, and a freeze-dried preparation of human fibrinogen (85 per cent "clottable" protein) separated from time-expired plasma and made available to us by the Lister Institute. The fibrinogen preparation was dissolved in distilled water to give the concentrations: "clottable" protein 9.0 mg/ml.; "non-clottable" protein 1.5 mg/ml.; sodium chloride 17.0 mg/ml.; trisodium citrate 7.4 mg/ml. This solution was filtered by means of a P_1 sintered glass filter before use.

A volume of 0.9 ml. of the substrate solution was placed in a 16 \times 120 mm test tube immersed in a water bath at a temperature of $37^{\circ} \pm 0.05^{\circ}$ C. A stirrer, made of a solid glass rod 3.5 mm in diameter with a 10 mm long T-piece, was placed in position with the cross-piece well immersed and the variable speed stirrer motor was started. The thrombin solution in a volume of 0.1 ml. in a syringe was immersed in the bath for a sufficient time to equilibrate its temperature and then added to the substrate solution in a single amount. The time to the appearance of the Weissenberg effect was measured with a stop watch. Fig. 1 gives the results obtained at a stirring speed of 2.8 r.p.s., for a range of thrombin concentrations in the final coagulating solutions between 0.25 and 5.0 NIH units/ml. The time to the appearance of the Weissenberg effect, when plotted against the thrombin concentration on a reciprocal scale, gives an excellent linear relationship: the deviations on the time axis from the best straight line are, in this case, within the limits ± 1 per cent ± 1 sec. (More generally the deviations from the best straight line were within the limits ± 3 per cent ± 1 sec.) The linear relationship between the concentration of thrombin and clotting time is well known4.

With the geometry and quantities described, we found that below the speed of stirring of 2.8 r.p.s., the Weissenberg effect was not reliable and occasionally failed for the highest thrombin concentrations within our experimental range; we presume that the shear rate in the neighbourhood of the stirrer was insufficient to develop an elastic force which could overcome the gravitational force before the gel was completely firm.

Above about 5.4 r.p.s., the effect was lost at the lowest concentrations of thrombin. When the volume of the solution was doubled in a 22×150 mm test tube, the upper limit was increased to about 9 r.p.s. With 20 ml. of substrate contained in a beaker with a slightly larger stirrer (5.5 mm diameter rod, 21 mm T-piece), it was possible to observe the Weissenberg effect throughout the whole experimental range up to a stirring speed of 16 r.p.s., at which point the stirrer started to draw bubbles of air into the solution. Presumably, in the narrower vessels, at the upper limits, a sufficiently high shear rate was



being developed at the stirrer to inhibit the formation of the molecular network.

In general, the faster the stirring rate, the sharper was the Weissenberg effect observed. For a fixed concentration of thrombin, the time to the appearance of the Weissenberg effect depended on the stirring rate: in the case of the large volume of substrate in a beaker with a thrombin concentration of 1.25 U/ml. of substrate, the time decreased from about 43 to 37 sec as the speed of the stirrer was increased from 2.4 to 16 r.p.s. The decrease of coagulation time with an increase in shear rate has already been noted^{5,6}. For determining curves, such as that in Fig. 1, we have used a stirring speed just above the lower limit.

The most commonly used method for determining the coagulation time is to note the point at which flow is no longer obtained when the container is tilted⁴. Another popular method depends on noting the time at which the optical density changes by a significant amount⁴. Other optical and mechanical methods have been described⁷⁻¹⁰. The observation of the Weissenberg effect provides a more objective and precise end-point than the first method and may be somewhat simpler than the others.

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PSYCHOLOGY

Conditions for Successful Transfer Effects

LEARNED behaviour is reported to be transmitted by injecting into untrained animals fractions¹⁻⁴ or homogenates⁵, containing RNA, from the cerebral hemispheres of trained rats. Others⁶⁻⁸ have been unable to confirm these positive transfer effects. The transfer problem is very important for the understanding of memory and learning, and we have carried out a series of experiments with rats to investigate certain preliminary conditions which may be important in the positive or negative results which have been reported.

A defensive motor response (jumping up to a shelf) was conditioned by pairing an acoustic stimulus (bell) with an electric shock applied through the floor grid of the training box, 12 cm below the shelf. The experimental session consisted of four successive trials with intertrial intervals of 1 min. The unconditional stimulus (electric shock) was delivered with a delay of 5 sec after the conditioned stimulus (bell) unless the rat jumped onto the shelf. Groups of trained animals which performed completely (100 per cent) the conditional response served

Table 1. CHRONOLOGICAL REPRESENTATION OF THE TWELVE TRANSFER EXPERIMENTAL SERIES as donors. In all experiments, the phenol Black rectangles indicate the preliminary treatment of the given experimental series. extraction procedure already described^{3,4}

HUMB ER OF	DAY OF	TRAINED DONORS .		RECIPIENTS					TED BY	SIGNIFICANCE LEVEL				
	TONEAL INJECTION	SHORT TERM	T MORMAL LONG		NUMBER	NUMBER HAIVE DI	HABI- TUATED TO CS	TRAINED TO JUMP TO US COOLN- SIS YOUNS		NAIWES	TRAI- NED NAITE DONORS		(WILCOXON TEST)	
1	1056 A PRIL 26				20	10					20	10	P > 0,05	
2	MAY 12				14	14					14	14	P < Q005	
3	MAY 12				14	14					14	14	P>0,05	
4	MAY 26				18	18					18	18	P < 0,025	
5	JUNE 16		,		16	16		٠.			16	16	P < 0,005	
6	SEPT 28		,		20	20					20	20	P <q01< td=""></q01<>	
7	OCT 12				10	10					10	10	P<0,005	
8	OCT 12		; ;		10	10					10	10	P>Q.05	
9	NOV 14				15	15					15	15	P>0,05	
10	NOV 26				14	14					14	14	P < 0,05	
11	NOV 28				20	10					20	10	P>Q05	
12	DEC 13		:		20	20					20	20	P<0,025	
			TOT	AL	191	171			TOTA	A L	191	171		

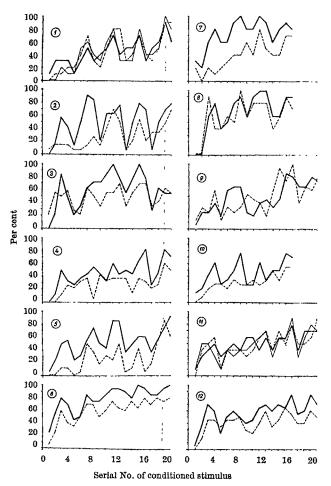


Fig. 1. Conditioned motor response of the groups of recipient rats injected intraperitoneally by brain extracts of trained (heavy lines) and untrained (dotted lines) donors. Diagrams 1 to 12 represent the chronological sequence of the experimental series. Abscissa: seriel number of conditioned stimulus. Ordinate: percentage of the recipient animals performing the conditioned response (jumping on to the shelf). In series 1 and 11 two groups of recipients were treated with extracts from trained rats, and one with extracts from untrained rats.

as donors. In all experiments, the phenol extraction procedure already described^{3,4} was used. Each recipient was given intraperitoneally 1 ml. of a solution containing total brain RNA extract from one donor (1·6-1·8 mg/ml. of RNA, determined spectrophotometrically).

Male Wistar rats (362) served as donors. Three different trained donor groups were used: (a) series 10 and 11 with a "low" ("short term") conditioning (five sessions in 3 days); (b) series 2, 3, 4, 5, 6, 7 and 9 with a "medium" ("normal term") conditioning (twelve sessions in 10 days); and (c) series 1 and 8 with a "high" ("long term") conditioning (twenty-four sessions in 20 days). The control donor groups consisted of untrained animals.

Another 362 male Wistar rats were used as recipients. The recipients were divided. before injection, into groups; (a) some groups were completely habituated to the conditioned stimulus and additionally trained to jump up to the shelf (series I and 7); (b) some groups were trained only to jump up to the shelf after electric shock, but not habituated to the conditioned stimulus (series 2.

3, 5, 6, 7, 9, 10, 11 and 12). Series 3 and 6 were young animals, 4 months old. Finally, (c) a third recipient group was trained to neither the conditioned nor the unconditioned stimulus. After intraperitoneal injection the recipient groups were tested in the same way with the trained donors, that is four successive trials in each session, 4, 8, 12, 28, 52, 76, 100 and so on hours after the injection of the brain extract. The experimental and control groups were conditioned and tested completely "blind", that is the experimenter did not know what previous treatment the animals had received.

The level of significance of the conditioned performance of the recipient groups injected with extracts of brain of trained donors, when compared with the recipient groups injected with brain extracts from untrained donors, was calculated by means of the Wilcoxon test, on the basis of the results of the first three sessions after injection.

A general trend is revealed in Fig. 1, which shows that injectees which received extracts of trained donors performed better than recipients of control brain extracts. The difference between the trained and the control group is most striking, however, in series 2, 4, 5, 6, 7, 10 and 12. This is explained by the following conditions (summarized in Table 1). (a) If the donors are poorly trained, the transfer effect is not excluded (series 10), but occurs with a lower level of significance (P < 0.05). In series 11 no transfer effect was observable. (b) If the donors are "over-conditioned". no significant transfer effect can be observed (series 1 and (c) If the recipients are habituated only to the unconditioned stimulus before injection, or if they receive no preliminary training, the memory transfer takes place. It does not occur, however, if they are habituated to the conditioned stimulus before the administration of the brain extract. (d) The young recipients first habituated to the unconditioned stimulus are probably less suitable for transfer studies than the mature animals (series 3, unsuccessful; series 6, successful).

Thus it seems that in our experimental conditions the optimal transfer effect occurs when donors are trained for 10 days (twelve experimental sessions) and when adult recipients are not habituated to the conditioned stimulus (series 2, 5, 7 and 12).

Our findings do not, of course, indicate whether they concern the transfer of some specific memory mechanism or simply of a certain kind of excitatory state. Furthermore, they are not directly relevant to the detailed mechanism, or to the nature of the chemical agent transmitted. These results may be valuable in filling the gap

between conflicting data¹⁻⁸ and in stimulating further investigation.

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GENERAL

Entropy and Evolution

CAMPBELL'S expressed aim1 is "to describe the entropy pump whereby the species of living matter not only prevent a drop into a position of greater positive entropy at each generation, but may in fact acquire more negentropy as their reproduction continues". This is attempted by recourse to the example of a codfish laying a million eggs the entropy content of whose genomes shows a normal distribution about a mean value in accordance with the second law of thermodynamics. On one tail of this distribution curve will be a small percentage of eggs with an entropy content equal to or less than that of the parents. There follows the crucial sentence: "These There follows the crucial sentence: remarkable ones are most likely to grow up and repeat the reproductive process". What follows in Campbell's communication is unexceptionable provided that this sentence is true. But what is the evidence that those eggs which are "most likely to grow and repeat the reproductive process" are those whose genomes have a lower entropy content than those of the parents? It is at this point that the author merely evades one of the most important questions at issue in the earlier correspondence²⁻⁴. The genome may be regarded as a series of DNA molecules functioning as templates. The thermodynamic entropy content of these molecules is a function of the arrangement of the atoms, conventionally expressed as $k.\log D$ where k is the Boltzmann constant and D is a measure of the atomic disorder. A single alteration in the relative positions of adjacent nucleotide bases could convert a crucial part of the genome code to nonsense so that its capacity to support development was lost, but this could occur without an increase, indeed even with a decrease, in the thermodynamic entropy content of the genome molecules. Stated in a more general way, this is the problem of the relationship between the amount of developmentally meaningful organization in the genome (we will call this the information content) and its entropy content. There are two problems here: first, the definition and quantization of the information content of the genome and, second, the nature of its relationship to the thermodynamic entropy content. One avenue of enquiry which offers hope in this situation would seem to be the information theory analogy.

Szilard⁶ pointed to the formal similarity between the equations defining information

$$H = -\sum_{i=1}^{i=n} \text{pi log. pi}$$

(where $0 \le pi \le 1$, $\sum_{i=1}^{n} pi = 1$ and pi is the relative probability of the *i*th symbol generated by a source), and entropy defined in statistical terms as

$$S = -i\sum_{i=1}^{i=n} pi \log_{i} pi$$

(where Σ_{i}^{n} pi = 1 and pi is in this case the probability of an idealized physical system being in the state i of n possible equivalent states).

The work of Shannon⁷ and Brillouin⁸ showed the fundamental relationship between information and entropy defined in these terms. It is the unwarranted extrapolation of this relationship to biological systems which leads to erroneous conclusions, although the warning was admirably given by Brillouin himself*: "The present theory of information completely ignores the value (or meaning) of the information handled, transmitted or processed. This point has been very carefully emphasized throughout this book. Many other writers seem not to have realized the importance of this restriction, and many misunderstandings about the possibilities of the present theory resulted from this situation". This is the biological problem because, as I have said, different arrangements of equal members of nucleotides may have the same thermodynamic entropy but a different value in the context of the development and viability of the organism. It may be that, as Lwoff⁵ has supposed, this functional order cannot be measured in terms of entropy units and is meaningless from a purely thermodynamical point of view. But this may be an over-pessimistic view. For example, one possibility which seems worth exploring arises from possible analogy with the work of Carnap and Bar-Hillel¹⁰, who have applied techniques of symbolic logic to define the information content of a sentence. Now if for a very simple organism such as Mycoplasma, one came to know the complete nucleotide sequences and the limits of their physical variation within each operon consistent with successful replication and development, and the essential sequence of operon functions necessary for development, it might be possible to define the information content of the organism in terms of the number of base sequences commensurate with reproduction and development. From this it is conceivable that one might obtain a "content measure" bearing at least a qualitative similarity to the information equation of statistical thermodynamics. If this were achieved one would then in effect be saying something in thermodynamic terms about what marked off the living from the non-living. This may seem a long shot, and indeed ignores the whole question of essential extra-genomic factors11, but in any event until this or other possible approaches to the problem are explored we cannot with any confidence pronounce on either the direction or the magnitude of the thermodynamic entropy changes involved in the acquisition of the "fuller informational systems" which are the products of evolution. This being so, it would seem premature to speak of an 'entropy pump''.

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BOOK REVIEWS

DALTONIAN REVOLUTION

Atoms and Elements

A Study of Theories of Matter in England in the Nineteenth Century. By David M. Knight. (The History of Scientific Ideas.) Pp. 167. (London: Hutchinson and Co. (Publishers), Ltd., 1967.) 30s. net.

It is customary to regard nineteenth century chemistry as proceeding smoothly in the wake of a chemical revolution initiated by Lavoisier; but, as this book shows, there was another revolution stemming from Dalton's atomic theory. It is not the case, of course, that Dalton's theory was universally accepted; it was not, even by English chemists. But the importance of Dalton's theory and its inclusion of the very useful and helpful theory of definite proportions made chemists necessarily more sensitive to the problem of the nature of matter, and caused physicists to realize that theories of matter must meet chemical as well as physical tests. That physicists like Kelvin were forced to take chemical problems into consideration when attempting to devise a new theory of matter is an indication both of the power of Dalton's work and of the growing maturity of chemistry.

The conventional view of the history of atomic theories in the nineteenth century has been that the Daltonian atom was unacceptable to physicists because of its "billiard ball" characteristics. As Dr Knight here shows, the real snag about the Daltonian atom was metaphysical, not physical: chemists and physicists alike generally preferred the view that matter is homogeneous to Dalton's view that the atom of each element is physically and therefore chemically distinct. They were also truer Newtonians than Dalton, and conscious of the existence of forces. For all these reasons, as recent work on the history of science has shown, English physicists and chemists of the nineteenth century found the atomic theory of Boscovich peculiarly satisfying. The attempts of Boscovich himself to apply his theory of point forces (endowed with inertia) to chemistry had not been very successful, but his Theoria naturalis philosophiae was too early (it was published at Venice in 1763) for his chemical knowledge to have anything to contribute to the nineteenth century; indeed his applications of his theory to physics were not noticeably more helpful. But the theory itself was wonderfully versatile and appealed particularly to English natural philosophers. (On the whole, continental scientists were more pragmatic.) Expounded in the supplement to the third edition of the Encyclopaedia Britannica (1803), discussed in early lectures at the Royal Institution by Thomas Garnett (1801), Boscovich's theory was adopted successively by Davy and Faraday and remained a dominant feature of English scientific thought in the nineteenth century, being used at the end of the century by J. J. Thomson.

In spite of its title, this book is mainly about the atom in chemistry; the author has not discussed the relation of theories of matter to the development of such physical problems as the kinetic theory of gases. It is also mainly concerned with the earlier half of the century. The sixth chapter does deal with the curious "debates" between officers of the Chemical Society in the late 1860s, and there is an epilogue on the next two decades, but these are specialized and not so easy to follow as the earlier chapters. Unlike the earlier chapters, these later ones presuppose considerable knowledge of the history of

chemistry in the period—of the development of the periodic table, of the long, tangled and slow growth of an understanding of organic compounds, and of the acceptance of the theory of valency. Two of the leading English protagonists in the development of the concepts of valency and organic structure, Alexander Williamson and William Odling, were active in these debates; the problems there raised have been more fully and therefore more lucidly developed in the accounts already published by the author in collaboration with W. H. Brock.

It is a fascinating subject for chemistry and physics alike, and one little studied so far. This book makes an admirable introduction to a subject worthy of much further study.

MARIE BOAS HALL

MATHEMATICAL LOGIC

Mathematical Logic

By Stephen Cole Kleene. Pp. xiii + 398. (New York and London: John Wiley and Sons, Inc., 1967.) 85s.

This book for undergraduate study is written by the author of a famous postgraduate text Introduction to Metamathematics written nearly twenty years ago. Like the more advanced work the present volume is characterized by its comprehensiveness and thorough treatment. Sentence and predicate logic are both presented in a variety of ways, from model theory to several versions of proof theory including axiomatic proof theory, natural inference and (in the last chapter) Gentzen's sequent calculus. The account of the difficult subject of substitution in predicate logic is particularly successful, but I do not consider the treatment of sentence formation which allows collision of variables and vacuous quantification suitable for a first course in logic. The simplicity gained in the definition must be paid for at too high a price in the interpretation of sentences like VxExPx in the model theory; I think a clearer distinction and separation between free and bound variables (and not just free and bound occurrences) is desirable for beginners in predicate logic even if this requires a definition of sentence by level of complexity.

As one would expect from one of the pioneers in general recursive function theory, the chapter on computability and decidability is extremely good and contains many references to recent work; for instance, on degrees of unsolvability and the arithmetical hierarchies (which the author initiated).

Completeness of predicate logic is established by means of Gentzen's sequent calculus and a proof of the equivalence of the axiomatic and the sequent formulations; the idea behind the completeness proof is to set up a systematic method of search which for any predicate sentence F leads either to a counter example to F if there is one or to a closing of all avenues to a counter example when F is valid. The equivalence of the axiomatic and sequent formulations is also exploited to give a simple proof of Craig's interpolation theorem for predicate calculus with equality which says that if $E \supset F$ is provable then there is a sentence I the parameters of which are common to E and F such that $E \supset I$ and $I \supset F$ are both provable.

R. L. GOODSTEIN

ELECTRON DIFFRACTION METHOD

Electron-Diffraction Analysis of Clay Mineral Structures By B. B. Zvyagin. Revised edition. Translated from the Russian by Simon Lyse. (Monographs in Geoscience.) Pp. xvi+364. (New York: Plenum Press, 1967.) \$19.50. For crystal structural studies, the method of singlecrystal X-ray diffraction is generally the most successful,

but because of the fine grained nature of clays, only powder

X-ray data can as a rule be obtained from these materials, with consequent loss of information. The usefulness of the X-ray powder method is, moreover, further limited for clays because of the difficulty of preparing a truly randomly oriented powder specimen from aggregates of platy particles. It has long been appreciated, therefore, that the method of diffraction from a single crystal using a fine beam of electrons would be a useful one for the study of the structures of clay minerals, but this also is not without difficulties. The first is again the problem of preferred orientation, the platy clay particles tending to lie parallel to the supporting substrate, and therefore limiting the amount of diffraction data that can be recorded. The second difficulty is the interpretation of the observed electron diffraction intensities, because these are considerably affected by dynamical interaction between the diffracted and transmitted beams and by other factors which are not easily estimated quantitatively. It is interesting to compare the rather different ways in which these two obstacles have been regarded and dealt with in the Soviet Union (illustrated by Zvyagin's book) as compared with the approach by most other workers.

The Russian school, exemplified by Pinsker, Vainstein, Zvyagin and co-workers, have overcome specimen orientation difficulties by using specially designed electron diffraction cameras. These have allowed the tilting of the specimen stage to quite high angles, and have led to the use of the "oblique texture" method which gives a reasonable coverage of the diffraction data in reciprocal space. Most workers elsewhere have tended to make do with their electron microscopes for electron diffraction work, and the requirement of high resolution microscopy has (until very recently) militated against the provision of high-tilt specimen stage facilities. Of course, the electron diffraction camera user cannot see an image of the crystal which is giving the electron diffraction pattern, but in the study of particular specimens of clay minerals which are known to be homogeneous this is not a great disadvantage.

The difficulties concerning electron diffraction intensities have daunted many, but the Russian school has pressed on regardless. There is no doubt that, except in very favourable circumstances, a highly accurate structure determination cannot be achieved using electron diffraction intensities, but crystallographers know well that even rough relative intensities can yield a close approximation to the correct atomic co-ordinates of a structure. It has thus been possible for Zvyagin and others to obtain useful structural information about clay minerals using electron diffraction which could not be determined in any other way.

The greater part of this book is concerned with the electron diffraction method and results, but a useful additional topic is dealt with in the first chapter, which describes the structures, polymorphs and polytypes of layered silicates. Here Zvyagin's systematic approach and specially devised notation are of great interest. The second chapter describes the electron diffraction apparatus, while the third and fourth chapters treat the theory of electron diffraction analysis in general, and in particular the "oblique texture" method applied to layered silicates. The fifth chapter gives examples of structure determinations of clay minerals which have been effected by the foregoing methods: these include kaolinite, celadonite, muscovite, phlogopite, nacrite, sepiolite and palygorskite.

For the reasons discussed here, relatively little mention is made of combined electron microscopy and diffraction. With the recent development of high resolution electron microscopes with facilities for high angles of specimen tilt, the researcher can have the best of both worlds. Furthermore, dark-field imaging, using selected diffraction spectra, is proving important for the study of short range detail rather than the long range average structural features examined by X-ray diffraction. Thus the advantages of electron diffraction camera work are far fewer now than

they were when this book was written in Russian in 1964. Others may now, using electron microscopes or cameras, take advantage of the methods and treatments set out so clearly by Zvyagin.

The author has taken the opportunity, on the occasion of the book's translation from the Russian, to make some revisions, and to add new material concerning the serpentine minerals, hallowsite, chlorites and disordered micas. The translation and technical reproduction of the book are both of a high standard, except in the case of the relatively few electron micrographs shown, which are less clear than the electron diffraction patterns.

This book can be read with profit by all those interested in the crystal structures of clay minerals or in the techniques of electron diffraction.

J. Zussman

NON-FERROUS HEAVY METALS

The Technology of Heavy Non-Ferrous Metals and Alloys

Copper, Nickel, Zinc, Tin, Lead. By J. H. Cairns and P. T. Gilbert. (Newnes International Monographs on Materials Science and Technology.) Pp. viii+319+2 plates. (London: George Newnes, Ltd., 1967.) 70s. net.

This book sets out to emphasize practical applications rather than scientific aspects, and thereby defines its usefulness to metallurgists, engineers and designers. It achieves these objectives very well, particularly in the excellent summary of mechanical working processes and the five chapters on applications.

It is least successful when summarizing the physical and mechanical properties, although, in fairness, the fly-leaf declares that this section is "not intended to be as detailed as the standard metallurgical textbooks". Typical of the surprising statements made here is that specific heat "increases slightly with rise of temperature", followed by a general approximation of "5 per cent per 100° C", which is hardly slight. Nowhere is there any incorrect statement, but the appeal is more to the old fashioned concepts of many engineers and designers than to the new approach of the Council of Engineering Institutions.

No mention of powder metallurgy processes or products is made, except for sintered bronze bearings, and in the section on welding, the friction process does not appear. In assessing the appropriate properties for bearings, the importance of fatigue strength is omitted. Unusual entries in the index include "beer" and "whisky", for production of which considerable quantities of copper and nickel are employed. One excellent feature of the index is a separate list of those maddening specification numbers for metallic materials and components made from them. Few books in this field are produced outside the publications of the metal development associations, and so the authors are to be congratulated on this volume.

C. R. TOTTLE

NO VITALISM FOR CRICK

Of Molecules and Men

By Francis Crick. Pp. xv+99. (Seattle and London: University of Washington Press, 1966.) 30s. net.

For his John Danz Lectures, given at the University of Washington, Francis Crick took a title very appropriate for the field in which his work is so well known; but it was, perhaps, rather a quirk to choose to develop his theme in terms of an attack on vitalism. Is this not flogging a dead horse? Crick is at pains to point out that the horse is still kicking, if faintly. "And so," he writes, "finally, we come to the question, 'Is vitalism dead?'. It

seems to me that, reluctantly, we must answer, 'No'. While there are intelligent people alive who sincerely believe in vitalistic ideas, even though they are fully acquainted with the scientific knowledge on the subject, we must conclude that vitalism is still alive. There remains the question how we can disprove it . . .".

The trouble is to define just what we mean by "vitalism".

Crick writes at one point (page 26), "We have seen that it means that there must be something else in a biological system which cannot be included under the heading of physics and chemistry". But what can, or cannot, be included under that heading? Crick often writes as though physics and chemistry are a complete systempresumably "classical", because quantum considerations are scarcely mentioned. "It is my argument that our present general knowledge of physics and chemistry is sufficient to act as an exceedingly solid foundation, though, let me add, much of the detailed chemistry is incomplete and needs much further study". One must ask, is a quark included? And if biologists should find it necessary to postulate an entity as odd as a quark, would that be vitalistic or not? The controversy about vitalism arose at a time when physicists thought they knew what the fundamental physical entities consist of. It takes a new form, which is much harder to define, at a time like the present when no physicist would make such a claim—when indeed it is becoming more plausible to suggest that there is no such thing as a truly fundamental physical entity, but that the universe is an open system, providing an infinite perspective for deeper and deeper study, probably in both directions, into the infinitely small sub-nuclear particles and the infinitely large cosmological systems.

In my opinion, the first step in any worthwhile discussion of vitalism is to make a distinction between what may be called the objective and subjective varieties of it1. The former holds that there is something not physically explicable about the observable behaviour of living systems; the latter makes the point that the difficulty arises in connexion with functions such as consciousness or awareness, which we can experience within ourselves but which we cannot actually observe in any other biological entities, although we may postulate their existence there. These are really two quite different points, although often confused, both by Crick and by many of the authors

he accuses of vitalism.

Elsasser, who is one of Crick's targets, is perhaps an objective vitalist. Crick has little difficulty in disposing of his arguments in the form they were first put forward² in which Elsasser was deceived by the ballyhoo about information theory to argue that there is some quite mysterious increase in information content between the egg and the adult. As Crick correctly points out, we have at present no possible way of estimating how much "information" is required to build up an organ like a hand, so Elsasser's dilemma does not arise. In point of fact, of course, the basis for the development of an organ is not properly called "information" at all; it consists of "instructions" or "algorithms" (that is, genes that make proteins that do something, to put it very crudely). In a recent book, however, Elsasser³ has put his point in a different form which cannot be so easily dismissed. Again putting it shortly and crudely, he claims that biological systems involve interactions between elementary units (genes, for example) numbered in 105's at least; the number of interactions will rise into the realm of "immense numbers", where end-states become uncomputable, and, he claims, uncomputable in principle. This is a difficulty very analogous to that known in physics as the problem of quantum measurement, or the translation from a quantum system to a classical system. I think there probably is a real difficulty here, although I am not a good enough physicist to understand it fully; but there seems little reason to feel that the biologists are much worse off than the physicists, who have not solved their own problem yet to everyone's satisfac-

As Crick points out (page 26), biology has available a category of explanation to deal with complex systems which is not used in the physical sciences, namely. natural selection. I agree with him that this principle might operate, in simple forms, in non-living systems, but in the biological world it has been developed into an exceedingly subtle and powerful process for producing highly organized entities. In my opinion, the concept has been somewhat debased in current biology by premature mathematization in terms of rather simple algebra. In consequence, it tends to be called in as a deus ex machina. all-powerful in principle but yielding little understanding in detail. It can certainly help us meet some of the arguments of the vitalists, but we still need to think a good deal harder about precisely how it operates.

Most of those Crick accuses of vitalism are more or less influenced by the subjective experience of consciousness. The problems presented by the central nervous system are, I think, of two quite different kinds. Consider running downstairs to answer the telephone; you may be conscious of nothing but "will the message be yes or no"; neurones are, of course, firing like mad, and fantastic feats of computation being performed as you go from step to step and around the corner. An objective vitalist would argue that these unconscious processes are for some reason, perhaps Elsasser's complexity point, not physically explicable. The subjective vitalist's point is that you may be consciously aware of something; possibly something complex, like planning your answer, or possibly something very simple, such as a single colour in which the wall is painted; and what is inexplicable, he maintains, is this awareness. This is quite independent of the complexity of the content of consciousness. In fact, because one can perform extremely complicated actions unconsciously, and can also be aware of extremely simple things, it is not plausible to argue that complexity has anything to do with the situation, one way or another.

The thesis that consciousness or awareness belongs to a different logical realm from that inhabited by present day science, which deals with observable behaviour. is, I think, irrefutable. This is not the place to discuss the philosophical problem of whether science could be modified so as to penetrate this realm as well. The point to make here is that Crick is quite right to insist, as he does (page 24), on the reciprocal of this; namely, that it is illegitimate to use the existence of consciousness as an argument for the necessity of non-physical laws to account for the behaviour of biological systems, as Wigner did. But it is, surely, a return to another of Crick's quirks to find the discussion of such matters leading to the old arguments—rather fully summarized in Needham's History of Embryology-of the time at which the soul enters the human foetus (page 85).

C. H. WADDINGTON

Waddington, C. H., The Nature of Life (Allen and Unwin, London, 1981). Elsasser, W. M., 1961-4, J. Theoret. Biol., 1, 27 (1961); 3, 164 (1962); 4, 166 (1963); 7, 53 (1964).

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BIRDS, BEES AND BACTERIA

Penguin Science Survey 1967

The Biology of Sex. Edited by Anthony Allison. Pp. 288+17 plates. (Harmondsworth, Middx.: Penguin Books, Ltd., 1967.) 12s. 6d.

What is sex? Many a treatise on the biology of sex has foundered in an attempt to define its subject, and because the Penguin Science Survey for 1967 has no less than sixteen contributors, one should, perhaps, not expect a unified approach. A glance at the table of contents will show that one's caution is justified.

This is an age of mini-fashions and biologists are nothing if not fashionable. The first chapter is on "Sex in Bacteria" by William Hayes and this is followed by "Sexual Reproduction in Protozoa" by G. H. Beale. From there we graduate to "Sex in Plants" by Kenneth Lewis and Bernard John, followed by "Sex Determination in Insects" by John Anderson. At last we are ready for "Sex Determination in Mammals" by R. A. Beatty, "The X Chromosomes and Gene Action in Mammals" by Mary Lyon and "The Sex-Ratio in Man" by A. S. Parkes. Now come four chapters on insects with varying connexions with sex: "The Chemical Basis of Courtship in the Insect World" by R. K. Callow, "Sound and Vision in the Sex Life of Insects" by H. Hurst, "The Rabbit Flea and Hormones" by Miriam Rothschild and "Control of Insects by Sexual Sterilization" by G. B. White. Then, after a chapter on "Sexual Behaviour in Mammals" by Paul Leyhausen, biology is left behind and we have "Sex in Psychoanalysis" by Irving Bieber, "Anthropology and the Sex Problem" by D. F. Pocock and "Sexual Deviation" by T. C. N. Gibbens.

To start at the beginning, Professor Hayes writes that "If we look for a common denominator of . . . sex . . It is an alternating cycle in which two haploid parental cells . . . pool their genetic material in a single diploid cell or zygote; this zygote ... subsequently segregates haploid progeny". True, but a common denominator is obviously less than the whole. Nevertheless, all the biological authors including the editor, who writes the introduction, seem to equate sex with sexual reproduction or basically the fusion of haploid nuclei followed by meiosis, the function of which is to provide new combinations of genes for natural selection to work on. Nobody seems to have thought it worth mentioning that, for instance, in man the volume of the egg cell is many thousand times that of the sperm. Not only is the large egg cell responsible for the complex development of the early embryo but the production of such diverse reproductive cells is associated with far-reaching physical and behavioural differences in the organisms which make them. Thus, sex is not just a mechanism to promote outbreeding, for which it is not necessarily very efficient and may indeed be downright useless, as pointed out by Professor Beale in connexion with autogamous protozoa. Self-fertilizing plants come into the same category. In its fully developed form, the sexual condition provides for the formation of highly complex organisms, which are themselves divided into two classes. It is surely the physical differences between males and females which provide the link between the biology of sex and its psychological and anthropological aspects. As it is, the different chapters are quite unconnected and, indeed, the word "sex" has entirely different meanings in different parts of the book.

Granted, then, that there is no unifying thread and that, moreover, the biology of sex has got rather a rough deal, the individual contributions are certainly worth reading. To mention only a few, the chapters on bacteria and protozoa are both models of clear exposition and, though their connexion with sex may be slight, this does not in any way detract from their interest. Nobody thinks any the less of prehistoric caves because they do not teach us much about architecture. "Sex Determination in Insects" is a useful summary of the classical cytogenetic facts of sex determination and also contains more recent work on experimental sex reversal. This chapter, however, is not always easy to follow, partly because the author uses such terms as "endomitosis" and "heterochromatization" without a word of explanation and also because of his attempt to explain the mode of action of sex chromo-This topic is still an unsolved problem and it would have been less painful to say so. The same problem crops up also in "Sex Determination in Mammals", which otherwise is a workmanlike account of the chromosomal and embryological bases of mammalian sex dimorphism. "The Sex-Ratio in Man" is a topic of great general

interest but one on which scientific data tend to be inadequate, and Professor Parkes is not afraid to say so. The subject of "The X Chromosomes and Gene Action in Mammals" would seem hardly ready for popular consumption but Dr Lyon has solved this problem by giving only one side of the story. The history of biology has many examples in which facts have eventually accommodated themselves to theory and time will show how far this will happen to the Lyon hypothesis.

One of the most absorbing stories in this book is squarely based on facts. It is about the rabbit flea whose life cycle is geared to the hormones of its host. It is a sordid story, co-ordinating rabbit blood and flea copulation. Here we have sex at its seamiest.

Given that its connexion with the biology of sex is rather weak, this Penguin Science Survey can be recommended as a miscellary of interesting topics in biology and allied subjects. URSULA MITTWOCH

MOLECULAR EMBRYOLOGY

Comprehensive Biochemistry

Vol. 28: Morphogenesis, Differentiation and Development. Edited by Marcel Florkin and Elmer H. Stotz. Pp. xii+276. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 90s. Pp. xii + 276.

THE contents of this book belie the title of the series to which it belongs. It is in no sense comprehensive, nor could it be at several times the length. Nevertheless it does offer useful reviews of six of the fields from which molecular embryology can expect its first

Monroy reflects an important shift in the biochemical centre of interest in fertilization. Egg-sperm recognition and the mechanisms of contact and penetration are ignored. Oogenesis, and in particular the elaboration of the protein synthetic machinery that will be called into play shortly (in mammals) or more tardily (in many other animals) after cleavage has begun, gets pride of place. Activation and its sequelae are discussed primarily in terms of the controlled de-repression of transcription and translation of genetic information.

Brachet, before discussing nucleic acid synthesis in embryos, provides a succinct and lucid account of the relevant background problems emerging from classical experimental embryology. He, too, is concerned with egg structure, but carries its consequences forward into later embryonic life. He stresses the value of single gene mutants for the analysis of gene regulation during development. For this reason alone the living mammalian egg, now so much more accessible to study than ever before, is certainly about to come into its own.

Both amphibian metamorphosis and embryonic induction have a long history of biological, physiological and chemical analysis. As helpful reviews by Weber and Yamada show, progress in both still seems to be piecemeal, but it is hard to believe that recent and refined biochemical work will not soon be rewarded by a real breakthrough. Articles by Gilbert on the biochemical concomitants of insect metamorphosis and by Scarano and Augusti-Tocco on biochemical pathways in embryos may each usefully draw the biochemist's attention to materials that may be unfamiliar to him.

A year ago Joshua Lederberg asked whether developmental biology might not be cleaned up within the next decade, and suggested that one of the things we should do, if such a timetable appealed, was to concentrate efforts on fewer kinds of animal. He was probably right, and if this volume gives little encouragement for believing that the end is so near, there are signs of increasing preoccupation with the animals and processes that offer the D. R. NEWTH best openings for biochemical attack.

MICROBIAL LIPIDS

The Chemistry and Metabolism of Microbial Lipids By William M. O'Leary. (Monographs in Microbiology.) Pp. xiv+210. (Cleveland and New York: The World Publishing Company, 1967.) \$8.

This monograph has been written primarily for the microbiologist but will also be useful for the biochemist interested in bacterial lipids. The first chapter will be a valuable guide for any microbiologist who considers entering the technically difficult field of lipid chemistry. In this chapter both the effects of varying culture conditions on bacterial lipid composition and the pitfalls associated with the commonly used methods of lipid extraction and identification are discussed in a refreshingly critical manner. Similarly the chapters describing the range of fatty acids and complex lipids occurring in micro-organisms are more than just a routine catalogue of reported data; the author discusses critically the validity of the analytical information that has been presented in the literature.

When Dr O'Leary moves from the subject of lipid chemistry to that of lipid metabolism there is a noticeable change in the standard of this book. The description of the biosynthesis of cyclopropane fatty acids stands out from the rest of this section, as would be expected in view of the author's interest and pioneering studies in this field. The description of the biosynthesis of the saturated and unsaturated fatty acids, however, could have been presented in more detail. During the past few years the individual enzymes of fatty acid synthesis in E. coli have been identified and isolated, and the role of acyl carrier protein in this system has been demonstrated by the elegant studies of Vagelos, Wakil and their coworkers. Whereas reference to this now classical series of investigations only occupies three lines, the discussion of cyclopropane biosynthesis extends to ten pages. Similarly the description of the synthesis of mono-unsaturated fatty acids is marred by the lack of discussion of the enzyme mechanisms involved in the formation of the double bond at the decanoate stage.

Dr O'Leary suggests that his book may well bridge the gap between the inadequate coverage of this field in the more important textbooks and the full review. If there had been a better balance between the sections describing the chemistry and biosynthesis of bacterial lipids the book would have fulfilled this function. The earlier chapters, however, will make an excellent introduction for microbiologists interested in investigating the lipid composition of the specific organisms they are studying. D. GOMPERTZ

PYRAMIDS OF POISON

Pesticides and Pollution

By Kenneth Mellanby. (The New Naturalist: a Survey of British Natural History.) Pp. 221+14 plates. (London: William Collins, Sons and Co., Ltd., 1967.) 30s. net.

DR MELLANBY has obviously used the past few years very valuably indeed and has now produced an informative, comprehensive and balanced account of the whole problem of pollution of our environment. He can say, with conviction backed by long term experiment and observation, just where the dangers of pollution lie now and in the future. Deleterious situations are often allowed to persist long after they have become a public nuisance. serious social dangers are brought to light, somebody, somewhere, has to shout very loudly indeed. Ralph Nader, Barbara Robb and Rachel Carson have, in their various spheres, performed this service for the community. Once public awareness has been aroused sufficiently to devote time and money to the problem, facts must be collected, experiments performed and the real enemy

sought out. To be fair, certain sections of the population and some official bodies were already aware of the problem of widespread pollution, and research in Great Britain was well under way long before Silent Spring was published: nevertheless, it focused public attention on a real and

growing problem.

Pollution of our environment has been with us ever since man lit his first fire. Nevertheless, it is an indictment of the criminal tolerance of man and his indifference to his environment that measures to control pollution are prosecuted with vigour and skill, not according to the level of danger inherent in any one source, but inversely according to the time they have been with us. Dr Mellanby performs another valuable service in showing how generalizations about pollutants may not apply across continents. The British Isles may have, to aggravate any situation. a dense population and an intense agriculture, but they are blessed with a less than fierce sunlight, which can do nasty things to "smog" in Los Angeles, and with insects rarely if ever as voracious as those of Africa or the Americas.

The herbicides and fungicides leave the court virtually unstained. Those responsible for the disposal of radioactive waste, Shell and BP, receive a well-merited pat on the back. The persistent insecticides are shown to be as dangerous as was first feared, for chronic poisoning and synergistic effects between two or more insecticides are now coming to light. A well-merited swipe is directed at Chemicals for the Gardener which recommends a host of dangerous chemicals for unskilled hands to use. garden, like the natural habitat which it begins to approach, does not need routine pest control; both need only the occasional and local rescue programme.

Some sources of pollution are, as Dr Mellanby points out, susceptible to legislation, but how do you legislate against a river which is half sewage effluent? It is staggering to learn that without effluent some rivers might run dry. A new water plan for Great Britain might allow us to use sewage in a more economic way by combining it with comminuted urban waste (the equipment is available) to produce soil compost. Small scale experiments suggest that both pulverized urban waste and pulverized cars can be re-used instead of being dropped on to sea beds to pollute an environment we have not yet reached.

Dr Mellanby is less than fair to the Thames Water Board which in the past three years has never allowed the Thames to become totally de-oxygenated. one-time sewer has shown a remarkable improvement during the past five years. We can be grateful that pollution is not worse than it is in Great Britain, and for this we thank those literate, voluble and knowledgeable conservationists who in the guise of ornithologists, anglers, entomologists and botanists cast a perceptive eye over our countryside at week-ends.

Collins, after this their fiftieth number of the New Naturalist series, should be encouraged to venture again into the constructive and emotive realms of natural history as well as the purely descriptive fields they have served so well in the past. I look forward to publications on such topics as "Recreation with Conservation" and "Water Supplies and the Environment". I hope that the authors of their future volumes will be as lucid and per-B. E. JUNIPER ceptive as Dr Mellanby.

CRAFT INTO TECHNOLOGY

Wood Finishing

By John W. Collier. (Pergamon Series of Monographs on Furniture and Timber, Vol. 6.) Pp. xxiii+306. (Oxford. London and New York: Pergamon Press, Ltd., 1967.) 35s. net.

It is commonly considered that one of the weaknesses of British industry is a failure to bridge the gap between

laboratory and shop floor. The technologist finds it almost impossible to see matters through the factory worker's eyes or to talk in language that he can understand, while the worker thinks of the technologist as remote from reality, with little to contribute to day to day problems.

How refreshing then to find a book written by one with a long practised skill in craft methods who is yet enthusiastic about modern innovations, and who welcomes scientific explanation of what many prefer to regard as craft secrets. In his foreword, addressed to apprentices, journeymen, foremen and managers, Mr Collier writes: "This book is intended to be practical. I do not accept the idea, however, that the practical man need be totally ignorant of technology. Nor do I think that a chemist cannot learn the techniques of application connected with the materials he formulates. I believe the line between theory and practice to be arbitrary and socially undesirable. I have found most chemists to be ready to come more than half way to meet the practical man and it is to be hoped that more practical men will make the necessary effort to understand his materials and the bases upon which they are formulated". These are admirable senti-

Wood finishing, particularly furniture finishing, is in rapid transition from craft to technology. As firms practising it grow larger in size and fewer in number, the leisurely processes of the past are superseded. French polish is today little used, and the stage of interlinked machines, with the various steps of finishing taking place along a conveyor, is now being reached. There is a continuing tendency for the machine to take over more and more from the human operator. In all this, finishing is but following a path long since trodden by more advanced industrial processes.

Until now the books devoted to wood finishing have been very much at the craft level, even when they have advanced to acknowledgment of spray guns and nitrocellulose lacquers. They have been more suited to the individual worker in a small shop than to the finisher involved in a modern production plant. Now we have a book to fill this gap. While not ignoring the older methods, Collier is equally thorough in his treatment of the materials that have emerged from the laboratory in the past dozen years or so, and of the methods of application that have been developed to reduce the manpower so prodigally used by the older ones.

This is a book for everyone concerned with finishing timber for interior use. It is not one to be skimmed at a sitting, but one to be dipped into, to be read in parts, to be digested and to be consulted time and again in the ROSTRON HINDLEY

DEGRADING TIMBER

Timber Pests and Diseases

By W. P. K. Findlay. (Pergamon Series of Monographs on Furniture and Timber, Vol. 5.) Pp. xi+280. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 30s. net.

In this small, handy book, the author attempts to deal with all the different types of agency which can degrade timber both in the growing tree and after felling. This is an immense task and because of the very wide compass dealt with there are inevitably inequalities in treatment. As might be expected from a co-author of Decay of Timber and Its Prevention, the chapters on timber staining, timber deterioration after felling, decay in buildings, in farms and gardens, and in boats and marine works are well written with good illustrations and straightforward prescriptions for control. Other sections seem less adequate and in the chapter on "Diseases of Standing Trees" for example, stump treatment against Fomes annosus is not well explained. The sections on damage to timber caused by insects are useful summaries of information. It is somewhat surprising, however, in a book where space is at a premium and which is presumably aimed at a mainly British readership, to find eighteen pages devoted to termites.

This book is essentially one for the non-specialist and practical man and it forms a good introduction to the subject of degradation of wood in its entirety.

> J. S. MURRAY Myles Crooke

ANCIENT MEDICINE

Primitive and Archaic Medicine

By Henry E. Sigerist. (History of Medicine I.) Pp. xxi+564. (London and New York: Oxford University Press, 1967.) \$3.50; 24s.

THE contributions of Henry Sigerist as a medical historian are well known. After he left the Chair at Leipzig in 1925 he went to the United States, where he subsequently occupied William H. Welch's Chair in the History of Medicine at Johns Hopkins University. By this time he had clarified his great plan for a history of medicine, global in scope; one that would place medicine in the broad setting of general history and give some indication of the vast part played by the medical sciences among the forces which have determined the progress of mankind. This is the basic explanation of his work as set out by John Fulton in the preface to this volume.

As part of his training to approach this vast task Dr Sigerist followed his classical education with a medical degree, training in historiography, and in addition to an expert knowledge of the languages of Western Europe he devoted several years to the study of Oriental languages and also of Russian.

After fifteen years in the United States he retired at the age of 56 from the Chair at Johns Hopkins and went to live in Switzerland so as to devote his whole efforts to the eight volume history of medicine that he had planned. By the time of his death in 1957 he had completed this first volume on Primitive and Archaic Medicine and also a second volume on early Greek, Hindu and Persian Medicine that was published in 1961.

The first edition of this present volume appeared in 1951 with hard covers. This is a notable second edition as a paper back. In this cheaper and more popular form it will command a wide audience, especially as this republication will draw new attention to the work.

The size and comprehensiveness alone make it very difficult to write a full appraisal of such a volume. It could also be an unwise thing to attempt to particularize details of such a vast and scholarly work.

In this volume of more than 500 pages we are able to read and appreciate a unique presentation of primitive and archaic medicine and to realize that until barely a century ago much of what could be thought archaic was still practised. Indeed, there are still relics and names of primitive medicine in our daily life and work.

The outstanding feature in this volume is the presentation of medicine in Ancient Egypt. Here alone one sees how the development of medicine is intimately mixed with the passage of history itself. This fascinating account of Ancient Egypt and the simple clear presentation of its history are followed by an equally enthralling account of Mesopotamian medicine. Each chapter in the work is followed by an invaluable and impressive biblio-

It is to be hoped that the second volume will in due course appear in the same form as this one. We can only regret that Dr Sigerist's death has prevented us from reading and enjoying the remaining volumes that would have completed his grand plan.

EVOLUTION FOR ALL

Understanding Evolution

By Herbert H. Ross. (A Spectrum Book.) Pp. ix+175. (Englewood Cliffs, N.J., and London: Prentice-Hall, 1966.) 20s.

This book is a remarkably successful attempt to give an account of evolution from the possible modes of beginning of the universe down to the production of man and such extremely recent phenomena as industrial melanism, all done in 165 pages of text with numerous illustrations, yet very readable. The ten principal divisions of the book deal with: history of evolutionary thought; evolution of the universe; life's nature and origin; the progress of life; passage through time (including opportunism, survival and extinction); variation and natural selection; increase in the number of species; the geotectonic factor (influence of the major geological events on evolution); evolution of ecological communities; and progression of change (summary). The width of treatment is unusual and extends to constituent topics as well—it is pleasant to find an author who does not spend his whole space for speciation on geographical speciation—yet in those topics of evolution that I know something about the treatment is remarkably comprehensive and clear although condensed, and very up to date. It would be easy, no doubt, to disagree over some points of emphasis or details of fact or omission, but most of those I have noted seem to me trivial in comparison with the good points of the book. It should be looked at by anyone seeking a good (and cheap) text for students and for non-biologists. A. J. CAIN

OBITUARIES

Dr Douglas McClean

Douglas McClean, bacteriologist in charge of the Vaccine Lymph Department of the Lister Institute of Preventive Medicine for twenty-five years, died in Oxford on July 10. He was born on May 13, 1896, in Constantinople, where his father was at the time medical superintendent at the British Seamen's Hospital. At the age of nine he came to England, where he was at school until he went to St. Thomas's Hospital in 1914. His medical training was interrupted by three years service as surgeon probationer in the Harwich Force of the Royal Navy, which left a permanent somewhat nautical stamp on him. He returned in 1919 to complete his training, and in 1924 took up the post of pathologist at Great Ormond Street Hospital for Sick Children.

Although he had a real interest in clinical problems he was irked by the inadequacy of current knowledge about the causation and especially the prevention of infective disease, and in 1928 he went to work under Dr (later Sir John) Ledingham at the Lister Institute at Chelsea. Here he was introduced to the study of viruses, especially vaccinia, and in 1930 he was appointed assistant bacteriologist in the Institute's serum department at Elstree. In producing vaccine lymph it was the practice to infect the skin of sheep with seed vaccine prepared in rabbit testicles, and McClean observed that uninfected testicular extracts had a remarkable capacity to enhance the diffusion of substances injected into the skin. His first paper on the phenomenon was published in 1930, and heralded a series of studies on the action of "spreading factor" and its possible role in microbial invasion, in fertilization and in therapeutics. He did not himself characterize the factor as a hyaluronidase, nor was he the first to demonstrate its production by bacteria, but his observation, which was contemporary with a similar independent observation by Duran-Reynals; opened up a new field of research and was a contribution to the recognition that bacterial toxins might be specific enzymes, which was first proved by M. G. Macfarlane and and B. C. J. G. Knight, also at the Lister Institute, in the case of the lecithinase of Clostridium perfringens. McClean became bacteriologist in charge of the vaccine lymph department in 1936, in which role he made a number of significant improvements in the production and stabilization of vaccine lymph, especially by devising a means of virtually eliminating contaminating bacteria by treatment with phenol.

Despite his critical yet enthusiastic approach to his scientific work, the execution of which he thoroughly enjoyed, he never wholly abandoned what might be called an amateur status, and refused to allow science (or administration, which he did well) to exclude his other interests. He enjoyed his garden, his bees, and good conversation—especially accompanied by good wine, of which he was a connoisseur. He shared with his wife (a well known writer and illustrator of children's books) a circle of artistic friends. Finally, he was a firm advocate of socialist principles—and an equally firm upholder of civil liberties—and he found an outlet for an important part of his energies in promoting the work of the Association of Scientific Workers.

J. H. Humphrey

University News:

Massachusetts Institute of Technology

PROFESSOR HARRY C. GATOS, at present both professor of electronic metallurgy in the Department of Metallurgy and Materials Science and professor of molecular engineering in the Department of Electrical Engineering, has been appointed associate director of the Center for Materials Science and Engineering.

Reading

DR P. G. Hall, at present reader in geography at the London School of Economics and Political Science, has been appointed to the chair of geography in succession to Professor T. G. Miller, who has been appointed principal of the University College of Rhodesia.

Appointments

SIR STANLEY BROWN, chairman of the Central Electricity Generating Board, has been elected president of the Institution of Electrical Engineers for the session 1967-68. The chairmen of the three divisions of the institution have also been elected as follows: DR E. EASTWOOD, director of research for English Electric, chairman of the Control and Automation Division; MR J. H. H. MERRIMAN, senior director of engineering in the Post Office, London, chairman of the Electronics Division; MR E. C. RIPPON, director of C. A. Parsons and Co., Ltd., Newcastle upon Tyne, chairman of the Power Division.

ADMIRAL O. A. QUIHILLALT of Argentina has been elected chairman of the new Board of Governors of the International Atomic Energy Agency, and Mr N. Ivanchev, governor from Bulgaria, and Miss L. Roesad, governor from Indonesia, have been elected vice-chairmen.

Announcements

THE Lalor Foundation makes annual awards to investigators for the study of basic phenomena and mechanisms in the field of reproductive physiology, and the 1968 programme of awards will give priority to applied scientific and clinical research on intra-uterine phenomena, uterine peristalsis in relation to implantation, early gestation and its control, clinical research on the physical sequelae of abortion, and the like. Further information about these awards can be obtained from the Director, Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware.

Meetings

476TH Meeting of the Biochemical Society, November 18, Medical Research Council Laboratories, Carshalton (Executive Secretary, The Biochemical Society, 7 Warwick Court, Holborn, London, WC1).

FLUORO-ORGANIC Chemistry, March 28-29, 1968, University of Birmingham (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, SW1).

AUTHORITY and Leadership Working Conference, March 29-April 11, 1968, Leicester (Conference Secretary, Centre of Applied Social Research, Tavistock Centre, Belsize Lane, London, NW3).

BIOLOGY of Reproduction in Mammals, April 9-11, 1968, Nairobi (Professor E. C. Amoroso, Department of Physiology, Royal Veterinary College, University of London, Royal College Street, London, NW1).

MARINE Food Chains, July 23-27, 1968, University of Aarhus (Dr J. H. Steele, Department of Agriculture and Fisheries for Scotland, Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen.

HAEMOPHILIA, August 26-28, 1968, Montreal (The World Federation of Haemophilia, 122 Arlington Avenue, Montreal 6, Quebec).

CORRESPONDENCE

Informed Chemists

Sm,—It is encouraging to those like myself working on the bibliographical side of science to read in a recent issue (Nature, 215, 1324; 1967) that British chemistry PhD students are to be kept up to date by computer. The students will all be in their third year and this should mean that they carry the resulting familiarity with mechanized information services over into their future jobs.

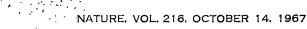
In planning this exercise, one wonders why the Office for Scientific and Technical Information thought it necessary to select and train six liaison officers to act as regional agents. Why not make use of university library staffs, already to some extent conversant with local research projects, with far better lines of communication into local chemistry departments and already working in the documentation field? Does OSTI not know that British university libraries already act as local agents for other computerized information services such as that for medical literature (MEDLARS) and that many of them are developing courses on documentation for PhD students, chemists included, which would make an ideal platform for promulgating schemes such as this?

I recognize that the scheme is an experiment, run in conjunction with the Chemical Society's Research Unit in Information Dissemination and Retrieval at Nottingham University. But in an experiment on such a scale and especially one designed also to influence people's future actions, why not normalize as far as possible the business of getting students to describe their research project in terms of an array of subject headings? This is rarely as painless as it sounds. It would be a pity if experience of a rather remotely controlled experiment in information dissemination like this were to put students off the kind of information services, based on libraries, which will be available to them later on in industry or Government research.

Yours faithfully,

R. J. DANNATT

University of Strathclyde, Andersonian Library, Glasgow, C1.



Towards a Broader Curriculum

SIR,—Few engineers would disagree with the spirit of the Declaration of the Cambridge Conference on a Broader Curriculum in sixth forms reported in your columns of 23rd September (Nature, 215, 1329; 1967), or underestimate the seriousness of any failure to solve the many educational problems involved. D. W. Hutchings in a recent survey for CRAC found that only 137 out of 2,006 science and technology students at 5 major universities had obtained an "A" level in an arts subject.

Priority appears to have been given at the Conference,

not unnaturally, to the interface between schools and university, but Dr. Nichol drew attention to looking at education as a whole, and this entails a closer look at the social needs of the country whose taxpayers, after all, foot the bill directly or indirectly. The end point of schools is too apt to be conditioned by the number of university places obtained, irrespective of the suitability of the discipline entered in relation to its later value. Equally important would seem to be the interface between university and the field of postgraduate employment.

It is so often said that universities would be ready to change curricula if they only knew what the customer really wanted, but that the industrial customer gives conflicting requirements. It is suggested that an extensive survey is needed over the whole field of technology on the lines of that conducted in 1961 by Professor Edgeworth Johnstone for chemical engineers, and in 1964 by Professor Hutton and Dr Gerstl for mechanical engineers. recent CEI/Ministry of Technology survey of 20,000 professional engineers has already shown the superior earning capacity over the £2,000 mark of graduates compared with non-graduates. A further survey conducted in conjunction with other learned societies could yield much of value.

The Hutton/Gerstl survey covering 387 mechanical engineering graduates revealed that the usage of subjects in the practice of their profession ranked in the ordermathematics, engineering drawing, technical report writing, applied mechanics, properties and strength of materials, industrial administration, followed by some fourteen technical subjects with foreign language rating last. But when asked to suggest an ideal course the general opinion favoured a time distribution of

Basic Engineering Sciences, e.g., strength of materials Fundamental Sciences (maths, physics, chemistry)	$\frac{27\%}{23\%}$
English and Humanities 7% Technical Report Writing 7% Foreign Languages 7%	21%
Foreign Languages 7%)	13%
Design Engineering Industrial administration, economics, social science	10%
Speciality engineering, e.g., instrument or textile	6%

which illustrates that these engineers themselves were well aware of the great importance of non-technical subjects, particularly of communication, and of the danger of blinkered curricula. Maybe the doctor provides the remedy, but the patient can at least indicate the symptoms; a similar survey of graduates qualified in the last 10 years, say, including Dip.Techs., might reveal a great many worrying symptoms, with the need for much greater liaison between universities and industry.

Mr Morrison's suggestion of five subjects for university entry prompts the question of whether consideration has been given to the suitability of the proposed European International Baccalaureat.

Any changes in curricula, however, will only be pipe dreams unless an adequate number of well qualified maths and science teachers is forthcoming. The CEI/Ministry of Technology survey indicates that the salaries of maths and science (men) graduate teachers in maintained schools were below those for graduate engineers, particularly after the age of 38.

Yours faithfully,

I. G. AYLEN

21 Ovington Square, London, SW3.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 16

ROYAL INSTITUTION (at 21 Albemaric Street, London, W1), at 1.15 p.m.—Professor George Porter, FRS: "The House of Michael Faraday".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, SW1), at 5 p.m.—Mr N. W. Hussey: "Prospects for Integrated Control in Protected Cultivation".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5 p.m.-Professor G. W. Robinson: "Why is Oxygen Blue?" (Bourke Lecture).

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (at the Institution of Civil Engineers, Great George Street, London, SW1), at 5.30 p.m.—Mr Harry Perry: "Current Coal Utilization Research in the USA" (Sixteenth Coal Perry: "Current Science Lecture).

Institution of Mechanical Engineers, Internal Combustion Engines Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "North Sea Gas—Internal Combustion Engines".

Tuesday, October 17

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (in the Department of Mechanical Engineering, Exhibition Road, London, SW7), at 5.80 p.m.—The Rt. Hon. Anthony Wedgwood Benn, MP: "The Government's Policy for Technology".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the AUTO-MATIC CONTROL GROUP of the INSTITUTION OF MECHANICAL ENGINEERS, at Savoy Place, London, WC2), at 5.30 p.m.—Discussion meeting on "Control Aspects of Synchronous Machine Dynamics" opened by Dr W. D. Humpage.

UNIVERSITY OF ASTON IN BIRMINGHAM (at Gosta Green, Birmingham, 4), at 5.30 p.m.—Professor A. J. Ede: "Blowing Hot and Cold—Some Reflections on Research in Heat Transfer" (Inaugural Lecture).

University of London (at King's College, Strand, London, WC2), at 5.30 p.m.—Professor R. M. H. McMinn: "The Cellular Anatomy of Tissue Repair" (Inaugural Lecture).

UNIVERSITY OF LONDON (at Senate House, London, WC1), at 5.30 p.m.— Lord Florey: "Elements of the Vascular System". (First of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

Institution of Mechanical Engineers, Railway Engineering Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Use of Fluidics in Control Systems".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, SW1), at 6 p.m.—Dr J. Heyboer: "Modulus and Damping of Polymers in Relation to their Structure".

UNIVERSITY OF LONDON (at the Linnean Society, Burlington House, Piccadilly, London, W1), at 6.30 p.m.—"The Historical Background to Modern Botany" by various lecturers. (Further lectures on October 24, 31, November 7, 14, 21, 28, December 5 and 12.)

Wednesday, October 18

INSTITUTION OF ENGINEERING INSPECTION (at the Royal Institution, The University of Liverpool), at 9.45 a.m.—Symposium on "APractical Approach to Reliability Engineering".

PHOTOBIOLOGY GROUP (in the Physics Department, Imperial College, London, SW7), at 2.30 p.m.—Meeting on "Vision and Photosynthesis".

GEOLOGICAL SOCIETY OF LONDON (at the Shell Centre, Belvedere Road, London, SE1), at 5 p.m.—Dr J. M. Harrison: "Geological Sciences in the World Scientific Community" (21st William Smith Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Road Heating with particular reference to UK Practice" opened by Mr W. M. Craig.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr J. H. H. Merriman, OBE: "Men, Circuits and Systems in Telecommunications" (Chairman's Inaugural Address).

SOCIETY OF INSTRUMENT TECHNOLOGY (at the A.E.I. Cinema, 33 Grosvenor Place, London, SW1), at 5.30 p.m.—Mr H. Edmonds-Brown: "Flame-proofing, Intrinsic Safety and their Engineering Implications".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, SW7), at 6 p.m.—Mr N. C. Cordingly: "The Design of a Clean Room for Making Special Vacuum Tubes".

INSTITUTE OF INFORMATION SCIENTISTS (at Knightway House, 20 Soho Square, London, W1), at 6.15 p.m.—Dr A. J. Harley: "The UK MEDLARS Service".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (in the Engineering Lecture Theatre, University College London, Torrington Place, London, WC1), at 6.30 p.m.—Dr F. L. Dolton: "The Irradiation Curing of Paint Films".

SOCIETY FOT ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP t "The Feathers", Tudor Street, London, EC4), at 6.30 p.m.—Discussion (at "The Meeting.

Wednesday, October 18-Friday, October 20

SOCIETY OF DYERS AND COLOURISTS (at the Palace Hotel, Torquay, Devonshire)—Symposium on "The Impact of Automation and Instrumentation in the Colour-using Industries".

Thursday, October 19

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the Royal College of Physicians, 11 St Andrew's Place, Regent's Park, London, NW1), at 10 a.m.—Symposium on "Aspects of Toxicity Testing".

University College London (in the Anatomy Theatre, Gower Street, London, WC1), at 1.20 p.m.—Professor J. Z. Young, FRS: "Memory".*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m.—Professor Ronald King: "Radiation and the Structure of Matter" (Civil Service Lecture).

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W1), at 5 p.m.—Professor M. R. W. Rey (Paris): "Early Development of the Copper Segregation Process"; Mr E. T. Pinkney and Mr N. Plint: "Treatment of Refractory Copper Ores by the Segregation Process".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W1), at 5 p.m.—Mr N. Jardine: "What is Homology?"; Mr J. D. Holloway and Mr N. Jardine: "New Methods in Zoogeography".

UNIVERSITY OF LONDON (at the Royal Postgraduate Medical School, Wolfson Institute, Du Cane Road, London, W12), at 5 p.m.—Professor E. B. Chain, FRS: "Biochemical Research and Progress in Medicine".

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W1), at 5.30 p.m.—Mr Richard L. Jodry "Pore Geometry of Carbonate Rocks".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the AUTO-MATIC CONTROL GROUP of the INSTITUTION OF MECHANICAL ENGINEERS, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 5.30 p.m.—Mr D. J. Kyte: "The Automation of Colour Reproduction in Printing".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Professor E. Jean Hanson: "The Molecular Structure of Muscles in Relation to the Mechanism of Contraction". (Second of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, SE1), at 6 p.m.—Mr T. J. R. Cooper: "The Application of Refrigeration to the Bacon Industry".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 8-9 Bedford Square, London, WC1), at 6 p.m.—Symposium on "Management, Methods and Media in Electronic Training at the School of Electronic Engineering, REME, Arbordeld". Speakers: The Commandant, Colonel H. G. Frost. and members of his staff.

SOCIETY OF CREMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, SW1), at 6 p.m.—Mr C. J. Keattch and Mr J. A. Reynolds: "The Analysis of Concrete—Problems in Determining the Fourth Ingredient".

BRITISH INSTITUTE OF RADIOLOGY (at 32 Welbeck Street, London, W1). at 6 p.m.—Discussion Group, 8 p.m.—Dr R. D. Hoare: Presidential address.

Friday, October 20

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W(2), at 10.30 a,m.—Colloquium on "Interface Problems in the Calibration of R.F. Instruments".

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Dr E. Vander Donckt (University of Brussels): "Charge Distributions and Free-Energy Relationships in Excited States".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, $W(^2)$, at 5.80 p.m.—Discussion Meeting on "Testing and Specification of Integrated Circuits".

BIOLOGICAL ENGINEERING SOCIETY (in the Botany Theatre, University College, Gower Street, London, WC1), at 6 p.m.—Professor A. F. Huxley, FRS: "Forgetting and Rediscovery in Physiology" (Woolmer Memorial Lecture)."

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 9 p m. - The Rt. Hon. George Woodcock, CBE: "Trade Unions".

Friday, October 20-Saturday, October 21

BIOCHEMICAL SCOIETY (at the National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7)—475th Meeting. Programme will include a Colloquium on "Functions of Nucleic Acids in the Cytoplasm" and sessions for the presentation of communications.

Saturday, October 21

FISHERIES SOCIETY OF THE BRITISH ISLES (at the Zoological Society of London, Regent's Park, London, NW1), at 2.30 p.m.—Inaugural Meeting.

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3.30 p.m.—Professor Johannes Nicolaisen (University of Copenhagen): "The Tuareg—Veiled Men of the Sahara".*

Monday, October 23

INSTITUTION OF INDUSTRIAL SAFETY OFFICERS (in the Lecture Theatre, Institution of Civil Engineers, Great George Street, London, SW1), at 2 p.m.—Mr W. A. Wood: "The Application to Industry of Recent Advances in Accident Prevention in the Mining Industry" (Alexander Redgrave Memorial Lecture).

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, SW7), at 5.30 p.m.—Mr J. D. North: "Eclipses and Eclipse Computers in the Ptolemaic Tradition".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr C. M. Van der Burgt: "Materials for Ultrasonic Delay Lines".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Professor A. Stratton: "Inertial Navigation".

UNIVERSITY COLLEGE LONDON (in the Botany Theatre, Gower Street, London, WC1), at 5.30 p.m.—Dr R. J. Smeed: "Traffic Studies and Urban Congestion".*

University of London Institute of Education (in the Great Hall, King's College, Strand, London, WC2), at 5.30 p.m.—Professor G. H. Bantock: "The Idea of a Liberal Education".*

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, WC2), at 6.30 p.m.—Dr A. H. Cookson "Compressed Gas as High Voltage Insulation" (Chairman's address).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the

APPLICATIONS are invited for the following appointments on or before the dates meutioned:

RESEAROH FELLOW (graduate with a higher degree or relevant research experience) in CONTROL ENGINEERING in the DEPARTMENT OF ENGINEERING, for studies in the development of methods of stability analysis of non-linear systems—The Registrar, The University, Leicester (October 25).

SENIOR LECTURER/LECTURER IN GEOGRAPHY in the School of Earth Sciences, Macquarie University (the appointment will be made within the field of human geography)—The Secretary-General, Association of Commonwealth Universities (Branch Office), Mariborough House, Pail Mall, London, S.W.1; or The Registrar, Macquarie University, Eastwood, New South Wales, Australia (October 30).

CHAIR OF PHYSICS—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (October 31).

LECTURER OF ASSISTANT LECTURER IN THE DEPARTMENT OF PSYCHOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (October 31).

RESEARCH ASSISTANT (honours graduate in engineering or science) in MECHANICAL ENGINEERING in the STRUCTURAL MECHANICS RESEARCH GROUP which is principally concerned with the study of the stable and unstable behaviour of thin-walled structural sections—The Assistant Registrar (Establishment), University of Sussex, Falmer, Brighton, Sussex (October 31).

SENIOR LECTURER or LECTURER in PLANT ECOLOGY in the DEPARTMENT OF BOTANY, University of the Witwatersrand, Johannesburg—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (South Africa and London, October 31).

RESEARCH ASSISTANT (graduate in metallurgy, geology or physics) in METALLURGY, to work on metallographic aspects of meteorite material using conventional metallographic methods in conjunction with a SEM 2 electron probe microanalyser—The Registrar, University of Manchester, Manchester 13, quoting Ref. 157/87/Na (November 9).

SENIOR LECTURER or SENIOR LECTURER in Sciolilogy in the DEPARTMENT o

JOHN INNES CHAIRS OF GENETICS AND APPLIED GENETICS in the School of Biological Sciences—The Registrat, University of East Anglia, Earlham Hall, Norwich, NOR 88C (November 30).

DEPARTMENTAL DEMONSTRATOR IN ANIMAL ECOLOGY—Professor J. W. S. Pringle, Department of Zoology, Parks Road, Oxford (November 30).

UNIVERSITY LECTURER IN ANIMAL ECOLOGY in the DEPARTMENT OF ZOOLOGY—The Secretary of Faculties, University Registry, Broad Street, Oxford (November 30).

LECTURER (with a special interest in power systems) in the DEPARTMENT OF ELECTRICAL ENGINEERING, to teach up to postgraduate level and to undertake original work—Professor J. H. Calderwood, University of Salford, Salford 5, Lancs.

PHYSICAL CHEMIST (preferably with research experience in the field of

undertake original work—Professor J. H. Canderwood, University of Sahotu, Salford 5, Lancs.

Physical Chemist (preferably with research experience in the field of polymer characterization, optical studies or electrical behaviour) to join a group concerned with long-range research into the study of new materials—The Personnel Officer, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Midlothian, Scotland.

Physiologist, Biochemist or Biologist (with a good honours degree) to assist with research in physiological, biochemical and cell culture studies of isolated mammalian nerve cells—Dr H. Hillman, Department of Biological Sciences, University of Surrey Annux, 14 Faicon Road, London, SW11.

RESEARCH ASSISTANT (with a degree or equivalent qualification and preferably previous experience in crystallography or electronic computing) in the Department of Hongamic and Surreyard Chemistry, to assist the group of research workers engaged in the field of X-ray crystallography—Professor H. M. N. H. Irving, The University, Leeds, 2.

SENIOR LECTURER (preferably with experience or interest in mineralogy, petrology or geochemistry) in Geology—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Fiftieth Annual Report of the Society for the Promotion of Nature Reserves. Pp. 8. (London: Society for the Promotion of Nature Reserves, c/o British Museum (Natural History), 1967.) [158]
Institute for Operational Research. The First Four Years, 1963–1967. Pp. 43. (London and Coventry: Institute for Operational Research, 1967.) [168]
Scottish Plant Breeding Station. Report to the Annual General Meeting of the Scottish Society for Research in Plant Breeding, 27th July, 1967, by the Board of Directors—Forty-sixth Annual Report, 1966–67. Pp. 44. (Pentlandfield, Roslin: Scottish Society for Research in Plant-Breeding, 1967.) 5s. [178]

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UNIVERSITY OF MANCHESTER MANCHESTER, 13

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Applications (three copies), stating age, qualifications and experience and naming three referees, should reach the Registrar and Secretary, the University, Leeds, 2 (from whom further particulars may be obtained), not later than October 23, 1967.

UNIVERSITY OF QUEENSLAND SENIOR LECTURER IN PSYCHOLOGY

SENIOR LECTURER IN PSYCHOLOGY

The University invites applications for the above-mentioned position. Applicants should hold a higher degree in psychology and preferably should have some training in applied or statistical mathematics. The applicant should have teaching and research experience in one or more of the following fields—test construction, multidimensional scaling, psychometric methods, mathematical psychology, factor analysis, computer applications, experimental design. The appointee will be responsible for the direct supervision of Ph.D. candidates in this area (4 at present), and assistance in the general supervision of experimental design for graduate and doctoral research. He will also be responsible for the overall supervision of teaching in statistics and psychometrics in the Department. The salary range for a Senior Lecturer is \$A6,400 by \$A200 (6) to \$A7,600. Academic salaries are at present under review and the proposed salary range for a Senior Lecturer is \$A7,550 to \$A8,750. The University provides Superannuation similar to F.S.S.U., Housing Assistance, Study Leave and Travel Grants.

Additional information and application forms will be supplied upon request to the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in London and Brisbane on November 17, 1967. (1231)

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or to MR. D. ANNIS, M.D., F.R.C.S., Department of Surgery, The University of Liverpool, 48-52 Bedford Street North, Liverpool, 7.

(1254)

NATURE Volume 216 OCTOBER 21, 1967

Dark Horse Run Well

THE appointment of Dr John McGregor Hill as chairman of the United Kingdom Atomic Energy Authority had not been widely anticipated, but it is none the less welcome on that account. To say the least of the occasion, it puts an end to the uncertainty there has been about a successor to Lord Penney and is thus, by extension, an assurance to those on the authority's payroll who may have feared that they would read one day in the newspapers that the anxieties of the past several years had suddenly culminated in the disappearance of the authority by means of some administrative sleight of hand. Now there is at least some tangible evidence that the authority will remain in being, for a time at least. Indeed, the fact that Dr Hill has been the member of the authority responsible for production will be taken by many people to be a sign that the authority will now be able to make come true some of the dreams which Lord Penney shared with the Select Committee on Science and Technology earlier this year. Dr Hill's appointment will, in particular, be regarded as a token that the authority is about to become the king-pin in a national organization for designing and even for constructing ever more advanced and successful nuclear power In the circumstances, it is wise if also obligatory that Dr Hill should have spent his first few days in office insisting that it is far too soon to know what will really happen in the years ahead. problem, of course, is plain enough, and Dr Hill will not have to spend much time on its definition. The real need is for a bright and quick solution.

The view that the authority has outlasted its original objectives is a truism but also an over-simplification, which implies that it is not a particularly valuable guide to action. Dr Hill's great difficulty stems from the way in which an organization which as recently as ten years ago was united-or reasonably well unitedin the pursuit of a few identifiable objectives has become fragmented. Military nuclear energy in Britain is no longer important enough to keep Aldermaston anything like fully occupied. The need for basic research as a support for reactor development is not urgent enough to occupy more than a part of the energy of Harwell. The people who make fuel rods seem happy enough, and the uranium enrichers are even increasing the scale of operations at Capenhurst so as to be able to manufacture fuel for advanced gas cooled reactors. But in spite of their successes, the design and development teams at Risley in Lancashire must be wondering what there will be for them to do in the mid-seventies, when the prototype advanced reactor will have been working for some years. It is no wonder that their ambition is to occupy the centre of the stage in the nuclear energy boom which may dominate the seventies. The trouble, of course, is that if Dr Hill tries to keep everybody happy, he will be perpetuating a form of organization the several parts of which can only be harmed, in some sense or another, by perpetual co-existence.

The problem of the research establishments is separable, almost in the literal sense, and the annual report of the authority for 1966-67 (see Nature last week) is by itself a reminder that vigorous steps must soon be taken to make more effective use of the great talents at present built into Harwell and Aldermaston. For some years the authority has been hoping to keep the Harwell laboratory in being by diversifying the work it does and, in particular, by encouraging the laboratory to take on research contracts with industrial companies. Further opportunities for diversification were provided by Section 4 of the Science and Technology Act (1965), for this enabled the Minister of Technology to direct the public laboratories in his charge to undertake specific programmes of research and development. It is only natural that the laboratories themselves have usually taken the initiative in suggesting suitable subjects for a ministerial directive. Expenditure under this heading amounted to £772,000 in the financial year just finished, while the cost of research on behalf of customers other than the British Government worked out at £383,000. On the face of things, this may seem a respectable achievement, although the sums involved are small compared with the annual expenditure on research and development which works out at £50 million. In reality, however, there is at least a danger that the programme of diversification may be a backward and not a forward

Although at this stage it would be wrong to write . off the policy of diversification as a failure, there is no doubt that it raises serious and unanswered questions about the relationship between industrial research and commercial success. As well as being a means of providing laboratories with work to do, diversification is also considered as a means of providing particular industries and even companies with research and development which are at present neglected. But the experience of the research associations, which are kept in being by the membership subscriptions of commercial enterprises, shows that in matters like these it is easier to produce the results of research than to persuade potential beneficiaries to take notice of them. It remains to be seen whether Harwell and Aldermaston will succeed where several experienced research associations have failed or almost failed. It is also hard for outsiders to appreciate what good purpose may be

served in the arrangement whereby Harwell has been asked by the Science Research Council to assist in the design of a radio-telescope, and by the Medical Research Council to undertake the development of an ultracentrifuge. Would it not have been better, in the long-term interests of the British economy, if contracts for these operations had been placed with companies in a position eventually to put the products concerned on the commercial market? There is at least a case to answer. And whatever the truth may be, there is no reason why uncertainty about the future of these laboratories should be perpetuated simply because one or two per cent of their combined activity is now occupied on projects brought in under Section 4.

The simple solution is to transfer both laboratories from the authority to the Ministry of Technology—although Aldermaston might go to the Ministry of Defence instead. But this is only half a solution. In the long run some means must also be found for encouraging people at the two laboratories to find jobs for themselves in industry. The Jones report on the brain drain had useful suggestions to make along lines like these. Dr Hill could do worse than spend half an hour reading what the Jones Committee had to say. The prize for a successful redeployment of the authority's unwanted talent would be a powerful and rapid strengthening of industrial research and development in Britain.

The reorganization of the industrial empire will be an even more hazardous undertaking. The penalties of serious error are potentially enormous. It is plain that the months ahead will be crucial for the development of nuclear energy in Britain and in the export trade. It is also clear that there are too many design teams chasing too few contracts for completed power stations. But does it follow that there is merit in the obvious and tidy solution of amalgamating all this effort under the umbrella of an atomic energy authority more deliberately oriented towards commercial con-

Boom in British Electronics

THE pace of advance in electronics is now quite remark-Lable. Fewer than twenty years have passed since the invention of the transistor, but already the electronics industry has crucial strategic importance even in the British economy. One result of the rapid expansion has been an extraordinary reduction in prices-a transistor which cost £15 in the early fifties can now be had for 1s. $7\frac{1}{2}d$. Another has been the complete domination of the market by American companies, selling to a home market which accounts for 65 per cent of all sales in electronics. The most recent development in electronics to come into production—integrated circuits—is dominated even more completely by the United States. The American market represents 90 per cent of the total market for integrated circuits, and American companies hold 80 per cent of the much smaller British market. One company based in Britain, Associated Semi-conductor Manufacturers, owned two-thirds by Mullard Ltd and one-third by the

siderations than it is at present because of its involvement in the supply of fuel and other materials? There would be obvious economies of manpower, and there is no doubt that the authority's prestige would be a great help in the export trade—whatever that may be. Indeed, making the authority into the linch-pin of the reactor construction industry would merely extend to the British market the kind of arrangement which is often used elsewhere. But one result would be to decrease still further the ability of most of the commercial builders of reactors to stand on their own feet in the design of nuclear equipment. There is also a possibility that the intervention of the authority in this role is not essential, if only because one of the commercial organizations seems well equipped to win orders for itself (see page 213).

A decision on this important issue cannot in decency be made until the Select Committee on Science and Technology has published its recommendations on the nuclear energy industry, but this is in one sense a formality. The proceedings of the committee were as much a process of self-education as of searching inquiry, which means that there will be a natural temptation for the committee to choose one (or even several) of the different solutions proffered by the various witnesses. The difficulty, of course, is that prescriptions for prosperity provided by vested interests such as the Atomic Energy Authority and the Central Electricity Generating Board may depart in several important ways from the ideal. Of the radical solutions with a claim to be taken seriously, Lord Penney's own proposal deserves quite serious study. Many of the opposing arguments which must be weighed against each other are, however, subtle and even unfamiliar. Immediate gains could easily be outweighed by more distant disadvantages. This, then, is the point at which a wise decision is most of all essential. The whole future of the nuclear energy industry in Britain may depend on it. There is hardly any time to wait.

General Electric Company, is now making a determined attempt to increase its share of the market, both in Britain and in the United States.

Although Associated Semi-conductor Manufacturers has been producing transistors for some time, its entry into the integrated circuit market has been delayed. Integrated circuits can perform most electronic functions, although they are no bigger than a pin head. They are manufactured from single crystals of silicon, with successive layers of oxide and other materials which are diffused onto the surface. A slice of silicon 1.5 in. in diameter will accommodate as many as 600 circuits. Production requires rigorous control of the factory environment, and most of the operations at the Southampton factory of ASM are conducted in clean rooms.

So far, the use of microcircuits has been restricted to the more esoteric parts of industry and defence. The first important market to be won over will be in computers where microcircuits, even if they are not actually cheaper than transistors, offer cheaper design because fewer circuits are needed. The telecommunications field is likely to follow, although in Britain this will involve winning over the General Post Office, which is still wedded to conventional technology for most applications. After that, much wider markets may be opened, in radio, colour television and even motor cars. Much will depend on the price of integrated circuits—their reliability is already proved. But price is falling rapidly, from about £1.5 for each component a year ago, to £1 each today, and perhaps £0.5 each next year. Apart from the understandable desire of manufacturers to encourage the use of microcircuits, it is also the case that the productive capacity already in operation is far greater than present demand justifies. There may therefore be an element of dumping in the rapid decrease in prices, as a discouragement to other firms to enter the field. As the price falls and technology advances, it becomes increasingly hard for new entrants to the industry to justify their capital expenditure. Companies now planning an investment in microcircuitry, for instance, would probably do better to forget it.

A very large investment has been made in the Southampton factory of ASM; £4 million has already been spent, and another £11 million is to come. The result is the largest semi-conductor and integrated

First AGR for Scotland

THE Nuclear Power Group last week won the contract for Hunterston B nuclear power station. The contract, which was awarded by the South of Scotland Electricity Board, is for a 1,250 MW(e) station to be sited next to Hunterston A, which is a 300 MW Magnox station. SSEB will be paying £87.5 million for the station, and in addition will have to pay the initial fuel charges. The price announced shows only a modest reduction from the price for Hinkley Point B, which was negotiated between TNPG and the Central Electricity Generating Board. TNPG says that the price for Hinkley Point B at the corresponding stage was £90 million.

It is hard to see how this tallies with the information given to the Select Committee on Science and Technology by Mr S. A. Ghalib, managing director of the Nuclear Power Group. Mr Ghalib explained that for direct replication of a design, a cost reduction of 10 per cent could be expected. For a development of the design, he went on, even greater savings could be achieved, and he quoted the difference between the Dungeness B contract, awarded to Atomic Power Constructions, and Hinkley Point B. If Hunterston B is to be a direct copy of Hinkley B, the SSEB should therefore have been expecting a tender of about £81 million; if it is not a direct copy, but a development, then by Mr Ghalib's argument the price should have been even less. Both stations are the same size and type; Hinkley will come into operation in 1972, and Hunterston in 1973.

The contract also makes Colonel O. W. Raby's remarks to the Select Committee look a little ill-judged. Colonel Raby, who is managing director of Atomic Power Constructions Ltd, claimed that his

circuit factory in Europe, built, ASM hopes, just in time for the boom in microcircuitry which it believes is on the horizon. Although the market is worth only £5 million in Britain this year, ASM believes it will be worth £20 million by 1970 and £50 million in 1975. Already the market in the United States is worth \$200 million, and ASM hopes to break into it through agreements between Westinghouse and Philips (the parent company of Mullard). Particular emphasis is being placed in the development of exceedingly fast digital circuits. Dr F. E. Jones, managing director of Mullard, stresses how difficult it is to attack the American market-first, it is necessary to obtain type approval from an American manufacturer and then it is necessary to bypass the Buy America Act, which discriminates in favour of the home producer.

Would a reorganization of the British electronics industry help British companies to become competitive? Mr Ronald Grierson of the Industrial Reorganization Corporation certainly believes so, and favours a merger which would produce two companies in the microcircuitry field. ASM has had talks with the IRC, and is now waiting for a review of the industry which IRC is producing. Not all is going IRC's way at the moment, however, and the take-over bid by GEC for Associated Electrical Industries is turning into a real battle. What seems logical to outsiders may not always be agreeable to those in the industry.

consortium could have undercut TNPG on the Hinkley contract if it had been allowed to tender. Despite this claim, APC has failed to win the Hunterston contract. There may be special difficulties in the Hunterston contract which have increased the price, but neither TNPG or SSEB has yet indicated any.

The contract leaves Nuclear Design and Construction, Ltd, the third consortium, very much out in the cold. Without an order for three years, NDC must now be favourites for the role of odd man out if the number of consortia is reduced to two. There are now talks of an amalgamation between APC and NDC, but both consortia claim that the Hunterston contract has nothing to do with it.

The end product seems likely to be that Scotland will generate electricity only marginally more cheaply than is possible at Hinkley Point B. The Hinkley price is quoted as 0.476d. per kWh; assuming that fuel and site costs are comparable at Hunterston, generation costs would be almost identical. This must be a very tentative conclusion, however, since both SSEB and TNPG are uncommunicative about exact costs. Both will be earnestly hoping that the price increases which have been a feature of the Dungeness B contract will not repeat themselves at Hunterston. At least the Hunterston design will incorporate proved design features, so it may be possible to avoid escalation. The station will have two reactors enclosed in prestressed concrete vessels with integral boilers and gas circulation. This design has already been used by TNPG at the Oldbury Magnox station which comes into operation late this year. Each reactor at Hunterston will supply steam at conventional temperature and pressure to a 660 MW turbo-alternator. The reactor will use enriched uranium in the form of uranium dioxide, and the design provides for the reactor to be refuelled on power by a single charge machine. Work will begin on the Hunterston site immediately.

The Nuclear Power Group is now in the happy position of having won contracts for at least £180 million worth of work. Power stations built by the group have generated a quarter of the world's nuclear electricity, and the group has the unique distinction of having successfully exported British nuclear technology, in the building of the Latina 200 MW station in Italy. (The other British export was to Japan, and is better not mentioned.) The group is bound to regard this latest order as a guarantee that it will still be in business after the Select Committee on Science and Technology publishes its report on the British nuclear power industry at the end of this month.

Redeploying Scientists

A TRENCHANT defence of the British Government's interventionist policy towards industry was given by Mr Anthony Wedgwood Benn, Minister of Technology, when he spoke at Imperial College on October 17. Mr Benn at first seemed eager to show how unexceptional intervention in Britain is—"The great military—industrial complex in the United States," he pointed out, "constitutes a public sector of formidable proportions which makes even the most radical British interventionist look like an amateur."

Despite its mildness, however, government intervention was inevitable and essential. In the computer industry, the ministry and NRDC had made available substantial amounts of money, and the next stage of the ministry's policy towards computers would concentrate on applications. Mr Benn hesitated to say where the British computer industry would have been without government support. The ministry's policy towards shipbuilding was openly interventionist, and the aid made possible by the setting up of the Shipbuilding Industry Board would only be made available to yards that would toe the government line by reorganizing or regrouping. In nuclear power, the task of finding the right industrial structure to allow Britain to cash in on the tremendous investment in civil research and development was almost a classic. Mr Benn gave away little of the Government's attitude towards the Atomic Energy Authority, merely observing that, having solved many of the technical problems it had set out to solve, it now faced some unresolved choices about its future.

Mr Benn did say something about his attitude towards the Government research establishments. There were two approaches, he thought; one would be to group the establishments under some sort of Technological Authority working closely with industry, and identifying and pursuing goals less expensive and more relevant than the US space programme but capable of establishing a tradition of excellence. would simply be to say that the establishments had outlived their usefulness, and run them down sharply, closing many in the process. But neither of these approaches would in fact be adopted-instead the ministry intended to pursue a carefully phased programme designed to break down the barriers separating research from production. The integration of research and production had proved to be the secret of industrial success abroad, Mr Benn thought, and it must become a major policy objective in Britain, too. "It would be quite wrong to find work inside Government establishments for its own sake just to keep scientists and engineers employed."

This aim might involve Government laboratories undertaking contract research from industrial firms on a basis of confidentiality. Alternatively, intimate connexions might be developed between establishments and the new large firms which will emerge—the Gas Turbine Establishment at Pyestock and Rolls Royce was an obvious example of this approach. Finally, the ministry would try to make it easier for people to move between the establishments and industry. "What we are engaged on is not just an attempt to transfer the emphasis from defence to civil work-although we are doing that—nor even to change the emphasis between intra-mural and extra-mural—though we shall do that as well, but to secure a re-distribution of more of our qualified scientists and engineers from research, wherever it is done, into design development production, marketing, and above all management.'

More Money for Innovation

THE National Research Development Corporation is learning to live with wealth. Three years ago its borrowing powers were limited to £10 million; they were then increased to £25 million, and a few months ago to £50 million. Last week the Minister of Technology was talking of increasing them yet again. But all this has not gone to the corporation's head, as its annual report for 1966–67 shows. It is the mixture as before—the development of inventions from industry, universities and government laboratories, with a liberal sprinkling of ideas from private inventors, some amusing, some improbable and some downright eccentric. What, for instance, is one to make of a semi-automatic jelly tester?

Increasingly, though, the corporation is becoming involved in industry's own problems, which call for commercial as well as technical judgments. This year 69 new projects have been taken on, against 28 for the 9 months covered by the last report, and the expenditure on development projects is up, to £3.65 million. Exploitation receipts are also up, from £430,000 to £793,000, and the total income is £1,307,000, against total outgoings of £4,705,000. As well as the 69 new projects taken on, support was continued for 115 existing projects. The corporation seems so far to have produced only one real money spinner-perhaps surprisingly it comes from the Medical Research Council and the University of Oxford. It is cephalosporin C, the antibiotic which the corporation has supported since 1952. Of the total income of the corporation, £493,000 came from overseas, and cephalosporin accounted for two-thirds of this. The corporation should be grateful to Professor Abraham and Dr Newton of the Sir William Dunn School of Pathology at Oxford, who discovered and isolated cephalosporin. The corporation continued to support the development of hovercraft, and records with pleasure that a patent dispute in the United States was resolved in favour of Mr Christopher Cockerell, the British inventor of the hovercraft.

There is one new idea in this year's report. The corporation is supporting a project which enables a

firm to install mechanically controlled machine tools and operate them for a period on approval. This project, the report points out, was undertaken at the specific request of the Ministry of Technology. Other new projects include plastic sacks for tropical produce, a new tower fermentation process, and transducer production, in collaboration with George Kent Ltd, for measuring instruments for the process control industry. That project, too, has the stamp of the Ministry of Technology. The corporation is also helping to develop microfilm equipment, and a high torque motor developed at International Research and Development in Newcastle upon Tyne. Although the report does not say so, it would be fair to guess that the motor makes use of superconducting magnets to produce a high torque at low speeds.

Promotion without Obligation

More scientists working in Government establishments in Britain have been awarded "special merit" promotions. These promotions, awarded to scientists who have produced a very high standard of original work, enable them to continue their research work without the administrative responsibility normally associated with their new grades. The promotions follow recommendations by a special committee which each year reviews the work of scientists doing research in Government and other public service establishments.

Of the twenty-six research workers awarded promotions this year, Mr K. C. Bowen, Dr J. Croney, Dr C. Hilsun, Dr O. Kubaschewski and Dr E. H. Mansfield have been promoted to Deputy Chief Scientific Officer. Dr G. H. Byford, Dr P. Dean, Dr A. Franks, Dr H. A. French. Dr P. H. Greenwood, Mr H. V. Hempleman, Dr J. M. Linke, Dr E. G. S. Paige, Mr P. H. Parkin, Dr E. R. Pike, Mr W. J. G. Pinsker and Mr D. E. Weston have become Senior Principal Scientific Officers and Dr J. H. Darbyshire is now a Senior Research Officer, Grade I. A number of promotions have also been made within the United Kingdom Atomic Energy Authority. Thus Dr P. G. Burke and Dr R. J. N. Phillips have become Senior Scientific Officers. In the Agricultural Research Council, Dr L. W. Mapson has been promoted to Deputy Chief Scientific Officer. Dr J. Bligh, Dr P. N. Hobson, Dr Daphne J. Osborne, Dr F. W. Robertson and Dr C. R. W. Spedding are now designated Senior Principal Scientific Officers.

Sad Gas

FOR an industry which has just found unexpected new wealth on its doorstep, the British gas industry is singularly long-faced. The annual report of the Gas Council (HMSO, 16s. 6d.) reveals that this year's surplus has fallen sharply from £11·1 million last year to only £3·9 million this year. In the days before natural gas was found beneath the North Sea, the council did much better—in 1964–65, profits were as high as £15·2 million. This year's results mean that the council has failed to maintain its running target of a return of 10·2 per cent on capital invested; the figure now stands at 9·1 per cent.

In fact these depressing results are understandable. The need for extensive re-equipment to cater for natural gas has combined with economic conditions to give the Gas Council a tricky year. Transitional costs have not yet justified themselves in increased revenue, and the Government-imposed standstill on prices has aggravated the situation. The council is now discussing with the Ministry of Power what its financial objectives should be over the next few years, and the report points out that future financial objectives must strike a balance between the transitional burdens in the short term and the undoubted benefits in the long term.

Research, testing and development cost £2.65 million in 1966–67, the report reveals, and the area gas boards spent a further £1.26 million on development. The basic research group has been working on catalysis, adsorption and chemisorption and reaction kinetics. The conversion to natural gas has involved the development of a natural gas substitute which can be burned in the same burners during the period of conversion and for peak loads later. Several underground storage sites have been investigated for porosity, permeability and pore-size distribution, and attempts are being made to assess the risk of contamination of gas held underground by hydrogen sulphide of bacterial origin.

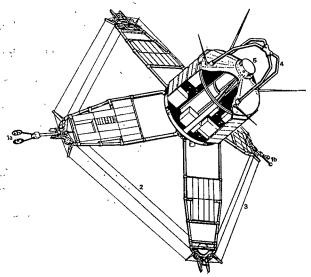
Snags in Space

ARIEL III, the first all-British satellite, has been a mixed success. Although all the sub-systems—power, data handling and telemetry—have worked well and show every sign of fulfilling their design life of one year, the data they have been sending back have been confused. One experiment, from the Nuffield Radio Astronomy Laboratory at Jodrell Bank, has so far yielded only interference.

There seems no doubt that the two experiments designed at the University of Birmingham are the cause of the interference. One measures electron density in space by means of a parallel plate capacitor consisting of two circular grids carried at the end of one of the solar cell paddles, and the other, attached to another paddle, measures electron temperature. Both these experiments are working well, according to Dr J. H. Wager of the University of Birmingham, who described them at a symposium organized by the Institution of Electronic and Radio Engineers on October 13. Unfortunately, both experiments interfere with the Jodrell Bank experiment, which is designed to measure absolute values of cosmic noise in Dr P. C. Gregory from Jodrell Bank has identified two distinct types of interference, one caused by the density probe and the other by the temperature probe. The first type is the more severe, saturating receiver output under all conditions, but the second is not quite so severe, and Dr Gregory philosophically hopes for at least some results from his experiment.

The Birmingham experiments have also upset the attempt by the Meteorological Office to measure the concentration of molecular oxygen in space. Dr P. J. L. Wildman described the experiment, in which the attenuation of the light of the Sun is studied as the satellite enters and leaves the Earth's shadow. In addition to the Birmingham interference, which in this case is not too severe, the experiment is sending back a spurious signal from the shadow side of the Earth, when it should be silent.

Professor Sayers from Birmingham is unrepentant. Other experimenters, he said at the symposium, paid too little attention to the problem of integrating their experiments into the satellite. Only the University of Sheffield was worried about possible interference from Birmingham; as a result, changes were made, and the Sheffield experiment has worked perfectly.



ARIEL III SATELLITE. 1, University of Birmingham: (a) electron density sensor; (b) electron temperature sensor. 2, University of Sheffield: VLIF loop antennae. 3, University of Manchester (Jodrell Bank): galactic noise loop antenna. 4, Radie and Space Research Station: terrestrial noise loop antennae. 5, Meteorological Office: oxygen distribution scanning mirror.

In all probability the interference could not have been predicted, except by testing the satellite with all experiments going in an ionized gas, a facility not available. A much simpler solution would have been to provide switching so that all the experiments could be turned on and off, instead of all operating all the time. NASA, which launched Ariel III for the Science Research Council, advised against this, because it increases complexity and all-up weight (Ariel III was already 25 per cent heavier than the target weight). Professor Sayers, however, believes that it would have been quite easy to switch off his experiment without increasing the number of commands. This is not the first time that interference of this sort has occurred, and clearly it would be convenient if experiments could be switched on and off. The British Black Arrow launcher should be able to offer more commands, space scientists hope.

Waiting for Answers

ALTHOUGH the Austrian Government has now declared its willingness to participate in the construction of the 300 GeV proton accelerator to be built within CERN, there is as yet no sign that the potentially important contributors to the cost of the project will make up their minds before the next meeting of the council of CERN, due to take place in Geneva on December 14. The British answer to the request by CERN for promises of contributions from the thirteen members of the organization is likely to come late in the day. Arrangements have apparently been made for a period of consultations within the Government, and it is unlikely that these will be completed much in advance of the timetable laid down by CERN. At the last

meeting of the CERN council at the end of September, the British delegation raised the problem of how to modify the draft convention for the construction of the 300 GeV machine in such a way that it would not be possible for countries making small contributions to the cost of the new machine to force an unwanted escalation of cost on the big subscribers. In the original version of the convention, as in the conventions which regulate the operation of the Meyrin Laboratory and of the proton storage rings which accompany it, the principle of one country one vote has been adopted. The British view that this might lead to irresponsible decisions in certain circumstances seems to have been accepted as an honest attempt to solve a common problem.

British fear of an escalation of cost seems to be shared by other European countries. At the last council meeting, the British delegation appears to have emphasized that its proposed contribution to the new CERN machine, estimated to amount to £37.5 million in the next ten years, is a substantial part of the annual budget of the Science Research Council. In circumstances like that, the argument goes, escalation would take funds from other fields of science and would for that reason be intolerable. So far, Belgium has agreed to participate, even on the original basis. So now has Austria. In spite of vigorous protestations of support by scientists among the French delegation to the CERN conference, however, there has so far been no official statement by the French authorities.

The eagerness the planners have shown has, however, been strengthened by reports of the speed with which the planning scheme under Professor R. Wilson for the 200 GeV machine being built in the United States is making progress. It is also now known that the proton accelerator at Serpukhov has been operated successfully to produce protons at an energy of 75 GeV. This is somewhat greater than the maximum design energy of 70 GeV, but, at the extreme energies reached in the past weeks of commissioning, the intensity of the beam has been such that each pulse has produced 10⁸ protons—one-thousandth of the number of protons in a pulse when the machine is operating successfully. Full operation cannot be long delayed. In spite of the agreement between CERN and the Soviet Union that will give European physicists access to the Russian machine, the news from Serpukhov will no doubt strengthen the sense of deprivation at Geneva.

European Physical Society

AGREEMENT has been reached on the formation of a European Physical Society and it is planned that there should be an inaugural meeting in Florence in the last week of September 1968. This will be a fitting tribute to the enthusiasm of the Italians for the new venture and, in particular, to that of Professor G. Bernardini, who is chairman of the steering committee. The draft constitution of the proposed society, which has already been circulated to the councils of national physical societies, is to be discussed and—no doubt—approved at a meeting of the steering committee due to take place early in 1968.

The view that the new society should begin as a loose federation will appeal not merely to those of

Gaulist temperament but also to those who are aware of the practical problems of integrating the physics community. One obvious obstacle is the variation in the character of physical societies from one country to another. Another is the obscurity of many of the organizations which should properly play a part in the development of an integrated society. No doubt it will be several years before the various member societies have enough experience of each other's needs and interests for them to know which parts of their activities can be integrated and which will have to be kept going on a national basis. Until then, however, the organizers of the society see plenty of scope for constructive and common activity, principally in the organization of meetings. It will be interesting to see whether there are funds for financing exchange visits on a significant scale. The problems of journals have not apparently been tackled yet, although there seems to be a view that the ideal solution would be the specialization of existing journals in certain fields of physics. The official headquarters will probably be in Geneva.

New Trend in Teaching Science

Is it possible to teach children engineering? This was the common question asked at the Institute of Electrical Engineers on October 12, when Mr G. B. Harrison, head of the department of creative design at Loughborough College of Education, opened a discussion entitled "The Influences of Changes in School Curricula on the Intake of Engineering Courses".

Mr Harrison, who is also director of the Schools Council engineering project, began by outlining the aims of the council, which helps children to understand what technology has to offer and trains them to meet the needs of growing up in a society dominated by technology. According to Mr Harrison, if technology is indeed accepted in schools, the traditional academic attitude of teachers will have to give way to the preparation of new material, apparatus, films, programmes and books that could be discarded when obsolete. He applauded the introduction of engineering as an A-level subject by five of the eight examining boards in the country, but is afraid that diversity might lead to confusion. A common A-level paper is essential so that universities, technical colleges and industry know what is being done at the schools.

An engineering science paper has been introduced by the Northern Universities Joint Matriculation Board to provide an alternative to physics which would meet university requirements. Although the syllabus is similar to traditional physics, it does include project work and practical exercises which demand design ability and creativity. The Oxford Examination Board also has an engineering course. This is, however, to be taken in addition to maths and physics in order to broaden the candidate's horizon. It includes the philosophy of engineering, design aspects and project work. The Cambridge course is essentially mechanical design but also includes the theory of structures and project work. Marks are awarded for project work and added as a bonus to the A-level physics mark. The value of all these courses will depend on the extent to which university courses are geared to work at school. According to Mr Harrison, the present flow from school to university is a series of acute mis-matches that can only be remedied by a long term reappraisal of the whole educational system.

In view of the importance of the topic, the discussion was sparsely attended, but there were several interesting points raised. Who, for example, is going to teach engineering in schools? Certainly not the traditionally trained teachers who have passed through training college and who are often accused of being out of touch. Furthermore, as one speaker noted, this is not merely the age of technology, but of change in general: we are no longer living in a static society, and developments should be made in all directions without over-emphasizing any one particular aspect. There was general agreement that a new approach to education is needed and that the attitude of universities and technical colleges towards the acceptance of engineering as an A-level subject should be made Obviously there are many arguments for and against the scheme, but several questions have yet to be answered. For example, have university students benefited by taking engineering at school? What evidence is there that children want technology and engineering in their general syllabus; have headmasters been approached by pupils anxious to study the additional subjects? Perhaps most important of all, has the introduction of engineering in the sixth form been reflected in the numbers reading engineering at establishments of further education? have a census of opinion at all major schools to take into consideration the views of children themselves? All this may be clarified by the working paper, prepared by the Schools Council, which is shortly to be distributed.

Indian Spoken Here

THE proposed change of language in Indian education away from English prompted Mr Chagla, the Foreign Secretary, to resign early in September. The following week the proposal was discussed at the Conference of Vice-Chancellors in New Delhi, and although the resulting statement published afterwards is in agreement, it contains a certain amount of qualification.

The intention is that English should cease to be the centre of the higher education system, but its importance as a tool is not underestimated. School teaching is at present carried out in regional languages and it is hoped to extend this to undergraduate level. It is realized that a link language is essential, and that the need for a foreign language increases year by year, particularly for library study. Postgraduate courses will continue to be based on English, as translation of books and journals at this level would be impossibly expensive. Regional universities could work on this basis, but all-India institutions raise another problem. Hindi is at present spoken by 40 per cent of the Indian population, but there are some states that are actively against it. Any decision to use it as the education medium in national establishments would therefore have to be made with agreement from non-Hindi states. It is also realized that in multi-lingual cities English may have to be retained as the education medium, alongside the regional languages. The speed and nature of the changes are expected to vary from one university to another, according to circumstances. but undergraduate courses should not take more than 10 years to be adapted.

The conference approved these proposals, and placed particular emphasis on its endorsement of the statement by the education minister that the criterion for any change-over decision should be an improvement in standards.

Broadcasting Abroad

LARGELY at the initiative of the Ford Foundation, plans are being canvassed for the setting up of an International Broadcasting Institute intended to ensure that the fullest use is made of technical innovations such as Earth satellites. The institute has a distinguished backing, with sponsors such as Dr J. Wiesner in the United States, and Mr Kenneth Younger and Professor Asa Briggs in the United Kingdom. A draft constitution is now circulating, and was indeed discussed in detail at a meeting in New York two weeks ago. The immediate problem is to find financial support for the enterprise, and several foundations have already been approached. To begin with, the institute might consist of a director supported by a small office staff and with a brief to define the field in which a more fully equipped institute might operate. For several reasons, one of which is that non-profit organizations are well treated by the taxation authorities in the United Kingdom, the official headquarters of the institute are likely to be in London.

For the time being, at least, the nucleus of the new institute thinks of itself more as a research organization than a pressure group. It would tackle a variety of problems, such as the way in which broadcasting (principally of television) might be used to provide educational services for developing countries and the legal questions which might arise in broadcasting from one country to another, possibly by means of satellites. Whether the institute would try to engage directly in the issue of just how the new international agreement for INTELSAT should be negotiated is another problem. It also remains to be seen how the institute will be able to define its relationships with bodies, official and otherwise, already operating in this field. Broadcasting networks in Britain and the United States seem to be cool but not hostile.

Social Medicine

THE only research unit of the Medical Research Council devoted to social medicine is just settling down in its new quarters at the London School of Hygiene and Tropical Medicine. Professor J. N. Morris, formerly the director, becomes honorary director of the unit on his appointment to the chair of public health in the University of London. The unit, consisting of a staff of eleven doctors, statisticians and social scientists as well as clerical staff, moves with him.

Three main topics are being investigated by the unit, which is using the same general methods of statistical analysis, individual and clinical studies for each. Heart disease, particularly coronary thrombosis, has been under examination for about twenty years, with three aims in view: first to discover causes, secondly to find individuals who might be particularly susceptible, and finally to attempt to reduce the risks for these individuals. Using the discovery that high cholesterol levels in the blood point to thrombosis, experiments are being carried out to see if lowering

these lipid levels in the blood reduces the incidence of thrombosis among otherwise healthy people. Trial groups in Edinburgh, Prague and Budapest are cooperating in this project. A study is about to be made of men in the civil service to see what effects exercise may have on the incidence of thrombosis. The unit has already established that occupational exercise has a protective value.

In its former home in the East End of London the unit involved itself in local affairs by beginning a survey of juvenile delinquency in that area. No obvious conclusions could be drawn from variations in housing backgrounds, and apparently similar schools produced widely differing delinquency rates. Attitudes within the schools may play an important part, but have yet to be studied. It is hoped that statistical and clinical analyses will lead eventually to a reasonably simple test that can be used by magistrates and probation officers to discover the likelihood of a first offender becoming one of the hard core of delinquents.

Operational research into various aspects of the Health Service forms the third activity of the unit. At present the quality and effectiveness of medical care in different types of hospital are being assessed. Why is it, for example, that teaching hospitals are more effective than others? In this field of everyday medicine it is important to discover causes for variations in the success rates, and a large scale survey of prostate treatment is now being made.

Connective Tissue Clubs Connected

AT the joint meeting of the French and British micropolysaccharide clubs, held on September 29 at the Institut Pasteur, Dr Robert, the secretary of the French club which initiated this first joint international meeting, proposed that a European Federation of Connective Tissue Clubs should be formed. This was agreed; Professor Kuhn of Munich pledged West German Under the federation the existing participation. national clubs will of course retain their identity and local interests, as well as participate in international meetings. This arrangement should improve on the present situation in which European workers meet each other, if at all, as visitors in the USA. Discussions are now under way in the hope that the return Anglo-French meeting in 1968, to be held in Britain, will be the first full meeting of the new federation.

At the Paris meeting the magnificent hospitality of Professor Delaunay and the French club provided a perfect background for much productive discussion. Seventeen communications, two of them full lectures by Dr J. T. Dingle and Professor J. Montreuil, were given during the day, on the chemistry, metabolism, morphology, embryology and pathology of connective tissue and its components.

The federation, by arranging regular international meetings, should provide new impetus for the European biochemists, pathologists, electron microscopists and many others involved in the expanding field of connective tissue research.

Human Physiology

THE National Institute for Medical Research seems particularly anxious to make known its work on the

physiology of normal man and the application of its results in industry and the services. A number of interesting and varied projects are currently in progress at the laboratories. In the Department of Human Biomechanics, for example, R. J. Whitney is studying movements of normal people. Photography is used for an exact analysis of the motion of different parts of the body, and the presence or absence of activity in specific muscles which may be involved in the motion is registered by electromyography. One experiment, completed nine months ago, was concerned with the effects of prohibition of movement on physiological and psychological activities of man. Several subjects under thirty were examined in four types of sitting positions varying from supine to upright during twenty-four hours of confinement. Among the changes recorded were EMG of limbs, heart pulse and posture change. A time lapse film indicated that principal movement of the head and feet occurred during the twenty-four hours. Although the results are difficult to analyse, there is evidence of a definite diurnal rhythm in temperature and in swelling of the ankles.

The function of the Division of Biomedical Engineering is to establish a link between modern technology and human biology. It has designed SAMI (Socially Acceptable Monitoring Instrument) to investigate "normal man leading a normal life". This heart beat counter, which derives its input signal from two adhesive chest electrodes, can store acquired information for prolonged periods by means of a reversible electrochemical integrator. Using SAMI, emotional stress has been measured in airline pilots, pregnant women, Peruvian women and schoolchildren. SAMI can also be used to estimate human activity and in clinical follow-up and in work study. A temperature SAMI is also becoming available and SAMIs for noise and posture are in preparation. The development of FAIR (Fast Access Information Retrieval) in the department should lead to full-text, special subject collections becoming available on a user's desk, together with a retrieval system which allows one to generate highly specific subject headings. By providing a list of key words for coding documents, it is hoped that a larger scientific population will consult literature sources of information.

Another interesting study at the laboratories is the investigation of anaemia during pregnancy in immigrant mothers with abnormal haemoglobins and thalassaemia. In the heterozygous conditions, these are both clinically mild but become more severe during increased physiological stress. The traits can be identified by starch-gel electrophoresis and treated with iron administered orally. In 1966 a Tristan da Cunha working party was set up through the council, to investigate the health of the islanders and to assess the balance of their diets. Results indicated that the islanders do not, in fact, live up to their reputation for good health.

Food Additives

THE use of food additives is difficult to control satisfactorily, for although many countries have agencies and scientific institutions with this special responsibility, others lack adequate facilities. The problem is being dealt with by a joint FAO/WHO programme which aims to make systematic evaluations of food

additives and to provide evidence to member states of FAO and WHO. The programme is implemented by the joint FAO/WHO Expert Committee on Food Additives which advises the Codex Committee on Food Additives, which in turn, through the Codex Alimentarius Commission, proposes to governments internationally acceptable tolerances for additives in various foods.

The Codex Committee selects the substances to be considered by the Expert Committee, whose terms of reference are to establish specifications for identity and purity for food additives and to evaluate toxicological data. It also recommends, if possible, acceptable daily intakes for man. This committee has just produced its tenth report, on "Some Emulsifiers and Stabilizers and Certain Other Substances" (WHO Technical Report Series, No. 373; 1967). The committee recommends that biochemical and metabolic studies be substituted sometimes for the more usual toxicological studies when the effects of food additives are evaluated. If the additive is completely broken down in normal metabolism to common dietary or body constituents, toxicological investigation is a wasted effort, for the additives are then behaving like foods. For such cases an acceptable daily intake has been calculated such that the additive should not increase the food component into which it is converted by more than about 5 per cent of the amount in an average diet. The fatty emulsifiers such as acetic, citric and lactic acid and fatty acid esters of glycerol are in this category, for in metabolism they are all hydrolysed to acceptable dietary constituents.

The committee says that toxic concentrations of arsenic, lead or heavy metals are unlikely to be found in food now, but it gives specifications to encourage the use of good quality raw materials and equipment. Most normal diets probably supply 1.5–2.0 mg of arsenic each day. The committee puts the maximum acceptable load at 0.05 mg/kg of body weight, and recommends that the maximum content of arsenic in food additives be 3 mg/kg; for heavy metals they recommend 40 mg/kg, and for lead 10 mg/kg in cases where the daily intake of the additive exceeds 1 g.

In several cases a decision on the use of an additive has had to be deferred because of lack of data. Of the natural stabilizers, furcelleran and its salts could not be evaluated in the absence of toxicological data. Concerning carrageen and its salts, now used as food additives, toxicological data are inadequate to establish an acceptable daily intake, and the committee recommends that this information be made available within the next four years so that a decision may be taken on permission to continue the use of this substance. Information is also inadequate concerning those long established additives gum arabic, karaya, tragacanth and carob bean gum, although tentative specifications have been prepared. Details of all specifications will be given in monographs containing biological data and toxicological evaluation of the various additives, and the committee suggests that a decision to use a particular additive should only be taken after consulting the detailed monographs.

Making Air Fresh

"TAKE a deep breath and be sick" is a wry comment sometimes heard in the United States. In Britain,

the National Society for Clean Air is trying to forestall such cynicism. The annual conference of the society is being held in Blackpool this week, and papers on various aspects of air pollution are being presented. The work of the society was described by Sir John Charrington in his presidential address on Tuesday. Sir John described the change of emphasis from industrial smoke to vehicle pollution and called for amendments to the Clean Air Act.

Air pollution from road vehicles was the subject of the report of the technical committee which was presented by Dr Albert Parker. Carbon monoxide is the principal pollutant in exhausts, although nitrogen oxides and smoke also occur. In Britain less than one-third of atmospheric carbon monoxide comes at present from vehicles, but if present rates of increase continue the amount discharged will have doubled by 1980 to more than 10 million tons a year. danger from carbon monoxide arises particularly in traffic jams, when there is little chance of dispersion. Fifty p.p.m. is the maximum concentration of carbon monoxide allowed for an eight hour day in industry, and yet in Oxford Street recently 360 p.p.m. was measured. There is as yet no legislation in Britain for controlling emission from petrol engine vehicles, and the British Standard Specification for diesel engines is above the present emission level set by most manufacturers. Work continues, however, on modifications to petrol engine design, for if it is ensured that all fuel is burnt, there will be little poisonous exhaust. Recirculation of crankcase fumes helps, and fuel injection systems and modified carburettors have been installed in some cars. The initial cost of the vehicle is increased, but fuel consumption is improved. It is hoped that preliminary murmurs from the Ministry of Transport on this subject will soon be transformed into effective schemes for checks and controls.

Industries using dangerous materials can keep checks on their employees, but waste substances that escape into the surrounding countryside can have serious effects. In a paper presented on Tuesday, beryllium, asbestos and fluorides were discussed. Illness and death can occur in both animals and humans living near processing plants, or from contact with industrial workers and their belongings, but no suggestions were made for improving the situation. The paper ended with a commendation of the care taken by those responsible for controls on radioactive waste.

By contrast, Dr E. F. Schumacher, economic adviser to the National Coal Board, considered the dangers of radioactive pollution. Too often these days decisions are based solely on economic considerations, with little reference to environmental factors. Pollution of the atmosphere by radioactive substances for the sake of cheaper electricity was the example Dr Schumacher used in his Des Voeux memorial lecture, when he related economics to clean air problems.

And so to the domestic scene, where keeping the home fires burning produces an estimated £280 million worth of damage every year. Working on an average figure of £16 for conversion to smokeless heating, the capital cost for cleaning up the black areas of England once and for all would come to £100 million. In giving these figures, Mr A. D. Smith described the situation as economically and technically crazy, because pollution from domestic smoke is the easiest

type to remove. If a date could be set when Treasury grants for conversion would be stopped, people might jump to get their money while they can.

Worthy Cause

This month, Mr Peter Scott will launch an appeal for £75,000 for development at Wicken Fen—the remnant of the once great fens of Cambridgeshire. Wicken Fen reserve, one of the oldest and most famous nature reserves in Britain, is approximately 700 acres in extent and has long been the site of biological and nature studies. But in recent years there has been a great increase in the use of the area by members of the general public, school parties and groups of students. To encourage these activities while at the same time conserving vegetation and wild life, the Wicken Fen Local Committee of the National Trust has made two sensible proposals. One, following the American National Parks pattern, is the erection of an open air laboratory with associated museum, lecture room and display unit and the provision of picnic and other facilities for visitors. A resident warden naturalist, appointed by the committee, will be in charge of the laboratory, but there will not be a permanent staff. Laboratory facilities, including exhibits and maps, will be available to interested parties, and school children will be allowed to examine in the laboratory their specimens at the end of a day's collecting. The other proposal is to create a marshland reserve in which a new range of aquatic and marsh communities can become established and provide a valuable habitat and migration refuge for many species of birds.

There has already been considerable progress in the management and conservation of the reserve. A ten acre mere, opened in 1955, is now the haunt of thousands of birds. A sixty acre reed field has also been established on the fen and, in the past three years, more than a mile of dyke has been cleared out. More manpower and modern implements are needed, however, to clear choked ditches and the wilderness of bushes—the result of years of financial difficulties. The present income of the fen, entirely composed of voluntary subscriptions and donations, is quite inadequate to meet these urgent needs.

The cause is particularly worthy. Instead of trying to restrict use of the reserve, the committee wishes to encourage public interest, while at the same time safeguarding the area.

Urban Grove for Academe

from Brenda Maddox

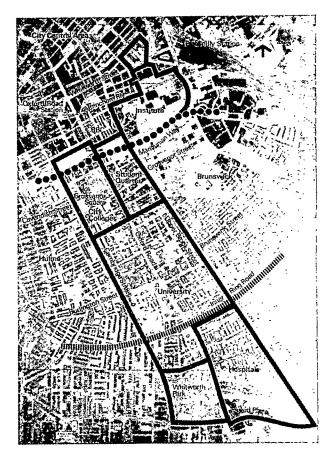
MANCHESTER is in the throes of a strenuous attempt to transform its academic community. The City Council, the University, the Institute of Science and Technology and the city's various colleges and hospitals are collaborating to create a green "educational precinct" in an area now engulfed by smog, traffic and slum. By 1984 the results of their efforts should be in place. There will be a strip, one and a quarter miles long and one-third of a mile wide, with the beautifully black university at its centre. Green grass and elevated pedestrian walkways will sweep from the site of the United Manchester Hospitals on the south to the group of city colleges on the north, and then east to take in

the Institute of Science and Technology. Cars, in the best modern manner, will be parked on the fringes, and new residential buildings will not only invite students into town, away from their suburban digs, but will screen the precinct from the noise of traffic.

The project may be what its planners (Hugh Wilson and Lewis Womersley) claim that it is: an opportunity unequalled in any city in Europe. Certainly the design critics are excited by it. The plan is grounded in realism and flexibility; it is considered to be a framework, not a master plan, the area being too large and susceptible to change for any kind of architectural uniformity to be stamped upon it. The raw material is planning.

What will create the precinct is the patterning of roads and grass and the mixture of different kinds of buildings-residence halls, academic departments, shops, restaurants and churches. The shape of any particular building is not crucial, although the planners insist on certain conformity from architects in building materials and in scale. (The centrepiece of the precinct -the university's new mathematics building—is being designed by the planners themselves.) The unifying element will be Mr Womersley's trademark, displayed before in his work in Sheffield and Nottingham—the first-floor footpath. People arriving at the precinct by bus—and there is little reason to hope that commuting will not continue to be a major characteristic of the Manchester academic scene—may take an escalator up to the walkways from the bus stop and continue, at first floor level, for the entire trip to their destination.

Will the precinct be beautiful ?—a burning question in such a renowned ugly spot as Manchester. answer, alas, is probably not. There is too much against The admirable realism behind the whole plan demands living with such obstacles as the Mancunian Way, the highway on stilts which, hardly finished, pours noise and darkness right across the heart of the precinct site. It isolates the university from the institute and the city colleges, creating a physical barrier to replace the invisible one which the precinct is supposed to eradicate. The whole scheme, however, depends really on the determination of the Manchester City Council to make the city more humane and attractive, even if it means sacrificing rateable land. This is a rare enough quality in city governments; one can hardly criticize the council for not wanting to tear down the Mancunian Way before it has barely risen. But the fact remains that this prior plan has doomed from the start any hope the precinct might have had of beauty and grandeur. The same, on a lesser scale, may be said of two city housing developments-



Brunswick and Hulme—which stand solidly on either side of the narrow strip. And, in an ideal world, the planners would have been able to remove Oxford Road, the north—south artery, altogether. As it must remain to carry buses and lorries, one wonders how successful Mr Womersley's pedestrian walkway will be in persuading people to walk up in the air. There is a danger that it will simply create more darkness at noon in a city which has more than its share.

That having been said, the precinct still looks like doing a lot for Manchester. It will draw the city and the university closer together; so should it make the city colleges, the adult education college and the medical school have a richer intellectual life because of association with the university and institute. If all goes according to plan, the precinct will be lively in the evening as well as during the day; it will be good for book-browsing and pram-pushing as well as for getting a degree.

Table 1. ESTIMATED CAPACITY OF THE ACADEMIC PRECINCT. IT HAS BEEN ASSUMED THAT THE SITE COULD NOT BE DEVELOPED TO THIS EXTENT UNTIL 1984

	Full time students	Part time students	Academic staff	$_{\rm staff}^{\rm Other}$	Hospital medical staff	Totals
University	15,000		2,500	3,750		21,250
Institute	5,000		850	1,250		7,100
John Dalton	500	800	300	170		1,770
College of Art	1,000	500	250	100		1,850
College of Music	600		100	20		720
College of Commerce	1,500	600	300	60		2,460
Teacher Training	1,000		110	50		1,160
Adult Education		730	90	40		860
Elizabeth Gaskell	850		90	80		1,020
Hospitals	1,400			1,620	1,950	4,970
Shops, offices, etc.			,	350		350
Totals	26,850	2,630	4,590	7,490	1.950	43.510

NEWS AND VIEWS

Hallucinations to Order

ONE of the most alarming features of the drug LSD is that it can be made in the laboratory. In other words, there is no natural physical limitation of the scale on which, in suitably bizarre circumstances, it could be supplied to the public. It follows that those who are concerned to see that the use of drugs is controlled by legislation are at least a little nonplussed by the appearance of synthetic processes for manufacturing drugs which were originally derived from natural sources and, more especially, by the application of synthetic processes to the design of new drugs. Although it will be a long time before the flower children and their like would be able to synthesize their own psychomimetic agents, it is entirely proper that there should now be considerable anxiety about problems of control.

The report by Smythies et al. (Nature, 216, 128; 1967) is a reminder of the difficulty of controlling the use of drugs which can be synthesized, if only because it shows that several derivatives of amphetamine have properties which are similar in a number of ways to those of the frankly hallucinogenic drugs-LSD and mescaline, for example. Although there is, of course, no prospect that any of these materials will be put on public sale, the sheer diversity of them is startling, to say the least of it. The preliminary report of the properties of paramethoxyamphetamine is particularly arresting. In rats, this material seems to produce signs of behavioural disturbance which are similar to those produced by LSD at a dose of 3.1 mg per kg. With a dose merely twice as great, however, profound changes in the behaviour of the rats seem to have been brought about. One rat died after a day without recovering its normal state. In another, the severe disturbance persisted unchanged for a week, when that rat also died. In other words, it looks as if the preliminary studies with rats suggest that sufficiently large doses can have irreversible effects. Making an objective assessment of how paramethoxyamphetamine affects the behaviour of human beings will be difficult enough. But it is also plain that if the uncontrolled use of LSD is dangerous, other materials may bring even greater risks.

It would, however, be unfortunate if the particular properties of one material were to obscure the wider interest of what Smythies et al. have done. Their immediate objective has been to assess the potency of several derivatives of amphetamine, which pharmacologists know to be a stimulant of the central nervous system and which others know as a "pep pill". It has been known for more than a decade that derivatives of amphetamine can sometimes be similar in their effects to the more familiar drugs which produce hallucinations, and this is not surprising. After all, mescaline is

derived from phenylethylamine while amphetamine itself is phenylisopropylamine. One of the generalizations which has come out of the work which Smythies et al. have done is that a paramethoxy-group seems to be necessary for hallucinogenic activity. This in itself is suggestive of how hallucinogenic drugs may function.

But how? Smythies and his colleagues have put forward at least two suggestions as to how paramethoxyamphetamine may function. They suggest, for example, that it may interfere with the activity of an enzyme which is usually responsible for sweeping nervous tissue clear of the catecholamines which serve as means of communication with nerve cells of certain kinds. Another possibility is that the presence of a methoxy-group in the para position may interfere with the biochemical processes which are able to get rid of unsubstituted materials such as amphetamine. Evidently there is plenty for the biochemists to do, and there will probably now be several attempts at a detailed study of the interactions between the hallucinogenic drugs and the various enzyme systems to be found in nervous tissues. The signs are, however, that this will be a back-breaking job. The detailed biochemistry of drug action seems most often to be frustrated by difficulties which are not yet fully understood. Nobody will be surprised if a detailed understanding of the way in which hallucinogenic drugs function has to wait on a description and understanding in molecular terms of the structures in nerve cells which function as receptors for the molecules which carry information between nerve cells.

There also remains the possibility of a connexion between the activity of the hallucinogenic drugs and the causation of mental disturbances, including schizophrenia. Such a possibility has been on the cards ever since it seemed as if the substance in the urine of schizophrenic patients producing a "pink spot" on chromatography might be a derivative of phenylethylamine and thus closely related to mescaline. Smythies et al. point out that the likelihood of a link between hallucinogenic drugs and schizophrenia may be even greater now that it seems more probable that the pink spot is really paratyramine—itself a parahydroxylated compound. But this, of course, is frank speculation. The real value of the work now being reported is that it will help vividly to suggest ways in which the function not merely of specific drugs but of nervous tissue as well may be opened up to new enquiries. In the long run, it is just possible that there will be something to suggest how problems of drug dependence may be dealt with, which is some recompense to those who will regard the description of new hallucinogens as a threat.

Colliding Beams

from a High Energy Physics Correspondent

An experiment which yields in all qualitative respects the theoretically predicted results and which has intrinsic novelty and elegance as well was reported at the recent High Energy Physics Conference in Heidelberg. H. Joos of the DESY group in West Germany reported the results of an experiment performed by a Russian group at Novosibirsk which has studied the annihilation of an electron and a positron and the subsequent production of a pair of π mesons with opposite This experiment, like similar experiments being performed at Orsay in France and Stanford in the United States, relies on the very recent experimental technique of making two beams of particles collide. The advantage of this method lies in the relativistic kinematics of the situation in which the square of the beam energy in the colliding beam is a true measure of the usable energy. In the more conventional situations, with a beam striking a stationary target, usable energy is proportional to the product of beam energy and the mass of the target particle.

In principle, it is quite simple to perform a colliding beam experiment such as that carried out at DESY. The first step is to build a storage ring, which consists of the field magnets and vacuum system for a small synchrotron. This device can then be fed with electrons from some conventional synchrotron or linear accelerator until a considerable current accumulates. A similar beam of positrons is then built up, either as a separate beam in the same storage ring (but travelling in the opposite direction because of the charge) or in another storage ring so arranged as to form a figure of eight with the first. The two beams can then be made to collide and conventional detection methods used to observe the reaction products. Although the relative probability of a collision between the beams is low compared with that when a solid or liquid target is used, this disadvantage is somewhat offset by the several chances of collision afforded by the repeated intersections of the orbiting particles. In practice, the difficulties, arising chiefly from resonance effects which cause beam instabilities, are enormous and have taken several years to understand, so that the new experimentthe first in which a strongly interacting final state has been studied—represents a landmark in the field and is a tribute to the skill of the experimentalists involved.

Several years ago, studies of nuclear form factors obtained from measurements on the scattering of electrons by nucleons suggested the existence of a neutral particle with unit spin and a mass of several hundred MeV. This was later confirmed by the discovery of the p meson at a mass of some 760 MeV in pion production experiments. By invoking what is believed to be one of the basic principles of physics known as crossing symmetry, it is possible to infer that if this p meson couples electromagnetically to the electrons, which would account for the form factors, then an electron-positron pair should annihilate electromagnetically to form such a ρ meson which would then decay, by strong interaction, into a pair of charged pions. The new experiment confirms this concept in a most striking manner. When the number of pion pairs is plotted against energy, an almost classic Breit-Wigner resonance decay curve is seen with a peak at a mass value of 764 MeV and a width of 93 ± 15 MeV.

Although the immediate interest lies in the novelty of the experimental technique, detailed experiments along these lines seem certain to suggest a major revision of current ideas.

Reliable Electricity

from a Correspondent

Can the economic consequences of unreliability of electricity supply be properly quantified? From whose viewpoint should this task be attempted? What is the place of statistical and probability methods in such studies? How can the experience of various countries in this field be collated and compared? These and many other related questions were discussed at a conference last week organized by the Power Division of the Institution of Electrical Engineers. About 250 delegates, including 40 from 14 overseas countries, participated in three full-day sessions devoted to the problems of generation, transmission and distribution connected with this issue. It must be said at once that the questions remained largely unanswered, although many preliminary suggestions were advanced.

It is rare, if not unprecedented, for the product of the electricity supply industry to be examined in this way. In both national and international forums, the manufacturers of electrical plant are accustomed to describe the design and performance of their products and to face questions and criticism from customers and competitors. Publications by electricity supply engineers are commonly restricted to planning and operational problems. The papers at this conference clearly showed the reluctance felt by some authors to unveil the basis for the cost-benefit calculations which they may have attempted. Unfortunately their major customers, the large electricity using industries, played only a small part in the conference and, despite some shrewd blows, were heavily outnumbered by the supply side. The plant manufacturers appeared uncertain as to their proper role in these unusual proceedings and remained rather quiet.

The conference might therefore have been a rather tame affair, but it was saved by the excellent, frank and good-humoured contributions from abroad. A Danish engineer drew some interesting contrasts between electronic and nuclear equipment design and that of power plant and systems. Might it perhaps be both cheaper and more reliable to employ a certain amount of duplication and redundancy while lowering the performance specification for individual items?

The same speaker added, quixotically, that he felt a power cut now and then might serve to remind us of our ever increasing dependence upon the machinery of civilization and progress. He was not supported in this by a Swedish colleague, who was sure that their job was to build the biggest possible market for electricity by offering consumers what they want to buy. (This speaker also made the possibly significant point that the Swedish State Power Board supplies only 40 per cent of the country's electricity. Comparisons with other undertakings thus remain possible and provide a valuable stimulus to efficiency.) An engineer from Electricité de France was the contributor who laid most stress upon the concept of "loss to the community" resulting from failures of supply, and who demonstrated how far l'esprit cartésien penetrates into technico-economic assessments of national issues. Other valuable papers and discussions came from Germany, Poland, Netherlands, the United States and Canada. The differences between countries having much hydro-electric power and those lacking it were brought out in several contributions.

The central problem is best illustrated by an extreme example. A short, or even momentary, interruption to a £10 million petro-chemical complex can occasion a loss to its owner of perhaps 1,000 times the sale price of the electricity not used during the incident. This results from the disturbance and need for restarting procedures on a process designed (and costed) to be continuously operating. A long outage in the small hours on a commercial or domestic supply might pass unnoticed.

Plastid Nucleic Acids Again

from our Correspondent in Cell Biology

Study of the nucleic acids of plastids is rapidly becoming one of the great band wagons of biology. Two weeks ago (Nature, 216, 14; 1967) in this column I wrote about some experiments done by Smillie and his collaborators indicating that the DNA of Euglena chloroplasts codes for chloroplast ribosomal RNA. Since then Fukuhara (Proc. US Nat. Acad. Sci., 58, 1065; 1967) and Suyama (Biochemistry, 6, 2839; 1967) have reported that, in all probability, the DNA of mitochondria in the yeast S. cerevisiae and Tetrahymena codes for the RNA of the mitochondrial ribosomes. Suyama also reports the unexpected result that very little of the 4-58 soluble RNA in Tetrahymena mitochondria hybridizes with the mitochondrial DNA and even more puzzling—that it does not hybridize with the nuclear DNA either. Earlier this year Barnett reported three mitochondrial specific species of tRNA in Neurospora, and Suyama and Eyer (Biochem. Biophys. Res. Comm., 28, 746; 1967) found mitochondrial specific leucyl tRNA in Tetrahymena, so it was confidently expected that mitochondrial DNA codes for these organelle specific tRNAs. Unless, however, Suyama is in error, this idea must now be questioned.

Recently Küntzel and Noll described the sedimentation properties and nucleotide composition of Neurospora mitochondrial ribosomes (Nature, 215, 1340; 1967). Now Luck, well known for elegant studies of Neurospora mitochondrial DNA, and collaborators report a virtually identical study (Proc. US Nat. Acad. Sci., 58, 1025; 1967). The two groups report analyses of the nucleotide composition of Neurospora mitochondrial and cytoplasmic ribosomes that agree to within 5 per cent and show the two classes are quite distinct; for example, the G+C contents of mitochondrial and cytoplasmic ribosomes are 38 per cent and 49 per cent according to Küntzel and Noll and 35 per cent and 50 per cent according to Luck et al. But Luck's values for the sedimentation coefficients of the ribosomes and ribosomal RNA are unusually high and very different from those given by Küntzel and Noll. For example, under their conditions, Luck et al. find E. coli ribosomes sediment at 81.9S and Neurospora cytoplasmic and mitochondrial ribosomes have virtually identical (and high) coefficients, 89.8S and 89.5S, whereas Küntzel and Noll's corresponding values are 70S (the conventional value for E. coli) and 77S and 73S for the

two classes of *Neurospora* ribosomes which are thus clearly distinguishable. Since the two groups find similar nucleotide compositions for *Neurospora* ribosomes, these perplexing differences must be attributed to differences in conditions of sedimentation, notably in the composition of the buffer solutions.

Also in the current Proc. US Nat. Acad. Sci. (58, 1051; 1967), Attardi and Attardi report the very interesting discovery that in HeLa cells that fraction of the cytoplasmic mRNA associated with ribosomes bound to the endoplasmic reticulum is of cytoplasmic origin. When cells are fractionated after a 30 min pulse of H³ uridine, about twice as much of the newly synthesized RNA is associated with a membrane fraction, which contains mitochondria, endoplasmic reticulum and 10-15 per cent of the cell ribosomes as with free poly-This membrane associated RNA differs in base composition from the mRNA associated with free polysomes; it has a higher adenine and lower G+Ccontent. Furthermore, the membrane associated RNA has a faster turnover than free polysome mRNA, sedimentation properties characteristic of mRNA and greater sequence homology to cytoplasmic, presumably mitochondrial, DNA than nuclear DNA. Bacterial mRNA is much less stable than eucell mRNA and mitochondria are thought to have arisen from symbiotic bacteria. It seems significant to this idea that the mRNA in HeLa cells which is thought to originate in mitochondria is metabolically unstable. Attardi and Attardi suggest that some of the mRNA transcribed off mitochondrial DNA migrates into the cytoplasm where it associates with ribosomes bound to the endoplasmic reticulum and is translated. It will be of great interest to see what proteins it specifies.

Recording Active Brains

from a Neurophysiology Correspondent

ONE of the outstanding problems in neurophysiology is the development of techniques for recording neural activity in alert, active animals so as to permit proper correlation of neurophysiology and behaviour. At present most knowledge of the neurophysiology of the central nervous system is derived from anaesthetized preparations in which it is most unlikely that cerebral processes are similar to those in alert animals. The problems to be solved include the rigid mounting of a microelectrode the tip position of which can be finely controlled, and the precise stimulation of, say, the retina of an unrestrained animal.

Recently MacLean (Electroenceph. Clin. Neurophysiol., 22, 180; 1967) described a platform which can be mounted firmly on the cranium of an experimental animal, so that it lies in the horizontal plane of the stereo-taxic co-ordinate system. Guide holes within the platform allow the insertion of micro- and macroelectrodes through holes drilled in the cranium. However, although it is possible to implant permanent recording and stimulating macro-electrodes, the use of micro-electrodes is more difficult. They are introduced at the start of a recording session during which the animal must be restrained, for head movements may cause movement of the brain relative to the electrode. The technique is therefore limited; nevertheless, intra-cellular recordings have now been made from units in the squirrel monkey's hippocampus

(Yokota, Reeves and MacLean, Science, 157, 1072; 1967). The hippocampal cortex with a much simpler and more ordered structure than the rest of the cerebral cortex is suitable for investigating single unit activity, particularly the relation of unit firing to specific inputs producing known patterns of synaptic activation. The authors investigated the effects of two inputs, one from electrical stimulation of the olfactory bulbs and the other from the septum. The septal pathway produced EPSPs which could lead to spike initiation, whereas olfactory bulb stimulation never leads to spike generation.

Marg and Adams (Electroenceph. Clin. Neurophysiol., 23, 277; 1967) also describe techniques for electrode implantation but in human subjects. They have obtained recordings from units (producing spikes of low amplitude and long time-course) in the striate cortex, and say that these show some of the properties found by Hubel and Wiesel for the primary visual cortex of the cat; furthermore, this activity was inhibited when the subject closed his eyes. This is an interesting observation, as reduction of the intensity of light reaching the retina usually leads to increased activity in the optic nerve, presumably because lateral inhibition in the retina decreases, making the retina more sensitive but reducing visual acuity (Arduini, Progr. Brain Res., 1, 184; 1963).

Immunology of Reproduction

from a Correspondent

An International Symposium on the Immunology of Spermatozoa and Fertilization was held in Varna, Bulgaria, from September 27-29, 1967. Organized by Academician K. Bratnov and his colleagues, it attracted more than 340 delegates from 20 countries, most of them from the Soviet Union and eastern Western scientists there took this excellent opportunity to meet people and evaluate data previously unknown. All the proceedings, entertainment and accommodation were provided in the International House of Scientists, which gave excellent opportunities for informal discussion.

Progress on various topics was reported, although major questions inevitably remained unanswered. Several important antigens extracted from tissues or secretions of the male tract have been characterized. Some of them in seminal plasma adhere so tightly to spermatozoa that they influence the antigenic properties of these cells. Immune aspermatogenesis received much attention, ranging from the identification of some of the antigens responsible to the physiological consequences of natural or experimental interference with normal processes. But the mechanism by which the antibodies interfere with spermatogenesis is still not fully understood. Removal of the site of sperm resorption in men with blocked vas deferens leads to a reduction in their serum titre of spermagglutinins. But it is still not clear why some men transmit these antibodies to their seminal plasma whereas others do

The effect of immunization with spermatozoa on fertility in females was dealt with by eastern and western workers. In several species including man there is now evidence of impairment of fertility in females due partly to the presence of antibodies in the repro-

ductive trace.

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Function of Histones

from a Corresponder.

THE relation of histones to the regulation is one of the most challenging and controvers. in developmental biology. Originally the Stedman. proposed that specific histones characterize specific cell types, but analyses of histones from different tissues do not support this idea. Genetic regulation must be at a more subtle level of histone chemistry. Allfrey and Mirsky and their colleagues have claimed that acetylation and phosphorylation of histones is a prerequisite for RNA synthesis at histone-repressed gene loci. Experiments by Gutierrez Hnilica (Science, 157, 1324; 1967) tend to support this idea. They have found that the differentiated cells of rat liver and spleen incorporate phosphate-32 into their lysine-rich and arginine-rich histones at a very much faster rate than the dividing cells of regenerating liver and transplanted Novikoff hepatoma, although the latter cells synthesize more histone than the differentiated cells. Furthermore, the degree of phosphorylation decreases with increasing rate of cell division. Thus it seems, on the basis of the degree of phosphorylation, that differentiated cells are more genetically active than the dividing cells.

It should be possible to detect changes in histone biochemistry at genetically active sites, like the "puffs" of Dipteran polytene chromosomes. This led Ellgard (Science, 157, 1070; 1967) to induce puffs on the polytene chromosomes of Drosophila larvae and to look for evidence of acetylation at the puffs. By autoradiography he found that the newly induced puffs were genetically active and they incorporated 3H-uridine into RNA, but he was unable to find any evidence of acetylation at these sites. So controversy remains.

Some pattern does seem to be emerging in the relation of histone type with division and differentiation. Holoubek and Hnilica (J. Nat. Cancer Inst., 39, 187; 1967) report that a histone very rich in lysine (F1) extracted from nuclei of the Walker carcinosarcoma of mouse, and normal thymus and liver cells, when injected into mice increases the rate of DNA synthesis in the nuclei of their liver and spleen. Other histones, including the arginine-rich fractions (F2a and F3), inhibit synthesis. It is interesting that the F1 fractions from thymus and liver nuclei have to be separated from some unspecified protein before being able to stimulate this protein. Other this protein. Other this protein. Other this protein. Other this protein of a lysine-atheris, is provided by Acta, 145, 531; 1967). Se ascites cells reduces DNA and cells end while synthesis of RNA and the synthesis about 35 per cent, but the very thore historis reduced by 60 per cent. So nich procee is an intimate association between the third in the synthesis of the very lysine-

ine-rich histones are considered to be ac of stable, non-dividing cells by Evans rs (Exp. Mol. Pathol., 7, 105; 1967). They some of the histones from the tumour induced Shope papilloma virus. The tumour contains ginase, and the histones, not surprisingly, contain educed arginine level. This has the effect of raising he relative level of lysine in the histone, and it is tempting to think that this leads to the onset of unregulated division and tumour initiation. Can the transition from division to differentiation really be mediated by the lysine-arginine balance?

Recognition and Conformation

from our Molecular Biology Correspondent

Some striking examples have been described which uphold the dogma of protein chemistry that the aminoacid sequence of a chain uniquely determines its conformation, and show in addition that the association of different chains may be required if the information contained in their sequence is to be expressed. The first example follows on one of the recent achievements of synthetic peptide chemistry, the total synthesis of insulin, reported simultaneously by Katsoyannis and his co-workers and by a group working in China. Although it has long been established that correct internal disulphide pairings are preferentially formed in relation to incorrect ones when a single chain is allowed to refold from the reduced unfolded state, insulin, which has two chains joined by two of its three disulphide bonds, for a long time eluded attempts at correct recombination. Until the successful syntheses reported last year it was indeed often surmised that proper refolding and correct disulphide pairing could not occur independently of biosynthesis of the chains. This is now known not to be the case, and further details of recombination experiments have just been reported by Katsoyannis et al. (Biochemistry, 6, 2642; 1967).

If all three disulphide bonds of insulin are broken and allowed to reform, several intra-chain and interchain combinations will clearly be feasible. In practice, under defined conditions, recombination of A and B chains gives rise to only six products, which have been separated by ion-exchange chromatography. Two of these have the composition of A chains, two others that of B chains. It is not reported whether these contain inter- or intra-chain bonds, but it is in any event clear that only a small number of the possible pairings are sterically favoured. The remaining two components are both hormonally active and one is indistinguishable from native insulin. The nature of the other is unknown, and its proportion is determined

by the precise conditions of recombination. It can, however, be converted into its native isomer by precipitation with picric acid. Katsoyannis *et al.* (*ibid.*, 2656) have also prepared hybrid insulins from chains of different species, natural or synthetic, and fully active products have been obtained in yields ranging up to 50 per cent.

An important case of specificity of association between polypeptide chains occurs in antibodies. Immunoglobulins contain four chains, which are of two types, known as heavy (H) and light (L). Hybrid immunoglobulin molecules can be prepared, but it has been shown by Mannik that there is preferential recombination of H and L chains originating from the same The antibody activity of the molecular species. homologous species is also higher than that of the hybrids. Dorrington, Zarlengo and Tanford (Proc. US Nat. Acad. Sci., 58, 996; 1967) have now found physical evidence of a specific conformational interaction between homologous chains by the use of optical rotatory dispersion. The separated H and L chains each show a single Cotton effect maximum at 220-230 m μ , but the intact immunoglobulin—as previously noted by Steiner and Lowey-shows a pattern in which the rotation is smaller, and at least three component Cotton effects can be discerned. Dorrington et al. find that this curve is completely regained when H and L chains from myeloma immunoglobulin are recombined. It is striking that recombination of heterologous chains—as in the case of a normal heterogeneous non-specific immunoglobulin-generates only a part of the pristine pattern, a minimum at about $240~\mathrm{m}\mu$ being lost. Thus \tilde{H} and L chains will recognize each other and combine in specific manner, but a further interaction occurs only in the homologous case. It should be noted that the Cotton effects are very small, and not of a kind to suggest the presence or involvement of, for example, α-helical structure. The conformational change could be confined to a very few residues, or such an effect could arise from large perturbation of a few chromophores, for example, by juxtaposition with charged groups.

Yet another instance of a recognition process between two chains leading to specific refolding is described by Taniuchi, Anfinsen and Sodja (*ibid.*, 1235), who find that a staphylococcal nuclease of 149 residues can be cleaved by trypsin in the presence of inhibitor and calcium ions into three fragments of 5, 44 and 100 residues. The two large fragments can then recombine non-covalently, when 8 per cent of the enzyme activity is regained. The system therefore recalls the subtilisin-modified ribonuclease of Richards, in which two fragments recombine to give substantially the activity of the native enzyme. The present case is particularly interesting because the structure is stabilized despite the absence of disulphide cross-links in this protein.

Chain Termination

from our Cell Biology Correspondent

Last year Adams and Capecchi discovered that the initiation of protein synthesis in *E. coli* requires the insertion of N-formylmethionine as the first aminoterminal amino-acid of the polypeptide chain. Since then, Capecchi has been working on the more intractable problem of how the synthesis of a polypeptide

chain is terminated and the completed chain released from the ribosome-mRNA complex to which it is bound by the tRNA molecule carrying the last amino-acid incorporated. There must be a mechanism for reading the chain terminating codon (three codons UAA, UAG, and UGA can code for chain termination) and cleaving the last peptidyl-tRNA bond. In Biochem. Biophys. Res. Comm., 28, 773 (1967) he describes an experimental system devised to give a rapid assay for chain termination, and in Proc. US Nat. Acad. Sci., 58, 1144 (1967) he reports the discovery of an enzyme required for termination.

The assay for termination employs the RNA from an amber mutant of the coat protein gene of the RNA bacteriophage R17. This mutant has an amber codon, UAG, at the position of the sixth amino-acid, a glutamine residue, in the R17 coat protein molecule. In a cell free system the mutant RNA directs the synthesis of a small N-terminal peptide of the coat protein with the sequence N-formylmet-ala-ser-aspn-phe-thr; the next amino-acid in the coat protein is glutamine, but with the mutant RNA the amber codon causes premature termination of synthesis and the release of the hexapeptide. The great virtue of this system is that the unterminated peptide still attached to the peptidyltRNA can be distinguished from the terminated peptide released from the tRNA and the amounts of each measured. Furthermore, starving the system for any one of the six amino-acids in the peptide arrests the translation machinery before it reaches the UAG codon so termination can be controlled.

Exploiting this procedure, Capecchi isolated a complex of ribosome, mRNA and pentapeptide

attached by the phenylalanyl tRNA simply by starving for threonine. With this he has been able to define the conditions necessary for termination; incubating the complex under suitable conditions with or without threonine or supernatant enzymes showed that to get release it is necessary but not sufficient to complete the peptide by incorporating threonine and that release is not solely dependent on the transfer enzymes required to move the ribosome from the threonyl codon to the UAG terminating codon. Release does, however, depend upon the presence of a supernatant factor. This factor, with a molecular weight of between 40,000 and 50,000, sediments between 3.5S and 4.5S. Because its releasing activity is not affected by RNase, it cannot be an RNA-protein complex. Apparently the substrate of the release enzyme is the ribosome-mRNA-peptidyl tRNA complex so that it must act directly at the ribosome and not cleave the tRNA from peptidyl-tRNA already released into the supernatant, which was the other possibility.

Capecchi does not yet know whether the release enzyme works alone or in conjunction with other factors, in particular a hypothetical chain termination tRNA which reads the termination codon but does not carry an amino-acid. Many people, including Capecchi, have searched in vain for such a tRNA species, and although negative results can never exclude the possibility that such tRNA exists it is reasonable to think about alternative models in which the terminating codons are read either by the ribosome or the release enzyme or in which nonsense codons function simply because they cannot be read at all. Further characterization of the release enzyme could well decide the issue.

Chlorinated Hydrocarbons in British Wildlife

by D. C. HOLMES J. H. SIMMONS J. O'G. TATTON

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Polychlorobiphenyl compounds have been detected in British wildlife. In birds' livers and eggs they are often in greater quantities than organochlorine pesticide residues. Polychlorobiphenyls are known to be toxic, and their detection in wildlife raises the question of the adverse effect they may have.

When wild birds' eggs or the livers of British wildlife are examined by gas-liquid chromatography (GLC) using electron-capture detection, peaks corresponding to such organochlorine pesticides as BHC, dieldrin and DDT, and their metabolites and breakdown products, often appear on the resultant chromatograms. These peaks sometimes represent amounts which are significant from a toxicological point of view, but the sensitivity of modern instruments also enables completely insignificant amounts to be detected. Analysts concerned with the determination of organochlorine pesticides in wildlife, however, have long been aware that it is not unusual for an additional series of as many as ten peaks, corresponding to unidentified compounds, also to appear on these chromatograms¹⁻⁴. Little is known of the structure and origin of these compounds, but Roburn¹ established that they are organo-

chlorine in nature, with a substantial chlorine content.

and consequently possess electron-capturing properties.

On silicone or 'Apiezon' GLC columns, the series of peaks for these compounds normally begins at the point at which pp'-TDE and pp'-DDT are emerging from the columns and extends, in terms of retention times, to four or five times that of pp'-DDT, which is the last of the commonly occurring pesticides to emerge.

Before examination by GLC, samples have normally been subjected to a fairly rigorous clean-up procedure, such as that of de Faubert Maunder et al.⁵, which includes a dimethylformamide—hexane partition and passage through a column of prepared alumina. Because these unknown compounds are similar in nature and properties to the organochlorine pesticides, they also pass through this and similar clean-up procedures; consequently, they

often interfere seriously with determinations of pp'-TDE and pp'-DDT in wildlife because of overlapping of the chromatographic peaks. Suitable procedures have been devised to overcome this difficulty of interfering peaks. One method is to effect a better resolution of these particular peaks by choice of a suitable stationary phase for GLČ6. Another method, which also demonstrates the presence of these compounds in the sample, is the use of a preliminary thin-layer chromatographic treatment?. The cleaned-up extracts are examined on silica gel chromatoplates using a 1 per cent solution of acetone in hexane as mobile phase2. Spots corresponding to the compounds can then be found at R_F values between 0.8 and 1.0, whereas the commonly occurring organochlorine pesticides and their metabolites produce spots of lower R_F values. A similar separation can also be shown with reverse phase paper chromatography⁸ whereby the unknown compounds move only a very short distance from the base line compared with the pesticides and their metabolites.

Gas-liquid chromatography using dual channel detection systems with separate electron-capture and flame-ionization detectors has also been used to demonstrate the presence of large amounts of weakly electron-capturing material, in addition to known pesticide residues, in the eggs of oyster catchers (Haematopus ostralegus)⁹.

Experience in this laboratory has shown that these compounds occur most frequently and in the largest proportions in the livers, fat and eggs of birds, particularly terrestrial predators such as sparrowhawks (Accipiter nisus) and kestrels (Falco tinnunculus) and marine feeders such as guillemots (Uria aalge) and kittiwakes (Rissa tridactyla); they also commonly occur in freshwater fish. We have detected small amounts in human and animal fat samples, but less frequently and in much smaller proportions than in avian samples.

A similar series of chromatographic peaks has been observed by Holden^{10,11} when examining extracts of seals and fish taken in Scottish waters. He found that the peaks given by the extracts of fresh and sea-water fish represented fairly low concentrations, but those given by extracts of seal blubber corresponded to much larger proportions, of the order found by this laboratory in birds' eggs.

Despite considerable curiosity about the identity of these compounds, little progress has been made in identifying them, but there has long been a general supposition that they were either further breakdown or condensation products of the organochlorine pesticides or possibly loose compounds of these with natural products such as protein matter.

Jensen, however, succeeded in identifying a series of such peaks as corresponding to polychlorobiphenyl compounds, in 200 pike taken in different parts of Sweden, and in an eagle¹². We have now been able to show that the long retention time compounds occurring in British wildlife are also polychlorobiphenyl compounds.

Polychlorinated biphenyl has been in common commercial use for some time as a plasticizer in paints, resins and plastics. It also has electrical insulating properties and can be used for a number of other protective purposes. Commercial preparations of this material are usually graded according to the degree of chlorination, but all of them are substantially complex mixtures of polychlorobiphenyl compounds that could be expected to resemble the organochlorine pesticides in their chemical and physical

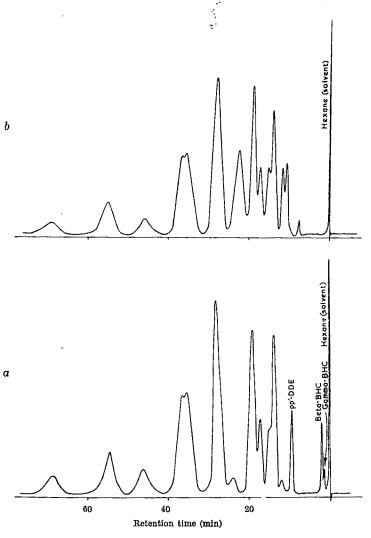


Fig. 1. Gas-liquid chromatogram of (a) extract of kestrel liver and (b) a commercial polychlorobiphenyl resin.

properties. In the prescribed extraction and GLC conditions, these mixtures produce a series of chromatographic peaks of the type in question.

The compounds occurring in British wildlife correspond to the more highly chlorinated biphenyl compounds rather than the more lightly chlorinated types. These highly chlorinated biphenyls also occur to some extent in the analogous polychloroterphenyl preparations which have commercial uses similar to those of polychlorobiphenyl. It is possible that some of the compounds with very long retention times occurring in British wildlife are lightly chlorinated polychloroterphenyl compounds.

Fig. 1a shows the gas-liquid chromatogram of an extract from a kestrel liver. The peaks corresponding to pp'-DDE (0.43 ng), beta-BHC (0.33 ng) and gamma-BHC (0.03 ng) are labelled. The other peaks form the series referred to above and until now would have been regarded simply as "unknown organochlorine compounds not corresponding to any known pesticide or its metabolites". Fig. 1b shows the analogous chromatogram of a commercial polychlorobiphenyl resin. So far as retention times are concerned and apart from the pesticide peaks, there is exact matching for all but a few peaks in the two chromatograms.

The chromatograms shown were obtained by use of a silicone gum (GE-SE 52) column, but this precise correspondence between the retention times of the peaks for the unknown compounds and those from the polychlorobiphenyl resin has also been demonstrated on 'Apiezon L'

Table 1. RELATIVE RETENTION TIMES ON THREE DIFFERENT GLO COLUMNS (Excluding times for known pesticides)

Silicone 6			1 = 1.00) $zon L'$ $zon n$	Cyanos GE-XE 60	
KLE	PCB	KLE	PCB	KLE	PCB
1·42 1·73 1·91 2·81 3·59 3·71 4·68 5·62 7·10	1·42 1·73 1·90 2·81 3·58 3·70 4·68 5·62 7·11	2·31 2·67 3·04 5·06 5·96 6·57	2·30 2·67 3·05 5·06 5·95 6·56	1·21 1·34 2·20 2·62 2·97 3·60 4·63 5·11	1-21 1-33 2-19 2-60 2-96 3-60 4-63 5-11

KLE, Kestrel liver extract; PCB, polychlorobiphenyl resin.

and cyanosilicone columns. The actual retention times recorded for the peaks on these three types of column are given in Table 1.

Further confirmation as to the identity of these compounds has been obtained by a study of their behaviour on thin-layer chromatoplates, alumina and silica gel absorption columns and reverse phase paper chromatograms. In addition, these compounds have been shown to resemble the polychlorobiphenyl compounds in their chemical inertness. Thus they are not easily modified by simple chemical reactions to produce readily identifiable shifts in their retention times on GLC as can be done, for instance, to pp'-DDT by hydrolysing it to pp'-DDE or to aldrin by oxidizing it to dieldrin.

A number of livers and eggs taken from birds in the British Isles or their coastal waters have been examined in this laboratory with results similar to those obtained for the kestrel liver referred to earlier. While the identification of these compounds in British wildlife may solve the mystery of these peaks on GLC chromatograms, it raises, however, many other questions. Why, for example, do these compounds seem to be accumulated, most frequently and in the largest proportions, in certain types of wildlife such as birds, seals and fish, and only at the most in very small proportions in man or his domestic animals such as cows or sheep? In this respect they seem to differ markedly from the organochlorine pesticides which seem to have invaded nearly all aspects of our environment and to which they bear a strong resemblance chemically and physically.

Some of the samples of birds examined in this laboratory have seemed to contain higher proportions of polychlorobiphenyl compounds than organochlorine pesticides. For example, the peaks on the chromatogram for the kestrel liver shown in Fig. 1 were estimated to be equivalent to about 12 ng of polychlorobiphenyl compounds. must be contrasted against the organochlorine pesticide residues listed above. These total only about 0.8 ng of which 0.33 ng is beta-BHC, an isomer of BHC which is generally regarded as non-toxic to wildlife.

Great concern has often been expressed by conservationists about the effects of pesticides on wildlife, particularly birds. The identification of these other organochlorine compounds in wildlife prompts enquiries as to the possible toxic effects they may be having. That these compounds are toxic is generally agreed^{13,14}; the polychlorobiphenyls are in fact regarded as an industrial hazard and threshold limits for them in air have been advised16.

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Quasi-stellar Sources, Supernovae and Novae

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By considering the opacity of a surface layer of a star, the author concludes that quasi-stellar sources, supernovae and novae may be different manifestations of the same mechanism.

Ir is usually considered that the outbursts of novae and supernovae are fundamentally different processes, but I shall here propose a mechanism which can account for both phenomena, and which at the same time can account for other problems concerned with quasi-stellar sources (quasars). In particular, I suggest that these outbursts are likely to be anisotropic (as indeed they are observed to be for novae). Because the energy released during a supernova outburst is comparable with the stellar rest energy, an anisotropic outburst could mean that the star is accelerated to relativistic velocity in order to conserve momentum, thus affording a Doppler explanation of the large red-shifts of the quasi-stellar sources.

Consider the opacity of a layer, L, at the surface of a star. Let ΔR be the thickness and R the radius of L, and let L contain plasma at temperature, T, with electron and positive ion densities N_t and N_t respectively. L will be opaque to radiation of wavelength λ provided that τ is about I, where τ is the optical depth of L. A major contribution to τ is τ_{ff} , the optical thickness due to free-free absorptions. It is known that $\tau_{ff} \propto \lambda^2 T^{-3/2} N_e N_f \Delta R$ (ref. 1). where induced emissions are included. Suppose, now, that L is compressed or dilated without changing the total numbers of ions; that is, ΔR is varied under conditions which maintain constant $4\pi R^2 \Delta R N_e$, so that $\Delta R N_e =$ constant. Evidently $\tau_{ff} \propto N_e N_i \Delta R \propto N_i \propto 1/\Delta R$. Thus compression of L increases its opacity, while dilation reduces the opacity.

Of course, the total number of ions will not remain constant in practice, because the recombination rate is proportional to N_eN_i . If the percentage of atoms ionized is large, the density of unionized atoms, N, will therefore be proportional to N_cN_i . This means that the contribution to the opacity made by bound-bound and bound-free transitions within unionized atoms is also proportional to $N_e N_i \Delta R$. This effect, recognized to be important for stellar pulsation^{2,3}, further increases the opacity of L on compression.

Some consequences of this variable surface opacity will now be examined. Under equilibrium conditions the luminosity of the star, S, must equal the power, P, which is generated by thermonuclear processes in the interior. The temperature of L will then be such that it radiates as much power as it absorbs. At this temperature and thickness, L has its equilibrium opacity. If, now, L is compressed, the decrease of ΔR increases the opacity of L, thus partially stemming the flow of radiation from the interior. As a result, S decreases below P, leading to an increase with time of the radiation density (and hence the temperature) inside the star. How is this situation relieved?

Ultimately L could regain its equilibrium opacity by absorbing heat from the interior (adiabatic heating during compression is quite insufficient). For radiation of wavelength λ , the rise in temperature of L would have to be such that $T^{-3} {}^{12} \Delta R = \text{constant}$; the temperature increase must be even greater if we consider a change of equilibrium opacity to the total radiation (all λ) which is proportional to $T^{-3.5}$ (ref. 4). Before, however, the opacity can return to equilibrium by this mechanism, which will be comparatively slow, the build-up of radiant energy within the star is likely to cause other changes.

The rise in temperature in the interior of the star, following the partial damming of the radiation flux by L, will in most cases cause thermal expansion of the whole star. L will be thrown outward, probably rather violently. Before it can fall back under gravity ΔR will increase, thus reducing the opacity of L and allowing the trapped radiation to escape. The interior now cools, so that the star contracts, and L returns to its original state of greater than equilibrium opacity. The cycle then recommences. L behaves as a valve for the control of the outflow of radiation, alternately opening a little and closing a little. Thus a pulsation is initiated and maintained by thermal energy. This process is well recognized^{2,3}; what is new is the important contribution made by free-free absorptions to the variable opacity of the surface layer.

A thermally driven pulsation, by its very nature, is likely to be rather irregular. The erratic light variations of the irregular variables can be thus explained. In order to account for the exceedingly regular variations of the cepheids and long period variables the thermal pulsation must be synchronized to a natural acoustical mode of the star; the occurrence of resonance between the thermal pulsation and a natural mode would both regularize and amplify the pulsation. The success of Eddington's calculations of the periods of pulsation is then understandable. The resonance condition could also account for the very sharp onset and cessation of pulsation in the H-R diagram.

The following observations lend strong support to the theory. (a) Maximum luminosity would be expected at the phase when L becomes transparent, which will be about one quarter of a period after maximum contraction and about the time when the outward velocity is a maximum. This is observed in cepheids. (b) As $\tau_{ff} \propto \lambda^2$, L will become transparent to short \(\lambda \) before long \(\lambda \). Thus, a progressive retardation of phase of the light curve with increasing λ is expected. This is observed in both cepheids and long period variables. (c) The amplitude of the light curve should be very much greater for wavelengths such that τ is about I than for longer or shorter wavelengths. Longer wavelength radiation will originate within L itself, and will not therefore be subject to valve Shorter wavelength radiation will not be appreciably absorbed by L and will not therefore be affected by any change in L's opacity. This effect is particularly marked in the long period variables.

(d) Particularly convincing are suppression phenomena observed in certain stars whose surface layer, L, happens to be subject to sporadic changes. The 387 day oscillation of luminosity in R Aquarii is sometimes suppressed for several years, during which time the star appears bluer and shows all the spectroscopic evidence of high temperature. Payne-Gaposchkin' mentions that an inner layer is probably exposed at such times as a result of dispersal of

surface absorbing material. The fact that the 387 day oscillation disappears with the dispersal of this materia indicates that the surface absorption is primarily respon sible for the oscillation. VV Pup, an RR Lyrae variable with a 100 minute oscillation, shows similar behaviour. In this case, however, the surface layer is not dispersed but thickened, or at least rendered too opaque to act as an effective valve, because the disappearance of the oscillation coincided with dimming of the star by 3 magnitudes; the oscillation eventually reappeared with continuity of phases. In RU Cam, a cepheid with an unusual phase relationship between the light and radial velocity curves, the variability has apparently disappeared altogethers.

Suppose, now, that the star is partially degenerate. Within a degenerate star (which is density ionized) the pressure is exerted by that relatively small fraction of the electrons which occupy states near the top of the Fermi distribution. As pressure is proportional to N_ckT , the smallness of N_c and the largeness of the pressure imply an exceedingly large T. In the core of such a star pressure is exerted by a low density of extremely high temperature electrons. Because most electrons cannot make transitions to unoccupied states and because the probability of transitions is very small for the small percentage that can (due to large T), a degenerate core has very low opacity and high thermal conductivity. The star consists of an isothermal core at high temperature overlaid by a thin stratum of non-degenerate gas which supports the entire temperature gradient.

Should the surface layer of such a star develop an opacity greater than the equilibrium opacity, the radiation density within the star will build up without an associated increase of temperature. The unstable situation cannot now be relieved by thermal expansion as in the case of the pulsators. I suggest that the internal radiation density increases gradually until radiation pressure is sufficient to blow away the opaque skin layer, L. This will bring about a sudden and very large increase of luminosity, which is sufficient to account for novae and supernovae. The increase in luminosity for ordinary novae is a factor of 105, and for type I supernovae 108. The broad spectrum of nova phenomena (the energies liberated vary from 10³⁸⁻⁵ erg for dwarf novae, through 1045 erg for ordinary novae, to 1048 erg and 1051 erg for types II and I supernovae) can have a common explanation in terms of a sharp reduction of surface opacity.

Spectroscopic evidence indicates that about 1028 g of material is expelled in a nova outburst. In order that radiation pressure should be able to lift off this mass against gravity (for a star of mass $1 M_{\odot}$ and radius $0.1 R_{\odot}$), the effective internal temperature must rise to 7×10^8 ° K. This will be the temperature of the exposed sub-layer immediately after the removal of L. If the initial effective temperature was 50,000° K, then it is a simple consequence of Stefan's law that the bolometric magnitude increases by $\Delta m_{bol} = -25$. This is probably very reasonable in relation to the observed increase in the photographic magnitude, namely $\Delta m_{pg} = -12.5$. Standard bolometric corrections cannot be applied, because at X-ray wavelengths the layer, L, is probably optically thin, a conclusion supported by the recent observation of X-rays from such a star¹⁰. In the case of a supernova it might be assumed that $\Delta m_{bol} = -30$ as $\Delta m_{pg} = -19$ is observed. This would imply that the effective temperature at the inner surface of L is 10^3 times that at the outer surface. The corresponding radiation pressure would be sufficient to lift off 1.5×10^{31} g.

The spectroscopic properties of novae are now easily understood: (i) Kopal¹¹ finds it difficult to understand why the largest violet shifts should be recorded for absorption lines rather than emission lines. As the temperature of the effective photosphere falls by a factor of only five or six while the radius expands by a factor of 1,000, Kopal argues that the emission must come from a shock front which preserves a high density. It is this conclusion that

introduces the absorption line problem. The present theory indicates that the broad emission comes from the underlying star, and that the most rapidly expanding material, at least initially, is the absorbing shell L.

(ii) Following the departure of L, there will be a strong stellar wind because of the large temperature gradient. Initially, this wind will be a fully ionized plasma which emits only a continuum. On cooling sufficiently for recombinations to occur, a spectacular change to a line spectrum is expected. This is observed in the emergence of the principal spectrum.

of the principal spectrum.

(iii) The impact of the stellar wind on the more slowly expanding shell, L, causes further spectroscopic changes. The diffuse enhanced spectrum of short duration bears evidence of the impact. A shock front caused by the impact may be reflected back and forth between L and the star, accounting for periodic dips in the light curve and

for oscillatory emission line shapes12.

The time scale for rise to maximum will be the time scale for L to become transparent. For novae the rise time is about a day, and because the expansion velocity of L (as estimated from the premaximum absorption spectrum) is of order 1,000 km/sec the shell evidently expands to $100\,R_\odot$ (a factor of 1,000) before becoming wholly transparent. Such a large expansion indicates that it is expelled as a shock front. The time scale for decline will be the time scale for the formation of a new surface layer, L, of high opacity, and will therefore depend on the rate of cooling. Decline times are observed to vary greatly; fast novae decline by 3 magnitudes in about 30 days, slow novae in about 200 days.

If the opacity of the newly formed surface layer is greater than the equilibrium opacity, the radiation density will again build up within the star, and the outburst will recur. Thus, the recurrence of novae is

easily explained.

The opaque skin layer, L, is unlikely to be of uniform thickness and density. Rotation, or surface magnetic fields, would disturb the spherical symmetry. Radiation pressure is therefore more likely to puncture the shell (as a bursting balloon) than to expand it uniformly; a window may be blown out of L at one or more localized regions. The patchy nature of the nebulosity surrounding old novae, and the knots which appear in the Doppler ellipses in spectrograms taken with the slit across the source¹², leave no doubt that many nova outbursts are anisotropic. This anisotropy is of the greatest significance in the following theory of quasi-stellar sources.

If some fraction of the radiant energy emitted during the outburst of a nova or supernova is anisotropic, then in order to balance the resultant momentum of the radiation, the star must acquire a recoil velocity. If $F(\theta, \varphi)$ is the flux through the surface in a direction defined (relative to the direction of resultant momentum) by the polar angles, θ and φ , then an anisotropic luminosity, S, can be defined by $S = R^2 \iint F(\theta, \varphi) \cos\theta \sin\theta d\theta d\varphi$. The energy liberated anisotropically is $E = \int S dt$. The resultant momentum of the radiation is E/o, and the recoil velocity of the star (mass M) is v = E/Mc. This velocity will be relativistic if $E \sim Mc^2$. For $M = 1M_{\odot}$, this implies $E \sim 1.8 \times 10^{54}$ erg. According to Zwicky13 the surface temperature at the initial stages of a supernova may reach 3 × 106 °K, and the total radiant energy liberated will lie in the range 1051-1055 erg. Thus, even neglecting the momentum carried off by expelled matter, it is possible for such stars to be accelerated to relativistic velocity.

A star which has been accelerated to a relativistic velocity will soon escape from the Galaxy. If the frequency of occurrence of supernovae is of order 1 per galaxy per 400 yr, and if the quasi-stellar sources are old supernovae now receding from the Galaxy with relativistic velocity, then in order to obtain say 10^5 quasi-stellar sources we must go back in time 4×10^7 yr and therefore out in distance 3–10 Mparsec. Another limitation on distance is imposed by the absence of detectable proper motions¹⁴.

If x is the perpendicular distance from the Earth to the trajectory of a quasi-stellar source whose distance is now d and velocity v, then the component of velocity transverse to the line of sight is xv/d, which corresponds to an angular motion of $\theta = 6.5 \times 10^{12} vx/d^2$ sec of are/yr. If x > 1 kparsec and $\theta < 0.0025$ sec of are/yr, one deduces d > 100 kparsec for v = 0.37 c.

The present flux from 3C 273 in the visible range is 1.3×10^{-10} erg sec⁻¹ cm⁻² (ref. 15). At a distance of 100 kparsec this implies a luminosity of 1.5×10^{38} erg/sec which is of the order of the luminosity of novae during outburst. Actually, 3C 273 is perhaps slightly exceptional; at the same distance most quasi-stellar sources would have only about one hundredth of 3C 273's luminosity of 3C 2

inosity.

The persistence of high luminosity suggests that there may be a new type of nova outburst—a stabilized nova. Suppose, for example, that the opaque skin layer, L, were punctured by a tube of magnetic flux, creating what is essentially a window to the hot interior. circumstances the cooling of the interior might become sufficiently slow to be balanced by the internal radiation build-up; by concentrating all its luminosity through the window in L the star may be able to keep this window open—any tendency for L to reform at the leak would be opposed by a strong stellar wind. The existence of objects like y Carinae, which remained at maximum for 20 years, gives plausibility to the suggestion. Assuming a constant luminosity confined to solid angle, $\delta\Omega$, the constant acceleration of the star will be S/Mc. Its red-shift after time t will be $z = St/Mc^2$, and when its red-shift is z its distance will be $d=z^2Mc^3/2S$. The total flux at Earth will be $f=z^2Mc^3/2d^3\delta\Omega$. Knowing f, z, and $\delta\Omega$, d can be deduced. For 3C 273 substitute $f = 1.3 \times 10^{-10}$ erg sec⁻¹ cm⁻² (neglecting flux outside the visible), z=0.158, and $\delta\Omega = 0.1$ (an assumption), which gives d = 1.2 Mparsec.

The stabilized nova hypothesis has other attractive consequences. (i) The red-shift builds up gradually and becomes large only at large distances. This introduces a correlation between red-shift and apparent magnitude, for which there is evidence 16.17. (ii) As the sources continue to radiate anisotropically, they will be visible only when viewed from the galaxy from which they have originated; thus large blue-shifts for quasi-stellar sources which have been emitted from a nearby galaxy have not been observed simply because they are not visible to us.

In two respects this theory is very successful. The first success is a natural explanation for the smaller red-shifts of absorption lines relative to emission lines in many sources¹⁸. The differences of red-shifts imply that absorbing material is escaping from the sources with velocities usually found only in novae or supernovae, for example 1,000 km/sec for 3C 191, 3,300 km/sec for PKS 0119-04, 17,000 km/sec for PKS 1116+12, 25,900 km/sec (and also 1,950 km/sec) for PKS 0237-23, and 33,000 km/sec for Ton 1,530. The expulsion of the material may be the result of minor outbursts; these have been witnessed (within the small time scale of our observations) in 3C 446¹⁹⁻²⁰, in 3C 345²⁷, and in 3C 273²⁸ (during 1927-29). These outbursts show that activity is still present, thus favouring the stabilized nova over the supernova hypothesis.

The second major success is that the variability of the quasi-stellar sources is accounted for, and would in fact be expected. The variability is remarkably similar to that found in old novae^{29,30}, a fact noted relatively early by Matthews and Sandage³¹. All fluctuations could be attributed to variable surface opacity. The rapid small amplitude variations (flickering) might be due to convection in the opaque skin layer of a partially degenerate star, because, as has been shown, such a layer has a very large temperature gradient. The more drastic outbursts mentioned above may have the same explanation as the outbursts of a dwarf nova (see text). It seems significant that the peculiar colour of the quasi-stellar sources, so important for their identification, should be matched only by the

colour of SS Cyg and U Gem eruptive variables (dwarf novae)32. The frequent association of faint nebulosity with quasi-stellar sources31,33 is also understandable in the nova theory.

To conclude, I would draw attention to what seems to be a satisfactory derivation of the distance of 3C 273. Burbidge et al.³⁴ have given evidence for broadening of the emission lines in 3C 273 by electron scattering; the only competing theory would be an expanding nebula with a large velocity gradient (3,000 km/sec between inner and outer edges for a 50 Å line width). This Doppler explanation of line broadening cannot really be excluded in view of the likelihood of a strong stellar wind, and the evidence for large expansion velocities provided by the absorption lines in some sources. If, however, an optical depth to electron scattering of 0.8 is adopted, as suggested by Burbidge et al., then $N_e R_{neb} \simeq 10^{24}$ cm⁻², where R_{neb} is the radius of the nebula. It is very likely that the line emissions from 3C 273 arise in the nebula around the central source—in 3C 279 and in 3C 446 the line emissions do not fluctuate with the continuum23. From the presence of [OIII] Greenstein and Schmidt³⁵ infer $N_e \le 3 \times 10^7$ cm⁻³; from the presence of FeII but absence of [FeII] Wampler and Oke¹⁵ infer $N_e > 10^7$ cm⁻³ and probably $N_e \ge 10^8$ cm⁻³. If $N_e = 10^8$ cm⁻³ is adopted, then $R_{neb} = 10^{16}$ cm. temperature of the nebula is probably no greater than 10^4 $^{\circ}{\rm K}$, as otherwise the absence of CII $\lambda 1335$ when MgII λ2798 is present would be difficult to explain³⁴. With this temperature the upper level of the H β line has 0.1 times the equilibrium population³⁶. Then, in order to obtain the observed flux of H β emission, $3\cdot4\times10^{-12}$ erg sec⁻¹ cm⁻², one requires that $N_e^2R_{neb}^3/d^2=4\cdot5\times10^{14}$ cm⁻⁵. With the above values for N_e and R_{neb} it can be deduced that d=1.5 Mparsec. This is in remarkably close agreement with the distance derived from the stabilized nova hypothesis. Burbidge et al.34 give such an argument, but it does depend on the electron scattering explanation of line broadening. If, however, the optical depth to electron scattering is not as great as 0.8, then d < 1.5 Mparsec.

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Acid Conversions of Hydrazoarenes

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In a historical review combined with a consideration of their new work, the authors discuss rearrangement and disproportionation in the acid conversion of hydrazoarenes with particular reference to the one- and two-proton mechanisms.

THE acid-catalysed conversions of hydrazoarenes comprise the isomeric changes, collectively known as benzidine rearrangements, by which hydrazoarenes give variously named diaminobiaryls and aminodiarylamines, as shown Together with these isomerizations, reactions of disproportionation may occur: some of the hydrazo-compound becomes reduced to aniline bases (fission amines) and some oxidized to the azo-compound

$$\begin{array}{c}
NH_{2} \\
A \\
NH \\
O - Semidine
\end{array}$$

$$\begin{array}{c}
NH_{2} \\
B \\
O - Benzidine
\end{array}$$

$$\begin{array}{c}
NH_{2} \\
B \\
NH_{2} \\
A \\
NH \\
Diphenyline
\end{array}$$

$$\begin{array}{c}
NH_{2} \\
B \\
NH_{2} \\
B \\
Diphenyline
\end{array}$$

$$\begin{array}{c}
H_{2}N \\
A \\
B \\
B \\
B \\
Diphenyline
\end{array}$$

$$\begin{array}{c}
NH_{2} \\
B \\
Diphenyline
\end{array}$$

$$\begin{array}{c}
NH_{2} \\
B \\
Diphenyline
\end{array}$$

$$\begin{array}{c}
NH_{2} \\
B \\
Diphenyline
\end{array}$$

$$2 \text{ A}_{NH\cdot NH} \text{ B} \longrightarrow \begin{cases} \text{A}_{NH_2} + NH_2 \text{ B} \\ \text{A}_{N=N} \text{ B} \end{cases}$$

The mechanism of the rearrangement has been debated for more than 60 years1. The primary difficulty has been to understand the drastic stereochemical convolutions that some of them involve. How does a hydrazobenzene turn itself inside out in its conversion to a benzidine without falling to pieces? The mechanism of disproportionation has been widely suspected to be dependent on that of rearrangement, and thus to present the second of two problems that have to be solved successively.

The first theories of the rearrangements, those of Tichwinsky and Stieglitz, both of 1903, assumed that the hydrazo-compound was split by the acid into independent fragments, which transferred their unsaturated bonds to other positions before they recombined. Tichwinsky assumed homolytic fission, and Stieglitz heterolytic fission. After 1911, when Wieland demonstrated extensive homolysis without benzidine rearrangement in tetra-arvlhydrazines, the theory of homolytic dissociation fell into disfavour. Before and in 1922, Jacobson showed, as others have since done more quantitatively, that ABtype hydrazobenzenes give only AB-type, and not AA-or BB-type, benzidines. Such demonstrations exclude homolytic dissociation, but they do not exclude heterolytic dissociations, because an unsymmetrical hydrazobenzene could break into parts whose charge distinctions would ensure that the same parts recombined. In 1933, however, Kidd and one of us observed, as Wheland and his co-workers have done since with greater precision, that mixed symmetrical hydrazobenzenes give only symmetrical rearrangement products. non-crossing in that form excludes all mechanisms based on dissociation, and from about that date it has been generally accepted that benzidine rearrangements are basically intramolecular. Thus after 30 years the escape route of dissociation from the original stereochemical difficulty became firmly closed.

Of the several theoretical approaches since tried, the most successful is that of the polar transition state. This idea was introduced by Hughes and one of us in 1941, and has been developed since, notably by the incorporation of a suggestion by Hammick and Mason in 1946. The only intermediate compounds which this theory assumes have ordinary benzenoid or quinonoid bonding: they are like the factor or the products, but with extra protons. But between such intermediates the theory provides transition states having at least two bonds of strongly polar character. This strong electrostatic bond content will confer on the bonds greater lengths and very much lower bending force constants than those possessed by ordinary bonds, characteristics which can be shown to permit energetically cheap shape changes drastic enough to meet the stereochemical requirements. The electrostatic bond character will shift as the system passes over its transition state, old bonds being finally broken in electrostatic form, and new bonds introduced electro-statically, to pass into covalencies as they shorten and stiffen.

Concerning the role of catalytic protons, van Loon showed in 1904, as Hammond and Shine did more precisely in 1950, that the rate of rearrangement of hydrazobenzene in an aqueous organic solvent is quadratic in hydrogen ions, that is, the rearrangement involves two catalytic protons. The same result was subsequently obtained by others for the conversions of a number of substituted hydrazobenzenes. This work of generalization threw up one anomalous result, obtained by Carlin and Odioso in 1954, namely an apparent order in acid of 1-6, instead of 2, in the rearrangement of o-hydrazotoluene.

Between 1962 and 1964 results were recorded²⁻⁴ which established the existence of independent, but occasionally concurrent, two-proton and one-proton mechanisms; isolated the one-proton mechanism in several examples; and established the intermediates of the early and late stages of both these mechanisms. It was shown that the additions of all catalytic protons, even the second when two are involved, are in pre-equilibria, that is, they are low-energy processes occurring near the beginning of the reaction path. It was also shown that those losses of aromatic protons which admit the formation of new bonds

are irreversible low-energy processes belonging to the end of the reaction path. The events of the central high-energy region of the reaction path, the region of transition states, could then be hypothetically but consistently described by the polar transition state theory. The same work brought the slow uncatalysed rearrangements of hydrazoarenes in polar solvents into the same family of rearrangement mechanisms.

The next steps were to establish more firmly the nature of the high-energy events in the acid-catalysed mechanisms and to define more closely the range of conversions, whether of isomerization or disproportionation, to which the descriptions given of them apply. These objects have now been advanced by the study of kinetics and products in correlation for the acid conversions of a selection of hydrazobenzenes, having substituents of known polar and steric quality.

Kinetic Forms of the Acid Conversions

The approach, in 1962, started by asking why the kinetic anomaly of o-hydrazotoluene had remained unique and why persistent attempts over 8 years to find a second example of an order in acid of less than 2 had failed. The tentative answer was that the polar transition state theory had not guided these attempts, though it tells one where to look for other low orders in acid, namely among hydrazobenzenes with substituents which supply electrons to their phenylimine rings and weaken an aniline base (by impairing charge solvation). Either factor promotes polarization of the protonated NN'-bond, and, if strong enough, could act sufficiently to do so without help from the other; but the two factors collaborate in the o-methyl substituent, and hence any other substituent in which both effects are stronger could be predicted to reduce kinetic order more effectively. The following two series of orders of effectiveness of aryl groups in hydrazoarenes were thus predicted:

α -naphthyl > β -naphthyl > o-tolyl > phenyl o-anisyl > o-tolyl > phenyl

The first of these series was experimentally verified in 1962. Two α -naphthyl groups gave pure, and two β -naphthyl nearly pure, one-proton kinetics; and two α -tolyl groups gave transitional kinetics of the quantitatively correct form^{2,4} for concurrent mechanisms.

Table 1. Kinetic orders in hydrogen ion for conversions of hydrazobenzenes by perchloric acid in 60 per cent aqueous dioxan

Substituents in Ph groups	Range of [H+] studled	Order* in H+	Notes
2-MeO, 2'-Me()	0.0001-0.05	1	
2-MeO, -	0.002-0.3	1.1-2.0	a
4-MeO, —	0.000,007-0.005	1	
4-NHAc,	0.007-0.1	1	
2-F, 2'-F	0-10-8	2	b
2-Cl, 2'-Cl	0-8-2-8	2	
4-Cl, 4'-Cl	0.1-1.0	2	
4-Cl, -	0.07-1.0	2	
2-Br, 2'-Br	0.2-2.0	1.2-1.9	b c
4-Br, 4'-Br	0-1-0-5	2	ь
2-I, 2'-I	0.7-1.6	1	1. d
4-I, 4'-I	0.05-0.5	~2	b
2-Ph, 2'-Ph	0.9-1.6	2	
4-Ph,	0.0040.6	2	
4-NO ₂ ,	2.0-4.0	2	

^{*} When using ranges of acidity rising to above 0.3 N, we replace [H+] by h_0 in the reckoning of kinetic orders.

a, Experiments by J. Roy. The apparent kinetic order rose with acidity, in accordance with the rate equation for concurrent independent mechanisms.

b. Experiments by M. O'Sullivan.

c. Variation of order as in note a.

d, Hydrochloric acld was substituted for perchloric acid.

Verification of the second series is contained in the subsequent results summarized in Table 1. One o-anisyl group acts like two o-tolyl groups in producing transitional kinetics. Two o-anisyl groups give one-proton kinetics.

In several of the examples in Table 1, we see the two factors of NN'-polarization acting independently. A single p-anisyl group, and a single p-acetaminophenyl group, give pure one-proton kinetics, because the substituents in these groups release electrons sufficiently strongly from that position (para) which is shown by the detailed theory of the polar transition state to be the most effective⁴. Two o-bromophenyl groups lead to transitional kinetics, and two o-iodophenyl groups to pure one-proton kinetics, because the substituents, increasingly in this order, reduce base strength by impairing charge solvation. In each of these complementary types of case, the second of the possible factors exerts either a weakly countervailing or an undecided effect.

Rearrangement Products

The now considerable area of agreement of the kinetic findings with the picture presented by polar transition state theory sufficiently increases our confidence in the theory to allow the question to be pursued of how widely its descriptions apply to the formation of the various types of products. The new studies of products formed in kinetically defined conditions provide evidence on the matter.

Table 2. products of conversions of hydrazobenzenes by perchloric acid in "60 per cent" aqueous dioxan

Substituents in Ph groups	Order in H+	4,4'- linked	2,4'- linked	2.N'- linked	N,4'- linked	Dispropor tionation
2-MeO, 2'-MeO	1	95	-			5
2-MeO,	1, 2	~100				+
4-MeO,	1			55	24	20
4-NHAc,	1		+*‡	+++*	++‡	70
2-F, 2'-F	2	86				14
2-Cl, 2'-Cl	2	94	-			6
4-Cl, 4'-Cl	2		*****	22		75
4-Cl,	2	parent,	~19	30	20	31
2-Br, 2'-Br	1, 2	95	-		******	5
4-Br, 4'-Br	2		******	~ 30		~ 70
2-I, 2'-I	1	100		******		
4-I, 4'-I	~2	*******				~100
2-Ph, 2'-Ph	2	90	*******			10
4-Ph,	2		+++*	+++†		38
4-NO ₂ , -	2		+++	+++†	~20	~40

(The figures are percentages. Jacobson's signs are used, as before', for major, minor and trace products, when the data do not warrant a figure.)

- Identity was not confirmed by direct comparison.
- † Whether 2, N'- or N,2'-linked was not determined.
- I Noticeable only at acidities well above the kinetic range.

As to products of rearrangement, the chief new point relates to p-semidine formation. In 1964, this particular rearrangement was left out of the scope of the theory, because Hammick and Munro had reported in 1950 that azo-compounds, which on reduction with stannous chloride or zinc and acid, gave both o- and p-semidines on catalytic reduction in acetic acid gave o-semidines but no p-semidines. They concluded that p-semidines are not formed in homogeneous rearrangements of hydrazocompounds by acids, unless heavy-metal ions are present which are involved in some vital way in p-semidine formation. Shine and Stanley recently remarked that the recorded evidence for this conclusion is not satisfactory. Certainly the conclusion itself is not correct; for, as shown in Table 2, we have found three kinetically controlled acid rearrangements of 4-monosubstituted hydrazobenzenes, without heavy-metal ions, which produce substantial amounts of p-semidines. The examples, 4-methoxy-, 4-chloro- and 4-nitro-hydrazobenzene, between them embrace both the one-proton and two-proton mechanisms. So we would now allow the polar transition state theory to include p-semidine formation, as it is easily able to do. An N-to-4' polar bond about 3 Å long in the transition state would have plenty of strength and, as has been explained, direction is no problem.

The displacement of NN'-bond electrons here is towards the 4-substituted ring, as it is in diphenyline formation. In o-semidine formation, it is in the opposite direction. It can be seen from this that what we often shortly call "the" transition state might in reference to one product be represented by a simple energy col, but when several products are collectively considered is better represented by a group of cols, all within a few times kT of one another. This concept has been experimentally supported for the uncatalysed reaction^{3,4}. For the theoretical reason given, we adopt it for the acid conversions, though the obvious check of the temperature dependence of the isomer proportions has not yet been applied.

Disproportionation Kinetics

We know that disproportionation has both base-catalysed and acid-catalysed mechanisms, and that, through the latter, it accompanies many acid-catalysed rearrangements. We also know, and have confirmation in the present work, that, like the rearrangements, the accompanying disproportionations are kinetically of first order in hydrazo-compound. This is notable, because the stoichiometry of rearrangement involves only one hydrazo-molecule, but that of disproportionation involves two. The kinetics show that only one of the two can be concerned in the rate-controlling step. The second must enter into a subsequent fast step. There must, then, be an intermediate compound characteristic of disproportionation.

We now extend the kinetic description of disproportionation with respect to its dependence on hydrogen ions. In all the examples in the tables in which rearrangement and disproportionation occur in comparable amounts, the ratio in which the two groups of products were formed was found to be independent of the concentration of the catalysing hydrogen ions over the investigated range. This was true whether the overall conversions were of second or first order in hydrogen ions. Thus the kinetic dichotomy of rearrangement applies quite identically to disproportionation, the latter process taking its whole kinetic form from the rearrangement which it is accompanying. Table 2 contains, in the conversions of 4-methoxy- and 4-acetamino-hydrazobenzene, the first examples of one-proton disproportionation.

These kinetics show that the mechanism of disproportionation is grafted on to that of rearrangement at a late point in the reaction path, after the region of transition states has been passed over. At this point, an intermediate must be provided to engage with a second hydrazo-molecule in the second and fast step of disproportionation. The kinetics show that this intermediate must be formed at low enough energies to preclude any reversal of its formation. The second and fast step must comprise the redox process characteristic of disproportionation.

Disproportionation Products

The study of products has shown in three ways that the redox process occurs without preliminary fission of either of the two diaryl species involved. First, the oxidizing fragment of a preliminary fission would be a nitrene (in an equilibrium degree of protonation), and thus the benzene rings of the resulting azo-compound would come from different original hydrazo-molecules. Consistently with all older work, our study has shown that an unsymmetrical hydrazo-molecule AB gives only the unsymmetrical azo-molecule AB and not the symmetrical azo-molecules AA or BB.

Secondly, if a nitrene had any free existence, it would pick up fragments of solvent molecules, to produce, for example, amino-phenols from aqueous solvents. product of this nature has ever been found. We found none, despite the routine searching of residues by chromatographic methods.

Thirdly, we carried out the following experiments. A rapidly reacting and a slowly reacting hydrazo-compound are treated together with acid in such conditions that, if treated separately, the former would be converted completely and the latter not at all. Treated together, the latter becomes oxidized to its azo-compound, and the former accepts an equivalent amount of reduction to fission amines. Shine and Stanley recently recorded such an experiment, using 4,4'-diphenyl- and 4,4'-dichlorohydrazobenzene 5 ; and our results contain a second example, which employs p-acetamino-hydrazobenzene and hydrazobenzene. Cross-oxidations without crossed products are consistent only with electron transfer between unsplit diaryl molecules. Thus the intermediate of disproportionation must be an unsplit molecule.

When we consider the intermediates established for rearrangement, we find one family of intermediates which might meet the requirements of the intermediate of disproportionation. They are the quinonoid, ring-linked,

but still protonated intermediates (one of which is illustrated in the scheme below), which come first on the reaction paths after the transition states. We must assume that they, or some of them, can be split by reduction, to an exclusive extent if the atom or group R is such that its loss does not compete.

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Decametric Radio Emission from Jupiter

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Observations of the decametric radio emission from Jupiter establish the existence of a hitherto undetected sub-millisecond component. Bursts of between 0.2 and 10 msec are not due to interplanetary scintillation but come from the planet itself.

During the winter of 1965-66, observations of the decametric pulses from Jupiter were carried out at the Royal Radar Establishment, Malvern, using spaced receiving aerials. The purpose of the experiment was to find out whether the millisecond burst structure could, like the bursts of a few seconds duration, be the result of interplanetary scintillations^{1,2} imposed on a relatively slowly varying Jovian radio emission. The investigation was made by observing differences in time of arrival of bursts from Jupiter at three spaced aerials. If the burst structure were the result of the interplanetary medium moving at solar wind velocities, time differences in the range 1-10 msec would have been observed. The results show that the millisecond bursts are not imposed by the interplanetary medium, but are caused by variation of amplitude or frequency in the source itself. In addition, the existence of a hitherto undetected sub-millisecond component has been established; its presence during all intense Jovian emission strongly suggests that production of this component is a basic property of the emission mechanism.

The equipment consisted of three aerials placed at the corners of a right-angled triangle with sides 1.52, 1.27 and 0.85 km. Each aerial was made up of two horizontal half-wave dipoles, linearly polarized and phased to give maximum response in the direction of Jupiter at transit. The aerials were connected by low-loss feeders of approximately equal length to a central position, where they were applied in pairs to two similar receivers tuned to the same frequency in the range 19.7 to 20.2 Mc/s; the receiver bandwidths were 10 kc/s. The detected outputs were applied to the vertical deflexion plates of a double-

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beam oscilloscope with the time-base length set to 20 msec. When intense Jovian emission was detected, the display was photographed on 35 mm film moving continuously in a direction perpendicular to the time bases at 20 cm/sec. As the Jupiter storm continued, the three aerials were applied cyclically in pairs to the two receivers, giving recordings on each of the three baselines. The magnitudes of the instrumental time delays were monitored by recording a sine-wave modulated signal from a common source at the beginning and end of each observing session. An auxiliary double channel pen recorder which ran continuously during times of expected Jovian emission was used to monitor the signals received by the aerials, and one of the receivers was also monitored aurally.

The baselines between the aerials used in the present observations were relatively short, and this made it necessary to resolve time differences of less than a millisecond between bursts received at different aerials (assuming the bursts to be due to interplanetary scintillations). The advantage of using short baselines is that the effects of the Earth's ionosphere will be reduced, because the correlation of the Jovian signals at the spaced aerials will be destroyed much less frequently than for long baselines. At the short separation the Faraday rotation at any aerial differs negligibly from that at any other, and the components of the ionospheric amplitude modulation pattern corresponding to spatial wavelengths around 1 km in the ground diffraction pattern are seldom strong enough to produce more than partial decorrelation of the signals.

During the Jupiter runs, some observations were made

of the 20 Mc/s continuous wave signal radiated by the S-66 satellite passing overhead at a height of 1,000 km.

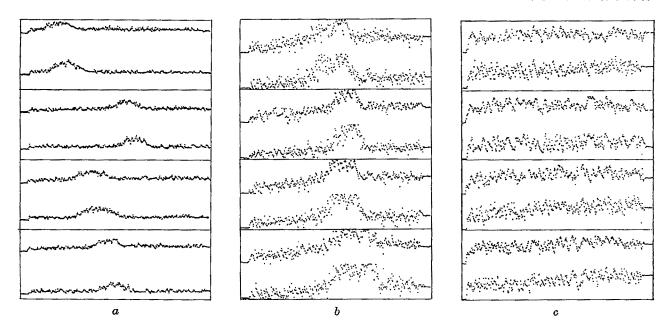


Fig. 1. Three examples of the 20 Mc/s radio emission from Jupiter recorded with spaced aerials connected to two receivers. The time-base lengths are 20 msec and time progresses from left to right and top to bottom. The traces are photographed in pairs, one receiver providing the vertical deflexion for each member. The original films have been digitized with 350 samples/time-base length. The receivers are suppressed during the short reference portions at the left of each trace. a, 4 mese pulses showing relative time shifts, north-south baseline, 1965 Jan. 23, 21h 83m ut. b, 5 msec pulses with much sub-millisecond structure, north-south baseline, 1965 Dec. 23, 00h 30m ut. c, Well developed sub-millisecond structure superimposed on a much slower 100 msec burst, south-east baseline, 1965 Dec. 8, 22h 34m ut.

Scintillations at frequencies corresponding to spatial wavelengths around 1 km on the ground never showed modulation depths of more than about 20 per cent, while frequencies corresponding to 5-10 km separation often showed 50-100 per cent depth of modulation.

On most nights after 20 h UT it was easy to find a band of about 100 kc/s width free of interference from terrestrial transmitters; the observing frequency usually used was about 20 Mc/s. Terrestrial interference could readily be identified (if present) by its aural characteristics, its narrow band and the general appearance of the chart record. Observations were made when Jupiter's hour angle was between \mp 02 h and when the longitude of the planet's central meridian and the position of the Jovian satellite Io were most favourable for the reception of decametric radiation³. During the interval November 1965 to February 1966 near opposition, strong Jovian emission was recorded photographically on twenty nights.

Some of the photographic records obtained during intense periods of Jovian emission were digitized by an electro-optical instrument of high resolving power for subsequent computer analysis; the sampling interval was 0.058 msec. An automatic graph plotter has been used to show some representative pulses from Jupiter in Fig. 1. Fig. 1a shows four consecutive pairs of traces, the two traces in each pair being triggered simultaneously. Pulses with durations of a few milliseconds are present, with a considerable amount of superimposed finer structure with durations as low as 0.2 msec. It is clear that the millisecond pulse envelope on the lower trace of each pair lags significantly behind the corresponding pulse on the upper trace. Similar time delays were recorded on all three baselines during this particular event. Fig. 1b shows four consecutive pairs of traces photographed on another night when the millisecond structure showed no significant time displacements: the sub-millisecond component is even more developed on these traces. In Fig. 1c we show four consecutive pairs of traces photographed on yet another night when a strong sub-millisecond component was superimposed on much slower variations with durations of about 100 milliseconds.

The average cross-correlation function between the pairs of traces of Fig. 1a is shown in Fig. 2a; Fig. 2b

and c are similarly related to Fig. 1b and c. The cross-correlations were calculated on the RREAC computer with a lag interval equal to the digitizing interval of 0.058 msec. Fig. 2a shows clearly that the millisecond pulses of Fig. 1a have suffered a relative shift of 0.38 msec between their arrival times at the two receivers. The small amplitude sub-millisecond component suffers no time lag, as is indicated by the presence of the small hump near 0 msec. In Fig. 2b, the more highly developed sub-millisecond structure shown in Fig. 1b is apparent: in this case neither the millisecond nor sub-millisecond pulses have suffered significant differences in arrival time. (The small difference of 0.029-0.058 msec apparent on the sub-millisecond structure in these cross-correlations is shown to be instrumental by the monitoring system.) Fig. 2c shows that the pronounced sub-millisecond pulses of Fig. 1c arrive together at the spaced aerials. In this case the average power spectrum of these pulses (computed from the Fourier transform of their autocorrelation function) shows that significant frequency components extend to about 6 kc/s.

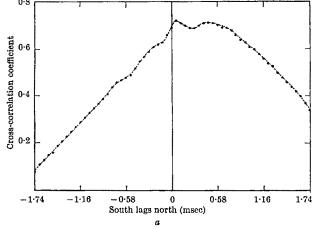
Amplitude variation with several different characteristic durations is present in the emissions. In Table 1 four categories of amplitude variation are listed, with the number of nights on which they occurred (out of the twenty active nights for which films were exposed). The table shows that the sub-millisecond structure was present during all periods of intense Jovian emission, although it is probable that the degree of modulation varied considerably. It is interesting that the sub-millisecond pulses appeared even when the bursts of intermediate length were absent.

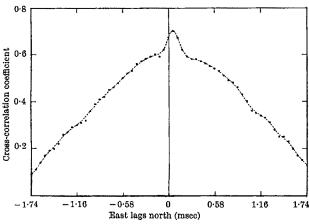
Table 1. TYPES OF STRUCTURE IN JOVIAN BURSTS

Component (msec duration) $0.1-1 \quad 1-10 \quad 10-100 > 100$ No. of nights present* $20 \quad 7 \quad 12 \quad 20$

* Photographic observations made on twenty active nights.

Because of the 20 msec length of time-base used throughout, bursts longer than about 10 msec cannot be subjected to digital analysis, and of the seven occurrences of 1–10 msec pulses only two were sufficiently prolonged to enable us to record the phenomenon on all three base





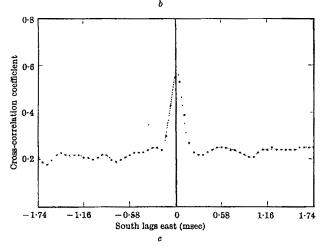


Fig. 2. Average cross-correlation coefficients between the members of the pairs of traces in Fig. 1. The lag interval is 0.058 msec and the total range 4.08 msec, giving an equivalent frequency resolution of 250 c/s. No corrections have been made for the non-zero means and slopes of the traces. a, b and c refer directly to the corresponding traces in Fig. 1.

lines. We discuss these cases (December 23, 1965, and January 23, 1966) in some detail.

The recorded millisecond pulses (see Fig. 1a and b) do not appear to possess the properties characteristic of irregular diffraction patterns drifting across the ground. Successive pulses are similar in shape and duration, tend to arrive at regular intervals of between 20-30 msec, and have widths of about 5 msec, giving a high interval to width ratio of about 6:1.

On the evening of January 23 the millisecond pulses showed consistent differences in arrival time at the receivers of about 0.5 msec for all three baselines: no

such differences were found on the records of December 23. On both occasions (as for nine other nights for which digitized results are available), the sub-millisecond structure did not suffer relative time shifts greater than 0.03 msec. The time delays measured on January 23 cannot be interpreted in terms of a drifting diffraction pattern across the spaced aerials because: (a) the delay was always present in the same receiver for all three combinations of pairs of aerials; (b) interchanging the aerial connexions to the receivers at a later stage of the same storm did not reverse the sense of the delay; and (c) the relative values of delay for the three baselines were not compatible with any velocity vector. Time delays with similar properties were measured during brief intervals of millisecond bursts on two other nights. On no occasion did we observe time differences which could be attributed to a velocity vector.

The time delays observed on January 23 are clearly associated with a difference between the two receivers used, a difference which did not appear when the instrumental time delays were monitored using a sine-wave modulated signal, from a single radio frequency source. The explanation is probably that the Jovian emission was drifting in frequency, and that there was a differential tuning error between the two receivers. Although the receivers were always tuned to within I ke/s of each other (tuning was checked at the beginning and end of each observation), delays of about 0.5 msec would result if a burst of Jovian emission drifted across the receiver pass bands at less than 2 Mc/s \sec^{-1} . The instantaneous bandwidth occupied by millisecond pulses has been shown by Riihamaa4 to be as low as 50 kc/s, the limiting frequency resolution of his radio spectrograph. Emissions of bandwidth between 10 kc/s and 50 kc/s drifting through the pass band of the receivers at rates of the order of 2 Mc/s sec-1 would produce millisecond pulses similar to those of Fig. 1a and b. Gordon and Warwick⁵ have observed bursts drifting at various rates, up to 35 Mc/s sec-1 for the bursts of largest rate. Such drifts are associated with fine structure in time: durations were less than 20 msec, the limiting time resolution of their equipment.

The sub-millisecond structure has shown no detectable time lag on any of our records. Such structure could be produced by emissions of less than 50 kc/s bandwidth drifting at rates of 20 Mc/s sec⁻¹ or by emissions without frequency drift modulated with sub-millisecond periodicities. The results of Gordon and Warwick⁵ show that both types of behaviour can occur when millisecond time structure is present; our results are consistent with similar behaviour for sub-millisecond time structure.

It seems unlikely that the sub-millisecond pulses could be caused by the sweeping across the Earth of a lobe pattern formed by coherent radio emission from large source regions on Jupiter, as suggested by Gordon and Warwick^{5,6} to account for the bursts of intermediate duration. Pulses of 0.2 msec duration imply a source region of 250,000 km in width, more than the diameter of the planet (140,000 km). Pulses formed by a sweeping lobe pattern should suffer arrival time differences of about 1 msec over distances of about 100 km in the direction of the velocity vector. An experiment aimed at detecting such a time difference would be worth performing.

The chief result of the present observations was that, for Jupiter bursts of duration between 0.2 and 10 mscc, time delays corresponding to solar wind velocities were not observed. Assuming that projected diffraction pattern velocities resulting from movements of the interplanetary medium are likely to lie in the range 100-1,000 km/sec, time delays between 10 and 1 msec should have been observed: the equipment used would have shown such delays very clearly. The millisecond and sub-millisecond bursts from Jupiter are hence not imposed by the interplanetary medium, and must originate from the planet itself. This conclusion is in agreement with the conclusions of Gordon and Warwick^{5,6} based on spectral observations. The

observed pulses may be the result of rapid variations in either amplitude or frequency or both. The evidence shows that some of the millisecond pulses are produced by frequency drift. The sub-millisecond structure is of relatively frequent occurrence, showing frequency components up to 6 kc/s. The absence of an interplanetary origin for pulses of less than 10 msec duration is consistent with recent observations of the spectra of interplanetary scintillations by Cohen et al., in which the power spectra were not found to extend much above 10 c/s.

Further investigations of Jupiter's decametric emission with higher time resolution are needed: it is also clear that studies of the frequency structure and drift rates are

required to elucidate the relative importance of time and frequency variations in producing the bursts of millisecond and shorter durations.

We thank Dr G. N. Taylor for assistance in observation and discussion

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Fission Products of Unusual Composition in Finland

by

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A cloud of fresh, highly fractionated fission products was observed in Finland between December 21 and 26, 1966. They seemed to come from Central Asia and were probably the product of an underground nuclear test.

In the morning of December 21, 1966, the radioactivity in the air unexpectedly rose in the eastern part of Finland. The front of active air moved westward at a speed of some 15 km/h and covered the whole country within 28 h (Fig. 1). The path of maximum ground level concentration, however, moved initially north-west, and later north-east at a speed of about 40 km/h.

Light snow with a specific β -activity of 1-3 nc./l. fell sparsely during this period. The β -activity, which was deposited through precipitation, amounted to about 10 nc./ m² in southern Finland and to about 2 nc./m² in northern Finland.

An autoradiograph of an air filter sample showed a very homogenous blackening of the film. No hot spots were detectable. Nearly all the previous samples in December had contained hot particles (originating apparently from the Chinese nuclear test of October 27) and so a new unknown source had to be considered.

The β -decay of an air filter sample (Whatman GF/A, glassfibre), collected at Nurmijärvi (see Fig. 1A) between December 22 at 15.30 GMT and December 23 at 08.00 GMT, was followed with a digital logger from December 24 to February 11 (ref. 1). Six 2.6 mg/cm² end-window counters took the measurements directly from the surface of the filter through different Al absorbers. Using the technique indicated in Fig. 2, we drew the following conclusions. The β -activity was chiefly a mixture of ⁸⁹Sr and ¹⁴⁰Ba- ¹⁴⁰La, with a ⁸⁹Sr/ ¹⁴⁰Ba activity ratio of 1.60 on December 24 at 00.00 gmr, and at least two additional β-emitters (half-value thickness ca. 10 mg/cm²) were present, with half-lives of about 3 days and between 20 and 40 days.

An air filter sample, collected at Nurmijärvi on December 22 between 06.00 and 13.30 GMT, was measured gamma spectrometrically several times between December 23, 1966 (Fig. 3), and April 1967 using a Ge(Li)-detector. The γ-peaks of the short lived fission products 140Ba-140La and 132Te-132I could be immediately identified in the γ-spectra (Table 1). A small peak at 365 keV indicated the presence of ¹³¹I, and a short lived peak at 280 keV remained unidentified. The half-lives given in Table 1 are

calculated from the successive γ-spectra, and the intensities are based on experimental efficiency curves and decay scheme data of the Nuclear Data Sheets².

A quarter of the same filter sample was leached with concentrated hydrochloric acid and I, Te, Zr, Ba and Ce carriers were added. The carriers were chemically separated and purified, and the y-activities were shown to follow the appropriate carrier. The 668 and 772 keV γ-peaks grew into the tellurium fraction, confirming that they were ¹³²I. No activity was observed in the zirconium fraction. This is in agreement with the absence of the

Table 1. Gamma emitters observed in the γ -spectrum of the air filter sample collected at nurmly arvi on december 22, 1966

Nuclide	Main γ-energies observed (keV)	Half-life γ of γ -lines	of activity in air on Dec. 22 (pc./m³)	Fractiona- tion factor (140Ba=1.0)
140Ba	537, 160, 305	9 to 13 d	14.4 ± 3.6	1.0
140La	1595, 485, 329	Ingrowth	13.2 ± 3.3	_
	815, 920, 750	observed		
131Te	230	3∙3 d	1.6 ± 0.4	0.086 ± 0.013
1931	667, 772	2.5 to 3.0 d	1.6 ± 0.4	0.086 ± 0.013
		(daughter of		
1317	365	~8 d	0.50 ± 0.15	0.054 ± 0.016
Unidenti		38 h	$1.0 \pm 0.3 \pm$	
141Ce*	145		0.37 ± 0.10	0.056 ± 0.015
137C8*	662	No decay	0.13 ± 0.03	6.2 ± 1.2
103Ru*	498		$\leq 0.15 \pm 0.05$	$\leq 0.06 \pm 0.02$
95Zr-95N			< 0.017	< 0.005
				1 IT 100H

*These nuclides were identified in γ -spectra taken in March-April 1967, after the short-lived activities had decayed. Radiochemical separations were not carried out.
†The y-lines of " ΣT - ΣN b were not seen in any γ -spectra, and only an upper limit is given for their concentration.

† Emission rate of 280 keV γ-rays.

Table 2. CONCENTRATION OF SOME RADIONUCLIDES IN SNOW COLLECTED IN HELSINKI ON DECEMBER 24, 1966

Nuclide	Activity on Dec. 24 (pc./l. of water)	Fractionation factor (140Ba = 1.0)
**Sr	640 ± 65	5.7 ± 1.0
POST	3 ± 1*	4·1* ± 1·5
140Ba	450 ± 70	1.0
181]	< 40	< 0.15

* The *OSr values have not been corrected for the activity from old tests.

732 keV γ-line of ⁹⁵Zr in the gross γ-spectrum. No ²³⁹Np could be detected either. Peaks corresponding to the γ-energies of ¹⁴¹Ce, ¹⁰³Ru and ¹³⁷Cs were visible after the short lived activities had decayed. None of these peaks could be detected in a reference sample collected at Nurmijärvi on December 20, 2 days before the fresh debris arrived.

A sample of the top layer snow was also collected on December 24 in Helsinki, and ¹⁴⁰Ba, ⁸⁸Sr, ⁹⁰Sr and ¹³¹I were determined radiochemically. The results are given in Table 2.

The presence of an appreciable amount of ¹³²Te in the aerosol sample suggests that the debris had a rather short age. Calculation of age was based on the fact that ¹⁴⁰La

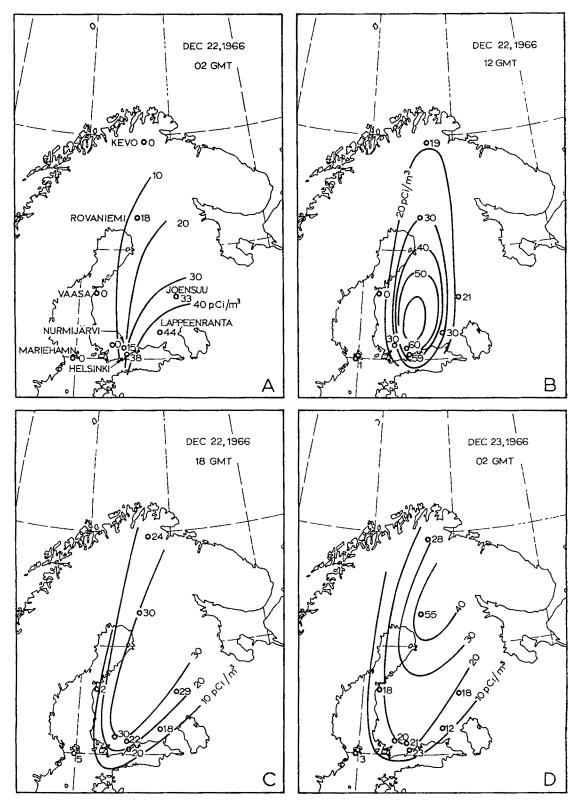


Fig. 1. Movement of the radioactive cloud over Finland during December 22.

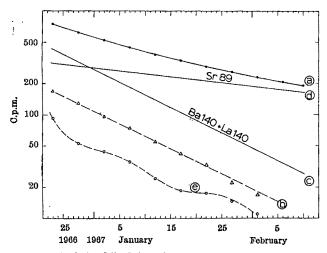


Fig. 2. Analysis of the beta emitters on the basis of decay curves measured through different absorbers. (a) Decay curve taken with a 2-6 mg/cm² end window counter. (b) Decay of an "excess" of soft betas, showing the half-life of ¹⁴⁰Ba. (Let the counting rate through an absorber of 100 mg/cm² be f and without an absorber a. The ratio alf approached 2-5 at the end of the measuring period. Curve b is obtained by plotting the expression a-2-5 f, (c) and (d) Main components of curve a. (e) A curve equivalent to curve b except that the absorber of 9 mg/cm² was employed. The influence of soft β -components of some 3 days and 30 days half-lives can be seen.

was not yet in equilibrium with 140 Ba in the first γ -spectra. An age of 5.5 ± 0.7 days, counted from December 23, 12.00 gMT, was obtained.

For investigation of the fractionation of fallout it is usual to use 95 Zr as a reference (compare refs. 3, 4 and 5). We did not find this nuclide in our samples and so have defined the fractionation factor f(A) in relation to 140 Ba.

$$f(A) = \left[\frac{N (A)}{N (^{140}\text{Ba})}\right]_{\text{exp}}^{\bullet} \left[\frac{N (^{140}\text{Ba})}{N (A)}\right]_{\text{theor}}$$

where N(A) is the amount of nuclide A at the time of production. The theoretical ratio refers to the fission yields of the thermal neutron fission of ²³⁵U. Our fractionation factors are given in Tables 1 and 2. The strongest enrichment is shown by ⁸⁹Sr and ¹³⁷Cs; these nuclides

also have rare gas precursors with the longest half-live (3·2 min and 3·9 min, respectively). Strontium-90 we apparently enriched to a lower degree (**0Kr has a half-liver of 33 see), while all the other nuclides observed were depleted in relation to **140Ba* (which has a 16 sec precursor **140Xe). Tellurium-132 has no gaseous precursor; howeve it is known that tellurium is relatively volatile at hig temperatures*.

It is interesting that fresh nuclear debris showing similar enrichment of the "volatile" fission chains we observed during January 1965 in Japan by Koyamet al.". They conclude that the activity originated in leak at an underground nuclear test near Semipalatins USSR.

Fig. 4 gives a good picture of the weather during th period; it shows an analysis of the mean isohypses a 850 mbar. The period covered is from 12.00 GMT o December 17 to 12.00 GMT on December 22. In Europea Russia and the western Asiatic Russia it was dominate by an area of high pressure, but there was a trough extending from Scandinavia to the Black Sea.

Surface winds were ageostrophic, and their direction in southern Finland was east and south-east. The difference between the surface winds and those at 850 mba first suggested that the radioactive air came with a fascurrent at the 850 mbar level to areas lying east and south-east of Finland. While travelling in this direction radioactive air subsided and was carried westwards by surface winds. Radioactivity at ground level first roswhen this air from the lower troposphere reached Joensus (Fig. 1A). Maximum beta activity was observed when radioactive air arrived with a surface wind on a longe route parallel with the radioactive flow in the troposphere.

Using this hypothesis, a CAV trajectory, calculated backwards from the White Sea, has been drawn in Fig. 4 Measured winds and 12 h steps were used. The time a the other end of the trajectory was 12.00 GMT on Decembe 18. There was a trough in the troposphere over the areast of the end point of the trajectory, and this led to divergence of flow patterns, and the calculation of the trajectory eastwards was not continued. Along the calculated trajectory the weather was extremely stable Many authors (for example, refs. 8 and 9) state that a centre

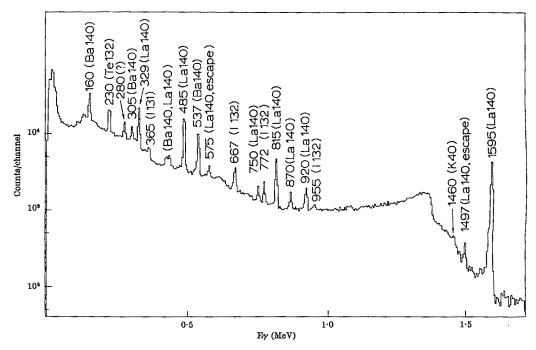


Fig. 3. Gamma spectrum of an air filter collected on December 22 at Nurmijärvi. The spectrum was measured by means of a 4 cm³ Ge(Li)-detector. Gamma-energies are given in keV.

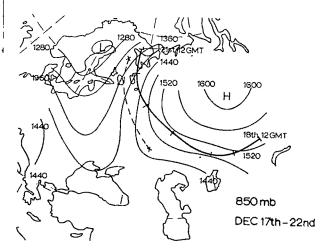


Fig. 4. 850 mbar mean contours for December 17-22. Heavy line is a CAV trajectory, calculated backwards from the White Sea. Broken line $(\times --- \times)$ is the cross section in Fig. 5.

of high pressure associated with stability in the troposphere is conducive to long-distance transportation.

Fig. 5 is a thermal cross section at 00.00 GMT on December 20, 1966. The cross section in this diagram lies along the line x - - x in Fig. 4. Fig. 5 shows that at 850 mbar there was relatively warm air (-10° C) with a layer of colder (-20° C) air under it. The troposphere from the inversion layer to ground level was sharply separated from the upper troposphere. In this case, a stable inversion at roughly 850 mbars made the preservation of high concentration possible.

The winds over Saratov and Moscow shown in Fig. 5 explain how the radioactive air moved so far in such a short time. Inversion wind maximum, first presented by Blackadar^{10,11}, increased at the stable inversion layer, and the maximum wind observed at 850 mbar was 55 knots.

The strong fractionation of the debris can be explained by assuming a sudden release of volatile (particularly rare-gas) fission products, such as would be expected from an underground nuclear detonation. Meteorological considerations strongly point to a central Asian origin. According to seismological information¹², an underground nuclear test was carried out in the Semipalatinsk area on December 18 at 04.58 gmt. In view of the air trajectories and the calculated age of the fission products, this event could be the source of the radioactivity reported here.

No health hazards were involved in the deposition of the activity in Finland¹³.

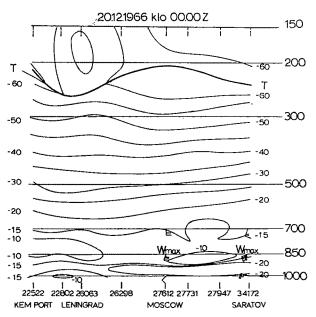


Fig. 5. Thermal cross section at 00.00 gMT on December 20, 1966. $W_{\rm max}$ refers to the lower troposphere.

We thank Professor J. K. Miettinen for his interest in this work, and Mr Henry Kalm for his help and for use of the Ge(Li)-detector of the Department of Physics for the first crucial γ-spectra. We also thank Miss Eira Akkanen for technical assistance.

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Inorganic Pyrophosphate and ATP as Energy Donors in Chromatophores from Rhodospirillum rubrum

by
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Bioenergetics Group, Department of Plant Physiology, University of Stockholm Both inorganic pyrophosphate and ATP may function as biological energy donors in chromatophores from the photosynthetic bacterium. Rhodospirillum rubrum, causing energy requiring spectral changes in endogenous cytochrome(s) and carotenoid(s).

PHOTOPHOSPHORYLATION in chromatophores from the photosynthetic bacterium *Rhodospirillum rubrum* yields inorganic pyrophosphate (PPi) as the end product, in the absence of added adenine nucleotides¹. We have

reported earlier that these chromatophores contain an inorganic pyrophosphatase (PPase) which is usually more active than the corresponding ATPase, and that both these activities are stimulated by certain uncouplers

of oxidative phosphorylation and photophosphorylation^{2,3}. After these findings, we became interested in whether PPi, as an energy rich compound, could be used to drive energy requiring reactions in a manner similar to ATP.

ATP has long been known to serve as an energy source for pushing reactions of oxidative phosphorylation in animal mitochondria in the reversed direction, resulting in changes of the steady state oxidation levels of electron carriers4,5, including cytochromes8,7. In agreement with our working hypothesis, based on the similarities between PPi and ATP, we have obtained evidence, briefly reported elsewhere³, that not only ATP but also PPi can serve as an energy donor, causing an energy requiring spectral change at the level of endogenous cytochrome in chromatophores from R. rubrum. Results from further experiments with PPi as an energy donor in this system, and a discussion of the more detailed picture thus obtained about the energy transfer reactions involved, are given here.

The bacteria (Rhodospirillum rubrum Strain S1) were grown on a synthetic medium described by Bose et al.8 and the chromatophores were prepared according to the standard procedures of this laboratory. The "chromatophore fragments" fraction was secured and stored at 0°C after one washing in 0.2 molar glycylglycine, pH 7.4, in concentrated suspension. Concentration of preparations was measured at 800 m μ (OD_{800}). The energy requiring spectral changes were measured in a suitable dual wavelength spectrophotometer, after appropriate dilution with the buffer and addition of 3.3 mmolar magnesium chloride (final concentration). The Soret region of the spectrum was used for most of the experiments, because the strong absorption of carotenoids and bacteriochlorophyll rendered observation of specific changes of cytochromes at longer wavelengths extremely difficult.

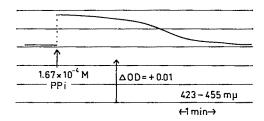


Fig. 1. Reduction of b-type cytochrome induced by addition of PPi. Reaction mixture contained in a final volume of 3 ml. : 0·2 molar glycyl-glycine buffer, pH 7·4, 3·3 mmolar magnesium chloride and chromatophores equivalent to $OD_{800} = 0\cdot900$.

A recording of a typical increase in absorbance induced by PPi at 423 mu is shown in Fig. 1. The change essentially represents the reduction of a b-type cytochrome. A return to the original level occurs when enough of the added PPi has been broken down by the strong PPase activity of the chromatophores. It will be shown here that the change induced by PPi is more rapid and greater than the corresponding change obtained with ĂTP.

As is shown in the difference spectrum given in Fig. 2A, the absorbance change obtained with PPi is greatest in the region 427-429 mu, which is the location of the Soret peak of the reduced form of b-type cytochrome in R. $rubrum^{10-12}$. It is further seen, in Fig. 2B, that PPi causes very distinct changes between 480 and 600 mµchanges which are known to be typical for carotenoids13. Similar changes were obtained when ATP was substituted for PPi.

Full extent of the cytochrome reduction can be obtained with as little as 6.7×10^{-5} molar PPi, as is shown in Fig. 3. The dashed line shows the trace obtained with 10-4 molar

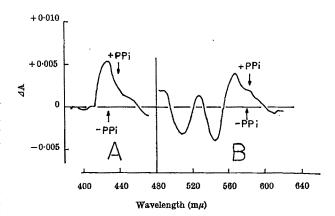


Fig. 2. Difference spectrum of changes induced by PPi in chromatophores. Both cuvettes contained in a final volume of 3 ml.: 0.2 molar glycyl-glycine buffer, pH 7.4, 3.3 mmolar magnesium chloride, 1.67 mmolar sodium succinate and chromatophores equivalent to $OD_{500} = 0.930$. 0.167 mmolar PPi (final concentration) was added to the measuring cuvette immediately before the spectrum was recorded.

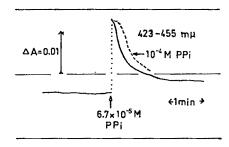


Fig. 3. Reduction of b-type cytochrome obtained by addition of low concentrations of PPi. Experimental conditions as in Fig. 1, except that the chromatophore concentration was equivalent to $OD_{800}=1.560$.

PPi in a subsequent control experiment, here superimposed for the sake of clarity.

Uncoupling agents such as gramicidin and desaspidin were found to inhibit the absorbance changes induced by both PPi and ATP. The inhibitory effect of gramicidin is shown in Fig. 4. In this system, the same low concentrations of gramicidin are needed as those required for interference with the photophosphorylation of ADP to ATP (ref. 14), or Pi to PPi (ref. 15).

If PPi is added after a maximum ATP-induced spectral change has been obtained and demonstrated by titration to excess ATP, there is always a further change, the total extent of which is the same as or greater than the change that can be obtained with PPi alone. In most preparations, also, addition of ATP to saturating concentrations of PPi gives some further change. These synergistic effects can be seen in Fig. 5. They appear to indicate that the ratelimiting steps of the reaction sequences between PPi or ATP and cytochrome are the reactions of these energy rich substrates with their respective enzymes, the reaction with ATP being the slower one. The reason why PPi gives both a more rapid and a more extensive reduction of cytochrome than ATP could be that PPi is more closely connected to the electron transport chain, so that fewer intermediate steps may be required to transfer the energy to the cytochrome level from PPi than from

Oligomycin is known not to inhibit the light induced formation of PPi (ref. 15), whereas the corresponding ATP formation is strongly inhibited by this compound¹⁴. In Fig. 6 it is seen that the reversed reactions behave in accordance with this, the cytochrome reduction induced by PPi being unaffected by oligomycin whereas the reduction induced by ATP is strongly inhibited.

It is now possible to construct a basic scheme including the metabolic connexion between PPi, ATP and electron transport, as well as the rate limiting steps in the "reversed" energy transfer from PPi and ATP to b-type cytochrome. $X \sim I$ is the hypothetical non-phosphory-lated, energy rich intermediate of the chemical theory for electron transport coupled phosphorylation. E_1 and E_2 indicate different enzymes (or possibly only different reaction sites on the same enzyme) mediating the initial reaction step from the respective energy donor towards the $X \sim I$ -level.

$$b$$
-type cytochrome \leftarrow b -type b -t

Keister et al. 16,17 have confirmed our first demonstration that PPi can function as an energy donor in chromatophores. They measured the energy-requiring pyridine nucleotide transhydrogenase reaction 18 and found that PPi, ATP or light could drive the reduction of added pyridine nucleotide. In their system also, oligomycin showed a strong inhibitory effect only on the ATP-driven reaction.

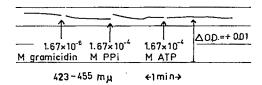


Fig. 4. Inhibition of both PPI- and ATP-induced cytochrome reduction with gramicidin. Experimental conditions as in Fig. 1.

$423-455 \text{m}\mu$

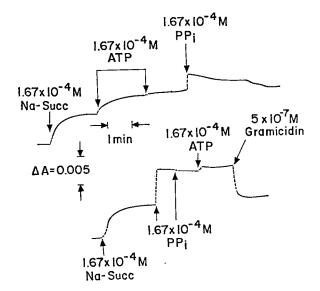


Fig. 5. The synergistic effects of PPI and ATP. Experimental conditions as in Fig. 1, except that 0-167 mmolar sodium succinate was included and that the chromatophore concentration was equivalent to $OD_{so} = 0.700$.

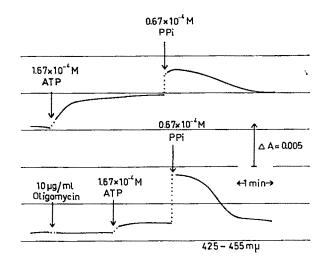


Fig. 6. Differential effect of oligomycin on PPI- and ATP-induced cytochrome reduction. Experimental conditions as in Fig. 5.

The assumption that the formation of PPi in photophosphorylation is of metabolic significance has been substantiated by the results, reported here, that not only ATP but also PPi can be used to drive energy requiring changes in the redox state of endogenous cytochrome. The ability of PPi to act as an energy donor in a pathway back to the level of endogenous electron transport is, however, not restricted to photosynthetic systems. We have now also been able to demonstrate PPi-induced energy requiring cytochrome changes in respiring mitochondria from both lower and higher organisms 19. In chromatophores, the unique response obtained has been that of endogenous carotenoid. Our finding that PPi and ATP change the absorption of carotenoid in the same direction as that which has been demonstrated earlier with light as energy donor¹³ indicates the existence of a metabolic connexion between light, PPi, ATP and carotenoid in bacterial photosynthesis.

I thank Dr Herrick Baltscheffsky for many valuable suggestions, and Professor Britton Chance and Dr Nils-Erik Saris for their help with the spectrophotometric experiments, which were performed in their laboratories.

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Parabiotic Intoxication in Germ-free Mice

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Department of Pathology, University of New Mexico Germ-free parabionts show greater shifts in erythrocyte mass between partners than do conventional parabionts. The germ-free condition also permits longer survival of both partners. This raises important questions as to the role of bacteria and viruses in the pathogenesis of parabiosis intoxication.

Even after extensive investigation, the pathogenesis of parabiosis intoxication remains unknown. Billingham¹ has called attention to the similarity of this phenomenon to homologous disease and has suggested that similar mechanisms may be involved. Experiments with germfree animals implicate bacteria and bacterial antigens in many of the manifestations of runt disease². We have tried to define the role of bacteria and viruses in the pathogenesis of parabiotic intoxication.

Ten female germ-free mice, derived by Caesarean section at the Charles River Breeding Laboratories, were maintained in Trexler isolators and given free access to sterile fortified 'Wayne Lab Blox' and water. Ten additional germ-free mice of the same random bred strain were housed in standard animal quarters and freely fed unsterilized 'Wayne Lab Blox' and tap water. Mice were acclimated to these conditions for eight weeks before any experiment was carried out.

Five pairs of non-littermate germ-free mice, subjected to 'Nembutal' anaesthesia, were placed in parabiotic union of the coelomic type, as described by Martinez's, and maintained in the germ-free state for the duration of the experiment.

Mice were bled from the retro-orbital sinus before surgery and 3, 8, 12, 15 and 21 days after parabiosis. Haematocrits were determined and the sera then subjected to standard cellulose acetate electrophoresis and immuno-electrophoresis.

On the death of one parabiont the partner was killed and both animals autopsied. Organs were fixed in 10 per cent formalin and sectioned and stained with haematoxylin and eosin. At fortnightly intervals, faeces, food and bedding, removed from the isolator, were cultured in fluid media and smears examined microscopically.

Five pairs of conventional mice were parabiosed, bled and their organs treated as described here.

Erythrocyte shifts occurred in all pairs. As shown in Fig. 1, germ-free pairs manifested earlier and greater red blood cell shifts than their conventional counterparts. Significant anaemia—polycythaemia was apparent in all germ-free parabionts by the fifth day after anastomosis with the first change noted in two pairs on the third day; on the average, significant shifts were present in this group on the fourth day. The haematocrit of the polycythaemic partner in the germ-free group increased on average 26 units from a pre-operative average value of 57.6 per cent (range = 53–60 per cent) with a corresponding average fall of 30 units in the haematocrits of the anaemic partners. Corresponding values for the conventional pairs were as follows: first indication of anaemia—polycythaemia (one pair) on third day, all animals by twelfth day, average first appearance on ninth day; average increase in haematocrit of polycythaemic partner

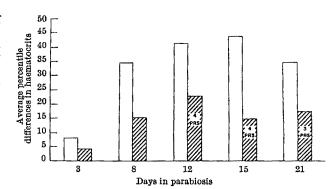


Fig. 1. Percentile haematocrit differences between individual parabionts in germ-free and conventional pairs as a function of time after anastomosis. White columns, germ-free parabiosis (5 pairs); hatched columns, conventional parabiosis (5 pairs).

12 units from a pre-anastomosis value of 47.5 per cent (range = 47-52 per cent); average decrease in haematocrit of anaemic partner was 18.0 units.

Additional haematological data for individual germ-free and conventional parabionts are summarized in Table 1, which also includes survival figures for each pair. Again it is apparent that maximum erythrocyte shifts were of greater magnitude in the germ-free than in the conventional group; germ-free parabionts became much more anaemic and polycythaemic than their conventional counterparts. Survival times, however, could not be predicted from the magnitude of the erythrocyte shifts.

Table 1. GERM-FREE VERSUS CONVENTIONAL PARABIONTS: SUMMARY OF HAEMATOLOGICAL AND SURVIVAL DATA

Pair	Lowest ha	ematocrit cent)	Highest ha		Survival (days)		
No.	Germ-free	Conven- tional	Germ-free	Conven- tional	Germ-free	Conven- tional	
$\frac{1}{2}$	6·8 24·5	14·8 32·5	80-0 80-0	60·0 79·5	34 22	15 27	
3 4 5	24·5 27·4 32·5	38·0 39·8 41·8	75·5 85·0 77·5	53·0 55·8 50·0	23 33 56	11 25 24	
Average	23-1	33-4	79•8	59•7	33-6	20-4	

Survival time in the germ-free parabionts averaged 33.6 days, with a range of 22 to 56 days and a median of 33 days; corresponding values for the conventional parabionts were as follows: an average survival of 20.4 days with a range of 11 to 27 days and a median of 24 days. In general, the anaemic partner died before the polycythaemic member in both the germ-free and conventional groups; however, in one germ-free combination, the polycythaemic member died first.

Gross autopsy examination revealed few differences. In both groups, the thymus of the anaemic member was

much smaller than that of the polycythaemic partner; the former was often difficult to identify while the latter seemed to be of normal size for the age of the mouse. Moderate splenomegaly occurred in all parabionts. The hearts of the polycythaemic mice were somewhat larger than normal while those of the anaemic animals seemed to be normal or slightly below normal in size. The large intestine of all germ-free mice was characteristically dilated. Displacement of portions of small and/or large intestine was present in four of five germ-free pairs and three of five conventional pairs; when present, such displacement always resulted in the presence of polycythaemic gastrointestinal tract in anaemic mice. In germ-free mice, such displacement, which included portions of the dilated large intestine, resulted in a distended, discoloured abdomen which was readily apparent before death. Germ-free pair No. 5, which survived for 56 days, did not show such displacement.

Microscopically, there were the expected differences between the lymphoid system of the germ-free and conventional mice. Spleens from germ-free mice contained only an extremely rare plasma cell, a paucity of large lymphocytes and in only one spleen were well developed germinal follieles evident. In contrast, conventional spleens contained numerous large lymphocytes which formed prominent germinal follieles; plasma cells were present in larger numbers than in the germ-free mice. Germ-free thymi contained few large thymocytes and lacked the numerous mitotic figures which were prominent in the conventional parabionts. Scattered nests of lymphocytes were found in assorted viscera of most conventional mice; these were not present in the germ-free parabionts.

There were few microscopic differences between conventional anaemic and polycythaemic partners. In general, the anaemic mouse died first and exhibited moderate autolysis while the polycythaemic member showed a modest degree of visceral congestion. In the germ-free group, comparable differences between partners were subtle but of interest. The polycythaemic partner appeared to have a greater number of large splenic lymphocytes than the anaemic member. Furthermore, survival appeared to be inversely proportional to the numbers of large lymphocytes contained in the spleen and thymus.

A comparison of the serum proteins in germ-free and conventional mice, as estimated by cellulose acetate electrophoresis and immunoelectrophoresis, revealed only the expected differences (Table 2); concentrations of serum gamma globulin and alpha-2 globulin were greater in conventional mice and concentrations of serum beta globulin were larger in the germ-free group.

Table 2. Germ-free versus conventional parabionts: summary of serum protein determinations*

	Germ-		
Serum protein	Anaemic	Polycythaemic	Conventiona
fraction	member	member	mice
Albumin	43.0	40·1	40-2
Alpha-1 globulin	11.3	10·0	10-0
Alpha-2 globulin	12.8	14·7	20-2
Beta globulin	31·3	26·4	19·6
Gamma globulin	0·4	5·4	8·7

^{*} Expressed as percentage of total proteins.

Serum gamma globulin concentrations in the germ-free group seemed roughly to correlate with survival, as shown in Table 3. Survival time was longest in those pairs (1 and 5) in which both mice showed markedly decreased or negligible gamma globulin. Furthermore, when there was a significant discrepancy between germ-free partners, the parabiont with more serum gamma globulin became polycythaemic with anaemia developing in the corresponding partner with the lower value (pair Nos. 2, 3, 4). There was no significant change in gamma globulin concentrations in any of the germ-free pairs during the course of the experiment with the possible

Table 3. RELATIONSHIP BETWEEN SURVIVAL AND PRE-ANASTOMOSIS GAMMA GLOBULIN DETERMINATIONS IN GERM-FREE PARABIONTS

	Serum gar	nma globulin*	
Pair No.	Anaemic member	Polycythaemic member	Survival (days)
1	0	3	34
2	0	10.6	22
3	0	8.35	23
4	2.2	7.9	33
5	0	0	56
Average	0.4	5·4	33-6

* Expressed as percentage of total proteins; comparable value for conventional mice = $8\cdot 7$ per cent.

exception of the polycythaemic member of pair No. 4; this animal exhibited a slight decrease on the fourteenth day after the operation.

Also in the germ-free group, there was a slight increase in concentrations of serum beta-2 globulin in all animals during the experiment. This change was slightly more pronounced in the anaemic parabionts. Serum protein determinations in conventional animals demonstrated no consistent differences between partners.

Much of the disagreement concerning parabiotic intoxication revolves around the putative role of immune mechanisms in the development and maintenance of the characteristic anaemia-polycythaemia. Hall and Hall's discount the importance of immunological factors in the pathogenesis of this phenomenon and suggest that the anaemia per se is responsible for the symptomatology of the affected partner. Others1,6 emphasize the similarities between the clinical expression of secondary disease and parabiotic intoxication in support of an immune actiology. Based on experiments involving F_1 hybrid mice joined to one of their parental strains, Hilgard et al.6 postulate that the anaemia-polycythaemia shift can be divided into two apparently independent phases. The experimental results of these investigators suggest that, although the initial erythrocyte exchange may be a reflexion of haemodynamic differences between the partners, the second phase is dependent on immunological differences between the parabionts.

This study reveals the following differences between germ-free and conventional parabionts of the same strain: the erythrocyte shift is much greater in the germ-free parabionts than in their conventional counterparts and the former pairs survive for a longer time post-anastomosis than do the conventional parabionts. The latter results may be unduly magnified by the presence of one germ-free pair which survived for an unusually long time (56 days); however, elimination of this pair still leaves an impressive survival differential (28-0 versus 20-4 days) favouring the germ-free group. It is tempting to attribute these differences directly to the absence of bacteria and viruses in the germ-free group. The present study, however, suggests that additional factors also may be operative.

Germ-free animals have poorly developed lymphoid systems, a paucity of immunocytes and low concentrations of serum gamma globulin. Their response to immunological stimuli and especially to tissue transplants⁷⁻⁹ is sluggish in comparison with conventional animals. If the second phase of parabiotic intoxication is on an immune basis, a reaction might be expected to be delayed or muted in germ-free animals. In this connexion, the prolonged survival of germ-free pair No. 5 is of interest; neither member of this combination had gamma globulin demonstrable by immunoelectrophoresis.

The association between prolonged survival and an increased erythrocyte shift in the germ-free parabionts was unexpected and is difficult to explain. A tardy response in the less sophisticated immune system of the germ-free animals might have modified release of vasoconstrictor substances and thus permitted the initial anaemia-polycythaemic response to continue for a prolonged period before restriction by the second (immune) phase of the reaction. Zweifach et al. 10 have shown that germ-free rats, after removal of blood, failed to rebound

to normotensive levels. A similar phenomenon might enlarge initial erythrocyte exchange caused by exaggerated blood pressure differentials between germ-free mouse parabionts.

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Computer Simulation of Biological Pattern Generation Processes

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Simulation of the growth patterns of plants on computers provides a new approach to the study of some basic problems of morphogenesis.

SEVERAL lines of evidence suggest that the overall pattern of differentiation and morphogenesis in living organisms is produced by a hierarchically ordered set of interactions between genes, gene products and the external environ-

In this article, we report an attempt to simulate the growth of some biological patterns with the help of a digital computer, using the simplest possible generation rules and the smallest number of parameters in the programmes. Earlier attempts in this direction have been made2, and a preliminary report of this work has already been published3.

A Fortran programme, consisting of an organized set of generation rules, was written for each class of biological patterns to be simulated. Each set of rules has been derived from plausible hypotheses of the natural mechanisms. The rules were modified and extended as the work progressed to perfect the model.

For the sake of simplicity, our work deals only with branching patterns in two dimensions. This class of pattern is characteristic of vascularization in leaves and other flat or layered structures, and of branching on a flat surface. In fact, our simulation models are restricted to growth occurring at the free ends of branches, and to branching behind the growing tip.

The following are the generation rules for some classes of two-dimensional branching patterns.

Growth Rules

(i) Growth occurs only at the tips of branches.

(ii) The amount and angle of growth are determined by local density field, the direction and magnitude of the gradient of this field, the previous angle of the free end of the branch, and the tendency of the growing branch to persist in its original direction in spite of the gradient in density.

(iii) The density field around a point is computed by taking thirty-six sample points at intervals of 10° at a unit distance from the central point, and taking, as a measure of the density at each sample point, the sum of the squares of the reciprocals of the distances to all neighbouring parts of the pattern. The direction of the negative gradient and its magnitude are then computed by interpolation.

(iv) The extent of growth UG is given by

$$UG = U \cdot \frac{DGT - DMIN}{DGT} \tag{1}$$

where U is a unit distance, DMIN is the local minimum in the density field, and DGT is a limiting density above which no growth can occur. (Negative growth is not allowed.) The value of DGT determines the limiting

density of the pattern as a whole. (v) Growth angle GA is given by

$$GA = \frac{SA \cdot ST + GRA \cdot GR}{ST + GR} \tag{2}$$

where SA is the angle of the free end of the branch, ST is the inertial factor for growth direction, GRA is the direction of the negative gradient of the local density field and GR is the magnitude of the gradient.

A continuous iterative application of these growth rules leads to unlimited growth except when the minimum local density exceeds the limiting density for growth, that is, when DMIN > DGT. In general, the growth will be directed toward the unoccupied space outside the already existing pattern.

(vi) Growth may be directed by biasing the density field in any one direction in space.

Branching Rules

The branching rules, to a large extent, determine the final form and texture of the generated pattern.

(i) The density is computed for each potential branching point in the pattern in exactly the same way as for the growing points.

(ii) Branching probability PRB is computed according to the equation

$$PRB = \frac{DBT - DMIN}{DBT} \cdot \frac{DB - DBL}{DB} \cdot \frac{DD - DDL}{DD} \quad (3)$$

where DBT is the limiting density for branching, DB and DD are the distance from base and apex of the segment, respectively, and DBL and DDL are the respective limiting distances. DMIN has the same meaning as in the growth equation.

(iii) A random trial decides at each iteration whether branching will actually occur.

(iv) Branching angle BRA is determined in the same way as growth angle with the addition of a standard angle BRAS and a persistence factor, STBR. Thus

$$BRA = \frac{BRAS \cdot STBR + GRA \cdot GR}{STBR + GR}$$
 (4)

A continuous application of these branching rules, together with the undirectional growth rules (i)-(v), will generate uniformly spreading patterns which will assume an approximately circular shape, irrespective of the initial direction and asymmetry.

(v) Branching may also be directed by introducing a bias in the local density field. Growth bias and branching bias may be entirely independent, although they tend to reinforce each other if they are in the same direction. The whole pattern may thus be made to assume a directionality.

Both growth and branching rules may have their parameters changed as a function of some local or general property of the pattern. This introduces a hierarchical element into the generation process. Thus a specific branching sub-routine, applied only once at an early stage in the growth of the pattern, will determine the shape and relations of the primary branches. Alternatively, the parameters may be a function of distance from a local disturbance in the growth space, and this may result in specific "morphogenetic" effects. Also, branching may be completely inhibited in segments that have some particular spatial characteristics.

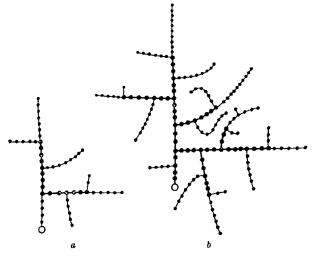


Fig. 1. Two stages in the growth of a simulated branching pattern. The persistence factors for growth and branching, ST and STBR, respectively, are both zero, so that both growth and branching always follow the negative gradient of the local density field. An intermediate spacing of the branches is caused by intermediate limiting distances between branching points, DBL for the distance from a basal branching point, and DDL for the distance from the distal end of the segment.

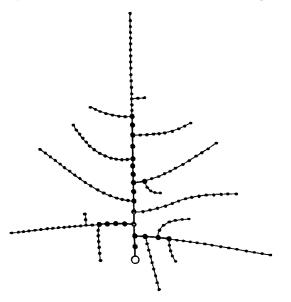


Fig. 2. This tree-like pattern is produced by increasing the limiting distance from the distal end of the segments, $DDL=10\cdot0$, while maintaining the limiting distance from the basal end at a low level, $DBL=1\cdot0$.

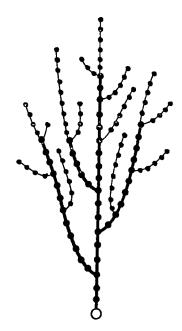


Fig. 3. A directed tree-like pattern is generated by blasing both growth and branching in the vertical direction.

We have introduced four principal effects:

(1) All the parameters for growth and branching may be different in branches of different ranks. This introduces an organization of branches into primary, secondary, etc.

(2) All the parameters may change their values during the growth process, depending on the total size of the pattern. As a measure of the total size, we have taken the total number of growth increments. This sequence of changes in the generation rules may result in marked differences between parts of the pattern formed at different stages of growth.

(3) The extent of growth and branching is made to depend on the distance from the basal and distal ends of each branch.

(4) Local disturbances in the growth space may increase or decrease growth and branching probability, and may do so to different extents in branches of different ranks. Branching may be directed towards or away from these local perturbations.

Some results of the simulations are shown in Figs. 1-6. In all the figures the starting point is represented by a circle. Those parts of the branching pattern which are no longer active in growth or in branching, that is, "old" segments, are represented by thick lines. The computed growth increments are indicated by the segments between the dots in Figs. 1-5.

In the simplest class of patterns there is no directional bias nor interaction with local disturbances. The growth and branching parameters remain the same in all parts of the pattern throughout growth (Figs. 1-2). The variety of patterns in this class depends on the distance between branching points, the standard angle of branching and its resistance to change by the local density gradient, and on the persistence of growth direction. This simple class is analogous to the growth of a mould in a uniform medium.

When a directional bias is introduced (Fig. 3), the effect is analogous to that of gravity on the growth of roots in a uniform medium, or to that of light on the growth of branching filamentous forms of algae and of moss protonema.

There are two types of hierarchical organization in our patterns, one according to the rank of the branches, and the other according to the total number of growth increments in the pattern. Both types may occur together. The first type of pattern takes the shape of certain kinds of tree. This is because the trunk, the major limbs, and

the secondary and tertiary branches all have their characteristic modes of growth and branching-a feature typical of many trees.

When the parameters vary as a function of the total size of the pattern, the modes of growth and branching differ in parts formed at different times during growth. This is analogous to the growth of moulds or trees when the external conditions change during growth, or when growth proceeds in a different way when the total size of the plant exceeds some critical value. This may be called a sequential morphogenes's (Fig. 4).

Patterns of a special kind result when a special branching sub-routine is applied only once early in the growth of the pattern. This sub-routine causes symmetrical branching, with equal angles between the branches, at the tip of the main branch when it reaches some critical length. The results simulate the venation pattern of maple leaves when five to seven branches are formed, or of clover leaves when there are only three branches (Fig. 5).

Patterns can be limited in extent, so that they resemble real plants more closely. This can be done by stipulating, for each rank, a minimum length beneath which branching cannot occur, and maximum lengths above which growth stops.

A most complex class of patterns resulted from the interaction of a growing pattern with local disturbances

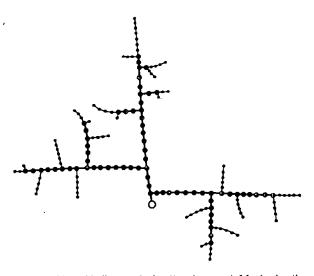


Fig. 4. A hierarchically organized pattern is generated by having the limiting distances for branching vary with the total number of points in the pattern. Both growth and branching are undirected.

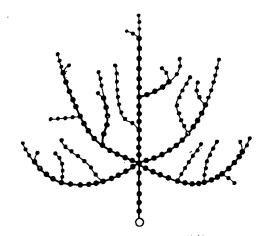


Fig. 5. This pattern is generated by the operation of a special branching rule once early in the generation process, arranged so that a number of branches are symmetrically spaced in relation to the primary axis. Growth is slightly biased upwards during some stages of the generation process, to give a leaf-like pattern.

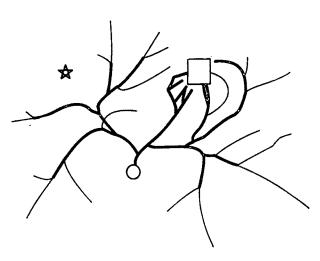


Fig. 8. This pattern is generated by the interaction of a growing pattern with a number of disturbances present in the growth space. Growth is directed towards attracting centres (squares) and away from repulsing centres (stars). The probability of branching increases near the attracting centres, and decreases near the repulsing centres. The growth of a branch which approaches an attracting centre closer than a critical distance is permanently terminated.

in the growth space (Fig. 6). These may be analogous to local sources of food in the growth of moulds, or to cell bodies of neurones which influence the growth and branching of other neurones. In this way, networks with functional characteristics can be produced.

A successful simulation of any one pattern is an analogue of the natural generation process. From such an analogue we can learn the following.

(1) The information content of a simulating computer programme is the upper limit of the minimal amount of information required to specify the simulated pattern. This limit can be expressed in binary bits, or in terms of the equivalent number of genes or the amount of DNA. The maximal information capacity of our programmes is $\sim 6\cdot 10^4$ bits, which corresponds to the DNA content of approximately 30 genes.

(2) The organized set of generation rules may be the most efficient language in which to describe and classify complex biological patterns. Any particular pattern in a class then corresponds to a particular set of values of the parameters in the generation programme for its class.

(3) Although the same pattern may be generated by several different simulation programmes, the characteristics of such programmes can suggest some general requirements for the natural generation process of any one class of pattern. For complex biological patterns, the simulating programmes may be useful in suggesting plausible mechanisms for the generation process. More definite conclusions can be reached about the minimal degree of complexity which is required in the generation of any class of pattern.

(4) A simulation programme which incorporates some hypotheses about the generation process of a natural pattern provides a method for an unambiguous rejection of incorrect hypotheses by comparing the natural pattern and its simulation.

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LETTERS TO THE EDITOR

ASTRONOMY

Friedman Cosmological Models with both Radiation and Matter

Jacobs1 has recently given an analytical solution of Einstein's field equations which represents a homogeneous and isotropic universe containing both radiation and dust; the space-like surface (on which the matter is comoving) is flat. Using arguments based on Mach's principle, however, Wheeler² argues that space is closed. This implies for a Friedman universe that the curvature is positive. But using statistical arguments and observational data, Chiu³ argues that the universe can be described by an open Friedman model with negative curvature. disagreement cannot be resolved at present because the energy density of the universe is not known to sufficient accuracy. Because of this, it seems reasonable to consider all three Friedman cosmological models.

A universe which is spatially homogeneous and isotropic but not necessarily flat can be described by the Robertson-Walker metric⁴

$$ds^2 = -dt^2 + a^2(t) \left[1 + (k/4)u^2 \right]^2$$

$$\left[du^2 + u^2 \left(d\theta^2 + \sin^2\theta d\varphi^2 \right) \right]$$
 (1)

where k=1, 0 or -1 correspond, respectively, to positive, zero and negative curvature of the space-like surface t = constant. We also assume that the radiation and dust expand independently and adiabatically and that $(8\pi/3)$ $\rho_m = K_m \ a^{-3}$ for the dust and $\rho_R = 3p_R = 3K_r a^{-4}/8\pi$ for the radiation, where K_m and K_r are constants. This choice satisfies the conservation law

$$T^{\mu\nu}$$
; $\mathbf{v} = 0 = (\rho a^3) \cdot + p(a^3) \cdot$

where the dot denotes differentiation with respect to time, ρ is the energy density, and p is the pressure. The only remaining equation to be solved is

$$\dot{a}^2 + k = K_m \ a^{-1} + K_r \ a^{-2}$$

If this equation and the conservation law are satisfied, the other Einstein equation is satisfied automatically.

The three solutions are

$$t - t_0 = (K_r + K_m a - a^2)^{\frac{1}{2}} + (K_m/2) \sin^{-1} \left[(K_m - 2a) (K^2_m + 4K_r)^{-\frac{1}{2}} \right]$$

for k=1 (positive curvature);

$$t-t_0=2 (K_m a-2K_r) (K_r+K_m a)^{\frac{1}{2}}/3K_m^2$$

for k=0 (zero curvature);

$$t - t_0 = (K_r + K_m a + a^2)^{\frac{1}{2}} - (K_m/2)$$

$$\log \left[(K_r + K_m a + a^2)^{\frac{1}{2}} + a + (K_m/2) \right]$$

for k = -1 (negative curvature).

The k=0 solution agrees with that of Jacobs¹, but for completeness I have given all three solutions. The requirement that a=0 at t=0 fixes the integration constant to

The Hubble constant $H = \dot{a}/a$ and the deceleration parameter $q=-\ddot{a}/aH^2$ are related to the total energy density and pressure through

$$3_q H^2 = 4\pi(\rho + 3_p)$$

Using the total energy density and the energy density of the radiation, one can (in principle) find the curvature constant k by means of

$$ka^{-2} = (8\pi/3) \rho - H^2$$

Because of the uncertainty in the density, however, there is no general agreement on the value of k (refs. 1-3). The situation is complicated still further because the mass density necessary for the binding of our cluster of galaxies is much larger than that observed.

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Evidence for Lattice Bands in Interstellar Grains

THERE is considerable evidence from the infrared photometry of Johnson¹⁻³ that the wavelength range 3-10μ is associated with strong emission and absorption of radiation by interstellar grains. Several early type stars which exhibit excess infrared emission have been shown³ to possess extended circumstellar envelopes. It has been proposed by Stein⁴ that the thermal radiation from a size distribution of grains heated to about 300° K could explain this excess radiation. In such a model, which contains several arbitrary parameters, the precise location of the maximum emission at about 10µ must be regarded as rather fortuitous.

It has also been claimed that strong absorption amounting to ~1 mag occurs for some stars in the same wavelength range 3-10μ. To explain these observations, Johnson¹ has suggested that a bimodal size-distribution of grains may be present with peaks at radii of 3µ and 0.3μ . We then require that a sufficient density of grains of 3μ radius are present to give an extinction in the $3{-}10\mu$ waveband of about 1 mag over a typical distance scale of ~ 1 kparsec. If a is the radius, Q is the extinction efficiency and s is the density of a grain, the mass extinction coefficient is

$$\varkappa \approx Q \pi a^2 / \frac{4}{3} \pi a^3 s \approx 3Q/4as \tag{1}$$

With $Q \approx 0.3$, $a \approx 3 \times 10^{-4}$ cm and $s \approx 2.5$ g/cm³, we get $\kappa \approx 300$ cm²/g for the large grains at $\lambda \approx 10\mu$. In order to produce an extinction of about 1 mag we thus require a mass density of about 3×10^{-3} g/cm² projected on the sky. Over a distance of about 1 kparsec, the space density required is about 10-24 g/cm³. Although current estimates of the "hidden mass" in the galaxy correspond to a density of this general order, such a high density of heavy elements such as carbon and oxygen cannot be permitted. If the density of these elements were indeed comparable with the neutral hydrogen density, the composition of the surface layers of stars would be drastically different from what is observed. One must therefore look for an alternative explanation of the infrared observations.

We consider here the possibility that grains with impurity induced emission and absorption bands in the $3-10\mu$ waveband may account for the observed effects. Although it does not seem that grains in the general interstellar medium possess strong absorption bands at these wavelengths, the grains in localized HII regions may be considerably different in this respect. grains are exposed to a high flux of energetic particles which could produce lattice defects and consequent changes in their optical characteristics. The solid curve of Fig. 1 shows the infrared absorption spectrum of silicon crystals doped with lithium and boron impurities. The dashed curve is the absorption spectrum of silicon crystals irradiated with thermal neutrons7. In either case, there are strong absorption bands at frequencies close to

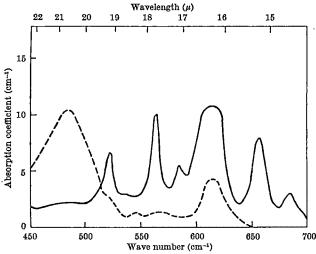


Fig. 1. The solid curve represents the infrared absorption spectrum of silicon crystals containing boron and lithium impurities in concentrations exceeding about 10¹² cm⁻³. The dashed curve is the absorption spectrum of silicon crystals at 77° K irradiated with about 10¹⁰ fast neutrons per cm².

about $k\Theta/h$, where Θ is the Debye temperature. For a typical absorption band the width is about 10 per cent of the central frequency and the oscillator strength is of order unity. A similar effect at about 3µ has been reported for quartz crystals with H-impurities8.

The vibrational spectra of pure crystals also possess peaks at frequencies close to about $k\Theta/h$. Pure quartz crystals show optically active lattice bands throughout the waveband 3-20 μ 9. In general, however, these modes are optically very weak in pure crystals, because the total linear electric moments tend to vanish owing to symmetry conditions. The presence of impurity atoms or defects destroys local symmetry and gives rise to optically active lattice modes. For irradiated graphite crystals, with $\theta \approx 1,000^{\circ}$ K, absorption bands similar to those in Fig. 1 may be expected to occur in a typical waveband 3-10µ. The possibility arises that such bands, in absorption or emission, may be picked up by Johnson's L, M and N

In the case of a star with an effective temperature of about 104 °K which is surrounded by a circumstellar shell, the total energy radiated in a 3µ wide waveband centred at about 10μ (appropriate for Johnson's N filter) is about 1031 ergs/sec. An excess flux of approximately 1 mag would result if the grains included in the field could radiate a comparable amount of energy over the same waveband. The radiating power of an excited impurity oscillator is about $8\pi^2e^2h/m\lambda^3$ ergs/sec, where λ is the wavelength and m is the mass of the oscillator. With $m \approx m_{\rm H}$ and $\lambda \approx 10\mu$, the radiating power is about 10^{-10} ergs/sec per oscillator, so an excess emission of about 1031 ergs/sec would result from approximately 1041 excited oscillators.

The focal plane diaphragm size used in Johnson's photometry is about 15". At the distance of the Trapezium stars this corresponds to a linear size of about 0.03 Johnson's infrared measures could therefore include the flux of an extended volume of about 1050 cm³, which may contain about 1039 grains. The observed excess emission would result if there are about 100 excited oscillators per grain.

Lattice bands occurring in absorption may account for the humps in the extinction curves at about 10µ reported by Johnson¹ for certain stars. The absorption cross-section of an atom near the centre of an atomic line of width y (in frequency units) is

$$\sigma_0 \approx \frac{4}{\gamma} \frac{\pi e^2}{mc} f \tag{2}$$

where f is the oscillator strength. For the usual case, where the line arises from an electronic transition, m is

the mass of an electron. In our case, the effective quantum oscillator is the entire atom so that m must be set equal to about $m_{\rm H}$. With $\gamma \approx 3 \times 10^{14} \, {\rm s}^{-1}$, and $f \approx 1$, we get from equation (2), $\sigma_0 \approx 2 \times 10^{-19}$ cm². In order to produce an extinction of ~1 mag/kparsec we require an oscillator density of about 10-3 cm-3. With a grain density of about 10⁻¹¹ cm⁻³ this corresponds to an impurity content of approximately 10⁸ atoms per grain, or about 1 per cent. It may not be unreasonable to find this high impurity content in grains in localized regions where severe particle irradiation may have taken place.

I gratefully acknowledge helpful discussions with Dr H. L. Johnson and Dr B. T. Lynds.

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PLANETARY SCIENCE

Concentrations of Beryllium-7 over Australia during 1965

In an attempt to provide more information about the behaviour of the southern stratosphere, the fallout research programme conducted by CSIRO has been extended to include the measurement of beryllium-7, a radioisotope produced by cosmic rays, the atmospheric production rates of which have been thoroughly investigated1.

During 1965, a study was made of the tropospheric and stratospheric concentrations of beryllium-7 over eastern Australia. Air filters were exposed at altitudes between 5 and 30 km. Air samples from lower heights (5 to 20 km) were provided for analysis by the US Air Force which, at the time, was operating from Sale, Victoria, as part of the US Defense Atomic Support Agency's Project Stardust. Air samples from greater heights were obtained by carrying an air filtration unit as extra payload on the balloon flights conducted by the Australian Department of Supply at Mildura, Victoria, for the US Atomic Energy Commission ("Project Hibal").

Analysis was by gamma-ray spectrometry after the digestion of the air filters in nitric acid and precipitation of beryllium as the hydroxide. A 2 in. × 2 in. NaI(Tl) well crystal and a Philips single channel analyser were used.

In the case of the Hibal measurements, air volumes were measured by a calibrated flowmeter. Flow rates through the aircraft filter systems were calculated from the reported air speed and density using calibrations provided by Mr G. E. Stout of the Illinois State Water Survey (private communication).

Fifty measurements were made between June and November 1965. Fig. 1 shows the results obtained. Most of the aircraft samples were obtained near Tasmania, while the rest were taken during flights north of Victoria. Accordingly, two sets of aircraft data are shown, corresponding to the mean latitudes 42° S. and 25° S. All the balloon flights were conducted from Mildura (34° S.).

The curves shown in Fig. 1 are the equilibrium steady state concentrations calculated from the production rate model of Lal, Malhotra and Peters, who gave production rates as functions of the geomagnetic latitude. average geomagnetic latitude around a parallel of latitude is, however, numerically the same as the geographic

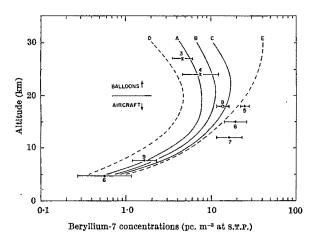


Fig. 1. Measured concentrations of beryllium-7 over eastern Australia at latitudes of 25° S. (○), 34° S. (×) and 42° S. (●) compared with the predicted equilibrium concentrations at the same latitudes (curves A, B and C respectively; see text). The number of measurements contributing to each point is shown, as is the logarithmic standard deviation. Curves D and E give the expected equilibrium concentrations at the equator and at 75° S., respectively.

latitude. Consequently, in the present calculations, the production rates have been weighted according to the range of geomagnetic latitude encountered during an assumed zonal flow.

In the derivation of the steady state concentration profiles of Fig. 1, no effects of mean motion or mixing were considered. Profiles north and south of the sampling latitudes are included for comparison. Errors in the estimated concentrations could be as great as 25 per cent.

The most striking feature of Fig. 1 is the large discrepancy between the measured concentrations of beryllium-7 between 10 and 20 km and the predicted equilibrium values. The four means plotted are about twice the values expected. The tropospheric measurements made using the same aircraft and sampling techniques and employing identical analysing procedures, however, agree well with the predictions of the model. It is therefore unlikely that the large concentrations at about 15 km are caused by errors of measurement alone.

An obvious explanation lies in the earlier disregard of stratospheric transport. Either of two processes, mean motion or mixing, can be invoked to explain the observations. Assuming a mean motion model, subsidence from greater heights and a drift towards the equator would be necessary. Studies of other tracers, however, suggest that if such a horizontal drift exists, its direction is towards the poles.

On the basis of a diffusion model, the large concentrations of beryllium-7 seen in the lower stratosphere can only be explained by stronger mixing poleward of 42° S. From stratospheric heat flux data of the northern hemisphere, Reed and German² derived values of the horizontal and vertical diffusion coefficients in 10° latitude steps. During the appropriate period (January to June in the northern hemisphere) and above 100 mbar, the average diffusion coefficients poleward of 40° were approximately three times those at lower latitudes. The aircraft measurements shown in Fig. 1 can be explained in these terms. The paucity of data and the experimental errors involved, however, make a lengthy discussion unwarrantable at this stage.

On the other hand, the average concentrations of beryllium-7 observed between 20 and 30 km were lower than the steady state equilibrium predictions. Because of the small volumes sampled, the total amounts of beryllium on 'Hibal' filters were small and the errors of measurement correspondingly large. It is possible that the filter material employed was not efficient for the low particle sizes known to predominate at about 30 km. Thus the obvious explanation that the small values observed imply

stronger mixing at lower latitudes should be considered with caution.

Other workers studying cosmic ray spallation products in the lower stratosphere have generally reported concentrations lower than the predicted equilibrium values. Drevinsky et al. measured beryllium-7 at about 35° N. during 1961 and 1962. Before the resumption of atomic weapons testing in October 1961, a mean beryllium-7 concentration of about 9 pc. m⁻³ at s.t.p. was obtained. This low concentration was explained in terms of the short residence time of the polar stratosphere.

Over southern Australia, low concentrations of beryllium-7 were found⁴, and these were attributed to either errors of measurement, errors in the production rate (of the order 25 per cent) or to mixing with the troposphere. Although the accuracy of the measurements now reported may be low, a systematic error of the order 100 per cent would be necessary to explain the results on this basis alone.

Determinations of beryllium-7 at between 20 and 30 km are continuing and attempts are being made to improve the accuracy of measurement. It is intended to expand the programme to include other cosmic-ray spallation products.

We thank Dr A. J. Dyer, Mr E. Curwood, Mr G. E. Stout and the personnel of the Fifty-eighth Weather Reconnaissance Squadron for their help. Mr G. Grauze performed the beryllium analyses.

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Thickness of Etnean Lavas

WALKER¹ has reported observations made on the volcano Etna of the thickness of solid lava flows and the inclination of the slopes on which they lie. He also made estimates of the viscosity of the lava flowing down the slopes of Etna in May 1966, by measuring the thickness and velocity of flow of the lava and the angle of inclination of the slope. The formulae used to determine the viscosity are those for a Newtonian fluid exhibiting laminar flow. Walker sought a relation between the viscosity of fluid lava, the thickness of solid lava and the inclination of the slopes on which the solid lava lies. It seems, however, that such a relation is unlikely because a lava flow having the properties of a Newtonian fluid would continue to flow until no lava at all remained on the sloping surface. Hot lava is observed to flow and cold lava is found to rest on sloping surfaces, however, so we may surmise that flowing lava is not Newtonian in its behaviour, at least as it approaches a fully solid state. Walker's valuable observations enable these questions to be investigated. If the thickness of solid lava flows were determined by the viscosity of the lava when liquid, if this had the properties of a Newtonian fluid, and if the flow were laminar, we should expect to observe a relation between angle of slope, α , and solid thickness, h, of the form

$$h^2 \sin \alpha = K$$

where K is a constant. In practice, Walker's observations do not support a relationship of this form but, rather, one of the form

$$(h_v \max) \sin \alpha = k$$

	Tab	le 1	•
(h max) (metres)	(degrees)	h, max	(h _v max) sin α
20.0	3.9	20.0	13.6
15.0	5.0	15·0	13·1
10.0	7.5	10.1	13.2
5.0	14.0	5.2	12.5
2.5	25.0	9.7	11.6

Values of (h max) and a have been taken from the upper dashed curve of Fig. 1 in Walker¹; $(h_{\tau} \text{ max})$ is taken to be $(h \text{ max})/\cos a$.

where $(h_v \max)$ is the maximum vertical thickness of lava found on any slope (see Table 1). Now $h_v \sin \alpha$ is proportional to the shear at the base of the flow caused by the weight of the overlying lava. That $(h_{\nu} \text{ max}) \sin \alpha$ should be constant for the solid lava flows on Etna suggests that in the final stages of solidification, as the final thickness is determined, the lava is non-Newtonian and approximates in its behaviour to a Bingham plastic2. If the stress acting on a Bingham plastic is smaller than a critical value, which is called the yield stress, no flow occurs, but if the stress acting on it exceeds this value then flow does occur. In the case of lava we may suppose that, as solidification occurs, flow continues until the thickness reaches a value such that, for the slope in question, the shear at the base of the lava caused by the weight of the overlying material becomes smaller than the yield stress, and that at this point flow ceases. If lava flows in the final stages of solidification do behave as Bingham plastics, it is clear that no lower limit to the thickness of a flow is set by this property and so flows may be of any thickness up to the limit set by the yield stress characteristic of the lava concerned and the inclination of the slope on which the lava lies. Walker's observations are consistent with these conclusions if the yield stress of Etnean lava in the final stages of solidification is about 2.5×10^6 dynes/cm². It follows that, in general, in the case of lavas tilted after their solidification, it is only the maximum thickness of lava at any locality which may supply information about the original slope.

I thank Mr C. A. W. Deane for drawing my attention to the properties of Bingham plastics.

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Origin of Round-body Structures in the Orgueil Meteorite

MICROBIOLOGICAL examinations of carbonaceous meteorites have been conducted by Oro and Tornabene¹. They discovered bacterial contamination on two carbonaceous chondrites but were unable to detect bacterial affinity for a third meteorite—the Orgueil. We therefore have chosen to examine the Orgueil meteorite2 because of this reported lack of bacterial contamination. Reports of biological materials and life-like objects associated with the Orgueil meteorite, as presented by Nagy and Claus and their co-workers^{3,4}, have, however, aroused speculation as to whether they could be indigenous. cautious, one can always entertain the possibility of terrestrial contamination or artefacts created during treatment of the meteorite. Because there is ample evidence for biogenic substances existing in the Orgueil meteorite⁵, evaluation of their origin would seem to be the next step to take. To investigate the problem of contamination, we have attempted ultra-thin sectioning of mineral pieces

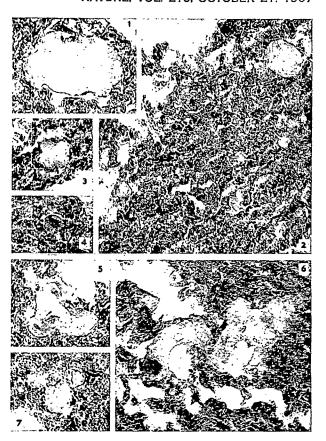


Fig. 1-7. Electron micrographs of ultra-thin sections of the Orgueil meteorite showing the round structures.

of the Orgueil meteorite. Nagy has advised us that any biological-like structures firmly embedded in the mineral matrix of the meteorite obviously could not be caused by contamination.

Small pieces of the Orgueil meteorite (from the Smithsonian Institution, Washington, DC, by courtesy of Dr G. Claus, Long Island University) were embedded in 'Epon' resin to facilitate sectioning. Being very hard, the meteorite specimens were sectioned with a diamond knife on an LKB microtome. The ultra-thin sections were then examined under a RCA EMU-3G electron microscope at 100 kV.

Electron micrographs of ultra-thin cross sections of the Orgueil meteorite reveal that the interior proper of the meteorite consists principally of ridges irregularly fitted together. These ridges are electron dense and are obviously the mineral materials. These mineral materials, although fitted together in one piece, are not structurally continuous—they are interspersed with gaps or fissures. The smaller holes are probably simply air pores. Among this vast area of mineral matrix, however, numerous objects or round structures can be readily observed. Contrary to the mineral materials, these round bodies are electron opaque. The surface, as well as the interior matrix of these round bodies, is continuous (Figs. 1-7). In addition, the surface of some of these round structures seems to be firmly and intimately enclosed by a thin layer of dark wall with an approximate thickness of 200 Å (Fig. 1). This enclosure might be contributed by the surrounding meteorite mineral matrix or might be an integral part of the round structure itself. The size of these objects is not uniform. They have various diameters of $1/8\mu$, $1/4\mu$, $1/2\mu$ and 1μ (Figs. 1-7). Most of them are round and single, but sometimes they appear together in close proximity to each other (Fig. 2) and are irregular in shape (Figs. 5 and 6).

The size, shape, electron density, interior matrix continuity, well defined surface contour and the thickness of the surface wall suggest that these round structures (bodies) possess some degree of organization (structural pattern). The mere fact that these structural bodies are located within the matrix of the meteorite mineral would render them relatively out of reach of factors which might produce artefacts or external contaminations. We are not suggesting that these organized structures are strictly biological bodies but we do wish to point out that they are very likely to be indigenous to the meteorite itself.

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Late Pliocene-Pleistocene Stratigraphy in Deep Sea Cores from the South-central North Atlantic

PALAEOMAGNETIC studies of deep sea cores have shown that the history of geomagnetic polarity reversal is recorded in sea floor sediments¹⁻⁸. The local extinction of certain species of Radiolaria can be related to palaeomagnetic polarity reversals1,4,8, and specifically there is an association of the Brunhes-Matuyama polarity reversal at 0.7×10^6 yr ago with the ψ/X radiolaria faunal boundary4,8. Another such extinction has been suggested^{4,9}; the Φ/X radiolaria boundary, occurring within the Olduvai Normal polarity event about 2×10^6 yr ago. approximates the Plio/Pleistocene chronostratigraphic boundary defined by Ericson^{10,11} for oceanic sediments. Although this chronostratigraphic age assignment may be correct, the relationship of the geomagnetic polarity time scale to the biostratigraphic units used to define Plio/Pleistocene geological history has not been demon-This results primarily from the lack of appropriate palaeontological criteria in deep sea cores which can be correlated with the standard (type) sections of the Pliocene/Pleistocene of Italy.

In this report we make a preliminary attempt to relate the "absolute" geomagnetic time scale to Late Pliocene! Pleistocene geological history on the basis of new palaeomagnetic and palaeontological evidence in three deep sea cores from the central North Atlantic. A more complete discussion of our investigation of these cores has been given elsewhere2; here we give a brief account of our results.

The cores contain a distinct planktonic foraminiferal fauna, elements of which are found in the type marine Plio/Pleistocene sediments of Calabria, Italy¹². Specifically, Banner and Blow12 in their biostratigraphic zonation of the Neogene have drawn the base of the Pleistocene at the base of their zone N22. This zone is defined on the first evolutionary appearance of Globorotalia truncatulinoides from its immediate ancestor G. tosaensis. This transition was observed to occur near the base of the holostratotype Calabria at Santa Maria di Catanzaro, Italy. The utility of this zonation was demonstrated¹³ in a study of deep sea cores from the tropical Indo-Pacific Ocean. The Calabrian stage has been shown to be older than the earliest Pleistocene continental glaciations and an age of 1.8 × 10° yr has been estimated for the Pliocene/ Pleistocene boundary.

Preliminary measurements of the natural remancut magnetization (NRM) in eight piston cores obtained during Chain Cruise 61 across the Mid-Atlantic Ridge were made with an astatic fluxgate gradient magnetometer15. Three of the plastic tube-lined cores (Chain 61 (171). (174) and (175)) showed reversals of remanent magnetization. These core tubes were split open and small oriented cylindrical samples were extruded into polyethylene

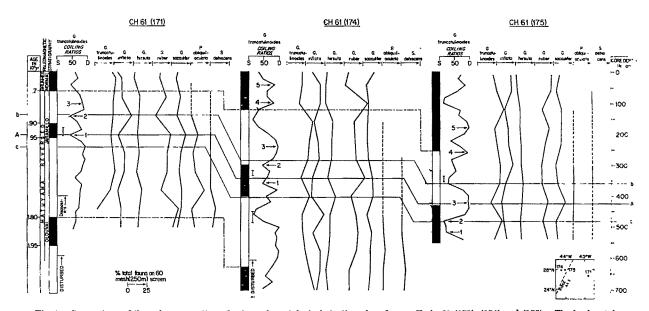
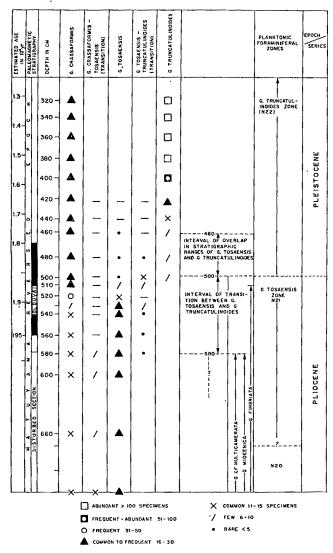


Fig. 1. Comparison of the palaeomagnetic and micropalaeontological stratigraphy of cores Chain 61 (171), (174) and (175). The horizontal lines show suggested levels of correlation among the cores based on distinctive fluctuations in the coiling ratio abundance of *G. truncatulinoides* and the relative abundance of the indicated foraminiferal species. The numbers are believed to mark points of general correlation in the coiling ratio curves for each core. The vertical bars adjacent to the magnetic stratigraphy column for each core delineate narrow bands of light grey brown foraminiferal lutite in the otherwise homogeneous pale brown foraminiferal lutite. The sketch map shows the location of the cores.



Relative abundance of Globorotalia truncatulinoides and Globorotalia tosaensis in the Chain 61 (171) core.

canisters and encapsulated with epoxy resin to form a rigid 5 c.c. sample. The NRM was measured again with a high frequency spinner magnetometer¹⁶.

The stability of each normal and reversely magnetized section along the cores was tested by partially demagnetizing representative specimens from each section in alternating magnetic fields¹⁷. The palaeomagnetic stratigraphy of the three cores after demagnetization at 150 oersted is shown in Fig. 1, together with curves showing the coiling ratio abundance of Globorotalia truncatulinoides and the relative abundances of certain foraminiferal species. All three cores appear to have penetrated the Jaramillo normal magnetic event at about 0.9 × 10° yr, the Chain 61 (171) core reaching the reversed interval below the Olduvai normal magnetic event about 2.0×10^6 yr ago.

In the Chain 61 (171) core, the transition from G. tosaensis to G. truncatulinoides was found to range from 580 to 500 cm and to occur within the Olduvai Normal event (Fig. 2). The first evolutionary appearance of G. truncatulinoides is at 500 cm, about 30 cm below the top of the Olduvai. Using the Pitman and Heirtzler¹⁸ time scale for palaeomagnetic stratigraphy, an age of 1.85×10^{6} yr is suggested for the base of the Pleistocene.

Globorotalia truncatulinoides develops from being a relatively rare form in Chain 61 (171) core at 500 cm to a dominant position in the microfauna above 400 cm.

This level was found to coincide approximately with the first disappearance of abundant discoasters in this core (between 420-400 cm); a brief reappearance of discoasters was found at 162-164 cm, within the upper part of the Jaramillo event.

The first appearance of Globorotalia inflata, a temperate water species, was found to coincide with the first evolutionary appearance of G. truncatulinoides. Globorotalia inflata has its maximum absolute abundance north of 40° N. latitude in the North Atlantic and maximum relative abundance approximately between 40° and 50° N. latitude.

The sudden appearance of G. inflata at 500 cm is interpreted to indicate a general decrease in the surface temperature of the sea. This climatic deteriorationmild though it probably was—may correspond to that recorded at the base of the Pleistocene at Le Castella, Calabria, in southern Italy¹⁹.

A comparative study was made of the three cores (Chain 61 (171), (174) and (175)) in an attempt to relate faunal changes to palaeomagnetic stratigraphy. results of our observations can be summarized as follows. (1) Although a normal magnetic event was recorded in the lower part of the Chain 61 (174) core, the planktonic foraminiferal fauna was wholly of Pleistocene character; the abundance of G. truncatulinoides and the absence of discoasters suggested correlation of the bottom of Chain 61 (174) core with a level above 400 cm in Chain 61 (171) core. Visual examination of the lower part of the Chain 61 (174) core suggests that vertical disturbance may be The resolution of this apparent conflict is left open at present. (2) A distinct inverse relationship was noted at a level within the Jaramillo event between the abundance of temperate species Globorotalia inflata, G. hirsuta and G. truncatulinoides on the one hand, and warm water Globigerinoides ruber, G. sacculifer and, in the case of the Chain 61 (171) core, Pulleniatina obliquiloculata. The marked increase in the former group is suggestive of a significant cooling at this level. The relatively common occurrence of G. inflata above the Jaramillo in all three cores is interpreted as indicating a continued important cooling trend within the Upper Pleistocene. In the light of recent evidence that the four "classic' glaciations of the Pleistocene may well have been preceded by one or more earlier glaciations, we suggest that this cooling event within the Jaramillo may indicate the onset of Pleistocene continental glaciation, or, at least, a marked cooling trend before the initial stage of glacia-

We thank Mr F. Jones and Mrs J. Brown, who made the measurements, Miss J. Golden for her help in the micropalaeontological analysis, the officers and crew aboard R.V. Chain for their co-operation and R. P. Von Herzen for helpful criticism.

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THE SOLID STATE

Debye-Scherrer Powder Diffraction using 55 Fe K X-rays on Lithium Fluoride

POWDER and single crystal diffraction of X-rays obtained from radioactive isotopes has been reported by this laboratory¹⁻³. The source was cyclotron-produced ⁵⁵Fe, laid down in a linear array on a platinum disk¹ (Fig. 1). Photons studied were the K_a X-rays from ⁵⁵Mn, the stable daughter product. The activity of the source was 100 mc., but this was reduced to about 19 mc. by self-attenuation and further reduced to about 13 mc. by natural decay at the time of this work. Experimental geometry used was that of the conventional diffractometer. Detection was by a proportional counter and relatively sophisticated electronics. Other workers (with the loan of this 55Fe source designed by this laboratory for use in the work reported) later also observed the more intensely diffracted beams from metallic (iron foil) samples4.

Concurrently, in Russia, Lobov and co-workers reported on film recording with an 55 Fe source in a Preston camera $^{5-7}$ of $25\cdot 4$ mm diameter. Their reported diffraction samples have all been metals, lead, copper and stainless steel, with the patterns describing the backscatter region and exhibiting one or two lines in evidence. Lobov's 55Fe source was produced by a reactor and was therefore contaminated with 59Fe as well as 54Mn, their gamma rays presenting, unfortunately, a serious film fogging problem.

A preliminary consideration was made of the relative source strengths, carrier-free character and potential

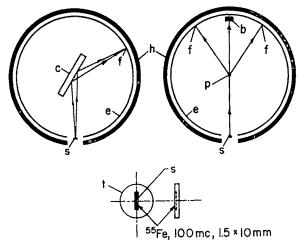
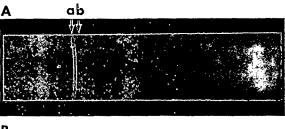


Fig. 1. The two geometries (single crystal (c) and powder (p)) used in this diffraction study. The linear **Fe source (s) is positioned, in both cases, within shielding, coincident with the circle defined by the emulsion (e). The focal point on the emulsion is indicated by (f). The emulsion (e) is mounted in a groove in the brass film housing (h). The **5Fe is laid down in a linear array 1-5 mm by 10 mm in a shallow recess in a 19 mm diameter platinum disk (t). The powder sample (p) is housed in a thin-walled glass capillary. The principal beam stop is at (b). A slit collimator, adjacent to the source (not designated), constrains the manganese K X-ray beam into a slightly divergent flat sheath; 2 mm wide at the sample and 4 mm at the beam stop.

exposure intensities. It was concluded that, with our uncontaminated, more intense 55Fe source, improved diffraction pattern recording by film exposure would be possible; and, more importantly, that nonmetallic powder samples might be analysed. A small (50.8 mm diameter) Debye-Scherrer camera was therefore designed and constructed.

The camera (Fig. 1) was constructed of brass. It consists of three parts: the base plate, with a centrally raised platform for the diffraction sample; the film housing, which fits on the base and is free to rotate through 360° about the stationary base; and the source holder and beam collimator, which fit into the film housing and turn with the film relative to the base plate, thus allowing for relative motion of the source and film with the sample. The source, when in place, is coincident with the diffraction circle. A slit collimation system is mounted in front of the source and inside the film circle. The collimator is 1 mm × 10 mm by 11 mm long. Its aperture is 14.4 mm from the centre of the system. The film fits in a recess around the inside of this housing.



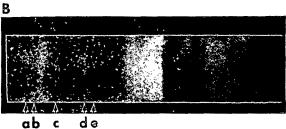


Fig. 2. A, A diffraction pattern of the 200 plane of a single lithium fluoride crystal produced by exposure for 2 h to the 13 mc. ⁵⁵Fe source in the camera previously described. a is the reflexion of the K_{α} doublet and b is the reflexion of the K_{β_1} radiation. B, A diffraction pattern from lithium fluoride powder produced with exposure for 118 h. The diffracted lines are identified as the reflexion of the K_{α} doublet on the following planes: a, the 222 plane; b, the 311 plane; c, the 220 plane; d, the 200 plane; and e, the 111 plane.

A single lithium fluoride crystal (Harshaw Chemical Co., Cleveland, Ohio) and 200 mesh lithium fluoride powder were selected as standards for the investigation. The preliminary studies carried out with the single lithium fluoride crystal immediately demonstrated photographic evidence of diffraction (Fig. 2A). The crystal was aligned to allow for diffraction from the 200 plane, and an exposure time of 2 h was used. The film was then developed for 4 min at 68° F in Kodak rapid X-ray developer and fixed in Kodak X-ray fixer. The emulsion was Kodak X-ray 'No-Screen'. The strong line is due to manganese K_a on the 200 plane, the weaker line is due to manganese K_{β_1} on the 200 plane.

After this single crystal success, lithium fluoride powder was mounted in a thin-walled (0.01 mm) Lindemann glass capillary with an inside diameter of 1 mm. The capillary was then mounted in a vertical position at the centre of the camera. The powder pattern obtained after exposure for 118 h is shown in Fig. 2B. Five of the diffracted lines caused by the K_a X-radiation of manganese are evident. The characteristic feathering of the line is due to the slit collimation. Developing procedures were the same as before. The various lines are identified in the caption.

These diffraction patterns obtained without the benefit of special intensification or developing techniques prove that recording of powder diffraction, by means of ordinary emulsion, is possible with an ⁵⁵Fe source of relatively low intensity. The exposure times used for the single lithium fluoride crystal (2 h) are in the range of practical work. The times necessary, however, for the powder diffraction patterns (118 h) are longer than desired. For both sample types, improved resolution would also be desirable, but analytical determinations can be made from the present data.

In order to see to what extent these conditions could be improved, a much more intense 55Fe source was studied (500 mc.) which had a self-attenuation of only about 25 per cent, compared with the 81 per cent for our source. On the basis of this comparison, it was found that an intensity improvement factor of 50 is currently obtainable. With such a source therefore exposure times could be reduced drastically or diffracted line width reduced appreciably. There is good evidence that the total output of such a source could be made still greater. In addition, the camera can obviously be made more efficient with a vacuum facility. Refinement in the alignment of this laboratory model is important and improvement is possible. It therefore seems that a zero power diffraction system of this type can be made truly competitive with standard techniques, especially where weight, portability, stability and power requirements are critical and are to be optimized.

We thank Jonathan Parsons for help in the development and densitometry of the diffraction patterns, G. Bartrum for the construction of the camera, and A. L. Klascius, Jet Propulsion Laboratory, for lending the 500 mc. source.

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Strain Polarizability Constant: a Measure of Ion Overlap in Crystals of Sodium Chloride Structure

It is well known that when a cubic crystal of sodium chloride structure is stressed the refractive index changes and is different in different directions. Mueller¹ pointed out that the observed difference between the refractive indices for mutually perpendicular directions—that is, along the direction of stress and a direction perpendicular to it—is always smaller in magnitude, and for some crystals even different in sign, than the calculated one. He explained this discrepancy by assuming that the polarizability of an ion changes with the application of stress. If a hydrostatic pressure is applied, the change in the polarizability is represented by the strain polarizability constant λ_0 . From the data then available Mueller assumed that λ_0 is constant for all the crystals.

Burstein and Smith² pointed out that λ_0 is not a constant but varies from crystal to crystal. They also pointed out that λ_0 is a measure of ion overlapping and homopolar bonding in the stressed state. According to them, the magnitude of λ_0 depends on the initial amount of overlapping and homopolar bonding present in the crystal. The larger the amount of overlapping in the unstressed state, the greater will be the value of λ_0 in the stressed state. As examples, they mentioned lithium fluoride and magnesium oxide.

The aim of this communication is to show that λ_0 is a measure of ion overlap even in ionic crystals of sodium chloride structure other than lithium fluoride and magnesium oxide.

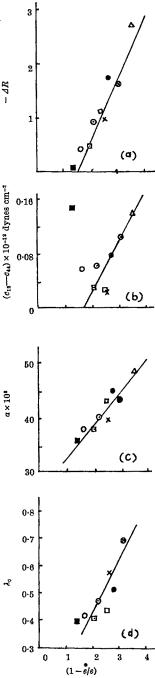


Fig. 1. $(1-\frac{e}{e}/e)$ versus (a) change in the molar refractivity ΔR , (b) difference between the elastic constants c_{17} and c_{44} , (c) coefficient of thermal expansion a, and (d) strain polarizability constant λ_{e} , respectively. \times , Sodium chloride; \otimes , sodium bromide; \triangle , sodium iodde; \bigcirc , potassium chloride; \bigcirc , potassium bromide; \bigcirc , potassium ioddide; $\boxed{\times}$, rubidium chloride; \bigcirc , rubidium bromide; $\boxed{\square}$, rubidium iodde.

Szigeti³ pointed out that Born's equation for the difference between the static and optical dielectric constants, which is based on the assumption of non-deformable and non-overlapping ions, is not in agreement with the observed values. In order to explain the discrepancy, he introduced the concept of "effective" ionic charge & which is less than e. He assumed that the electron cloud of the ions becomes distorted when these ions are in the solid state.

The magnitude of the electron charge, which resides in the distorted and overlapping regions, contributes to the homopolar bonding4,5, thus reducing the ionic strength of

the alkali halide crystals. Recently, Sirdeshmukhe, using the concept of effective ionic charge, showed that Megaw's relation for thermal expansion is valid for some of the alkali halide crystals.

It is well known that when ions change their state, that is, when they go from the gaseous state to the solid state, the molar refractivity changes and a deviation from the additive law is observed. The existence of non-central forces is represented by the fact that the elastic constant c12 is not equal to c_{44} . Similarly, Megaw's relation between thermal expansion and electrostatic share for alkali halides fails if the normal electronic

charge e is used. All these observations can be explained on the basis that part of the charge which resides in the distorted and overlapping region is responsible for the deviations. In order to show this, graphs have been plotted showing how the change in molar refractivity ΔR , the difference between the elastic constants $(c_{12}-c_{44})$, and the thermal expansion α , vary with the electric charge $(1 - \dot{e}/e)$, and are shown in Figs. 1(a), 1(b) and 1(c), respectively. These graphs clearly show that the change in the molar refractivity ΔR while going from the gaseous state to the solid state, the difference between the elastic constants c_{12} and c_{44} , and the thermal expansion of the alkali halide crystals increase with the decrease of the effective charge e.

In view of these observations, the strain polarizability constant of some of the alkali halides is plotted against (1 - e/e) and is shown in Fig. 1(d). This graph suggests that as the charge in the distorted and overlapping region increases, λ_0 increases. It means that the variation in λ_0 from crystal to crystal is an indication of the amount of ion overlapping present in these crystals in an unstressed state. This conclusion is in agreement with the views expressed by Burstein and Smith2.

Values of molar refractivity, elastic constants, thermal expansion, effective charge and λ_0 are taken from different sources and are listed in refs. 7–13.

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PHYSICS

Interaction of Self-trapped Light Beams

PRELIMINARY experiments have been carried out on the interaction of two self-trapped light beams in water. The 200 MW, 20 nsec output from a Q-switched ruby laser* was split by prisms and focused into a glass cell containing water to form two self-trapped beams (Fig. 1). Photographs were taken using the light scattered sideways from these beams.

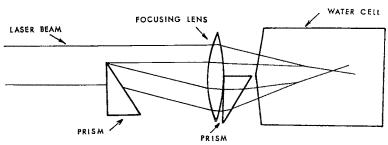


Fig. 1. Optical system used to produce two crossing self-trapped light beams.

Two types of scattering from self-trapped beams were observed; type A occurred when high purity water was used; type B was only produced in water which had been allowed to stand in an uncovered cell. When type A scattering was observed, the beams were characterized by fineness, short length, sharp cut-off and a tendency to scatter from a large number of discrete centres along the beam (Fig. 2). When type B scattering was observed, the beams were much longer than those associated with type A and the scattered light was much more diffuse, so that it was not always clear whether self-trapping had occurred (Fig. 3).



Fig. 2. Interaction of two crossing "type A" self-trapped beams. ($\times 1$.)

The apparatus used introduced distortion into the lower focused beam. The higher and lower edges of this beam were more intense than the centre, particularly the lower edge where self-trapping appeared to occur before the focal point was reached. Beyond the focal point two self-trapped beams were observed. The stronger beam travelled straight out from the end of the cone while the weaker appeared to be a continuation of the self-trapped lower edge of the cone. The weaker beam, after splitting from the stronger at the focus, bent and continued parallel

Using the optical system shown in Fig. 1, the interaction of the two crossing light beams was observed. In the case of type A scattering, interaction was shown as an intense

*Ruby laser Bradley type LH 351, delivering 200 MW in 20 nsec distributed among a number of transverse modes (between 1 and 8). Beam divergence, 8 milliradians.

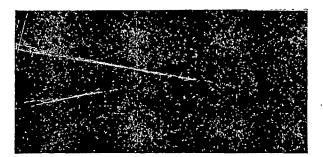


Fig. 3. Interaction of two crossing "type B" self-trapped beams. (×1.)

spot at the cross-over point (Fig. 2), but no significant effect on the beams could be observed because their scattering was not uniform. In the case of type B scattering, however, a considerable reduction in the intensity of the upper beam was always observed (Fig. 3). It is possible that this upper beam was not self-trapped, in which case interaction was between a high intensity light beam and self-trapped light. A considerable amount of energy was lost by the upper beam. This could have been reflected into the liquid or back along the pipe by the reflexion coefficient of the cross-over point, or could have been converted into shock energy. Whatever the mechanism, it is notable that the loss occurred only from the upper beam.

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Observations on the Shape of Chromium Oxide Particles in Hydrogen+Oxygen+Nitrogen Pre-mixed and Diffusion Flames

CERTAIN metals, when introduced into pre-mixed, hydrogen + oxygen + nitrogen flames ($T \sim 2,000^{\circ}$ K) burning above a Meker type burner, give a whitish, streaky appearance to the flame¹. Such streaks appear, for example, when chromium, vanadium, titanium, cobalt, nickel or iron (to mention but a few) are introduced in the form of fine sprays of dilute salt solutions. These streaks almost certainly originate from chemiluminescent reactions involving H and/or OH radicals on the surfaces of particles of involatile oxides¹, and their visible appearance alone clearly indicates that the particles consist of many molecular units. Light scattering and electron microscopy studies^{2,3} have shown similar particles to have sizes in the range $0.005-25\mu$.

An interesting effect observed—or rather heard—in such systems, with concentrated (~molar) aqueous solutions of chromic acid is a quiet but distinct decrepitation. This phenomenon could well arise as a side-effect of the manner in which these particles disintegrate in the flame, and what follows might reasonably be interpreted to support such a view.

Electron microscopy of carbon-coated copper grids passed through pre-mixed flames into which chromic acid had been sprayed revealed irregularly shaped particle fragments, of average size 50–100 Å. Similar experiments in diffusion flames, however, revealed larger fragments, and occasionally it was possible to pick up large, perfectly spherical particles like that shown in Fig. 1. Moreover, such particles were clearly hollow, and equally clearly

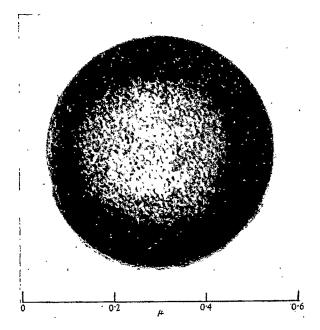


Fig. 1. Chromium oxide particle in hydrogen diffusion flame.

much of the debris on the grid originated from such hollow spheres.

Particles of size $\sim 1\mu$ (Fig. 1) contain the same number of chromium oxide molecules as would be present in an aqueous droplet of M chromic acid approximately ten times the particle in diameter. This predicted value of 10μ for the droplet size agrees very well with the size measured experimentally under comparable conditions⁴. Previous work in another context¹ has also shown that water droplets, once formed at the atomizer, do not significantly coalesce in the time taken (0.5 sec) to reach the flame, and it is therefore clear that each sphere observed on the grid represents molecules present in a particular water droplet at the instant of droplet forma-

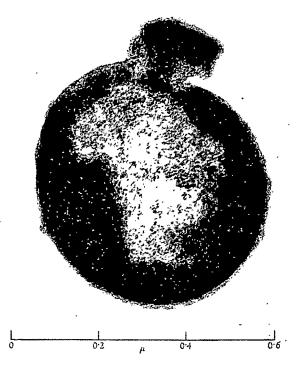


Fig. 2. Chromium oxide particle in hydrogen diffusion flame.

tion. If this were the case, then lowering the surface tension of the atomizer solution should cause the particle size to decrease; this effect has also been observed in the present work in experiments in which the chromic acid was made up in acctone and water mixtures.

The fate of a droplet bathed in flame gases may be visualized as one of rapid contraction of a spherical shape, the centre at least of which may retain a trace of solvent until contraction can no longer take place. At this stage, the temperature of the surface rises particularly rapidly by both physical and heterogeneous chemical processes, causing the particle to solidify, melt or vaporize (depending on temperature). Decrepitation might then result if the last traces of solvent were to cause particles which are molten on the surface to explode. Fig. 2 shows strikingly such a particle at the instant of disintegration.

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Table 2. VIBRATION PROGRAMMES (VIBRATORS APPLIED AT CENTRE BETWEEN CLAMPS)

Freq.	Magnetic		Reson-	Freq.	Hydrau Amp. (in.)	lie vibrate Loops	Reson- ance
(c/8)	(in.)	Loops	ance	(c/s)	•	TOODS	MACC
0	0	0		0	0	0	
23.3	0.188	1	Yes	8.3	0.031	1	No
35.0	0.032	1	No	16-7	0.031	1	No
46.7	0.125	3	Yes	25.0	0.050	1	No
70.0	0.020	3	No	31.7	0.250	1	Yes
93.3	0.093	š	Yes	40.0	0.031	1	No
160.0	0.015	5	No	50.0	0.031	3	No
187.0	0.063	5	Yes	58.3	0.031	3	No
250.0	0.010	ž	No	ő	Ô	Ō	*****
333.0	0.010	ģ	Ño	-	-		
000	0	ó					

Amplitudes are half the peak to peak displacement.

The experiments consisted essentially of establishing a given flow condition without vibration and measuring the discharge, pressure drop and temperature for that condition. Then, a programme of transverse vibration was followed noting the changes, if any, in the quantities. No changes were observed for any flow condition (except in a series of tests with water at Reynolds numbers above 2,200, as already noted). A typical set of data for aircraft hydraulic oil (AN-O-366) is quoted in Table 1 and vibration conditions are described in Table 2.

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¹ James, D. D., and Morton, A. S., Nature, 214, 692 (1967).

Comments on Effect of Vibrations on the Transition Regime in Pipe Flow

The results recently reported by James and Morton¹ disagree with results which I obtained in 1951 in research supported by the US Office of Naval Research. My work actually covered the effects of transverse and longitudinal vibrations on flow in circular tubes over a range of Reynolds numbers from 750 to a little more than 10,000, including the transition regime. I concluded that vibration had no effect on the friction factor except that at Reynolds numbers above about 2,200 flow which was laminar without vibration became turbulent with certain types of vibration. (Laminar flow was obtained to a Reynolds number of about 15,000 in the apparatus using a special head tank and water as fluid.)

My tests in the transition regime delineated by James and Morton were made in an aluminium alloy tube (12 ft. long, 0.5 in. outside diameter, 0.065 in. wall thickness), using chiefly aircraft hydraulic oil as fluid. The tube was clamped near both ends to give fixed end conditions about 9 ft. 2 in. apart. Piezometer taps were located 11.5 ft. apart, outside the clamps. The taps were carefully deburred by reaching in from the ends of the tube. An entrance length of about 30 in. was joined smoothly to the upstream end. A heat exchanger was provided to

maintain constant temperature.

CHEMISTRY

Variations in Ozone Formation across the Ozonizer Discharge Gap

I have studied, by means of the planar ozonizers with quartz windows, described previously1, the variations in the formation rate of ozone in the discharge gap. Double-glass and metal-glass ozonizers with 3 mm gaps were used with dried air and oxygen at a pressure of 760 mm of mercury and in the temperature range 20°-25° C.

A beam of radiation of wavelength 2537 Å, which is heavily absorbed by ozone, traverses the discharge gap with its axis parallel to the ozonizer electrodes, and a movable slit, 0.16 mm wide, selects the radiation which has passed through a known region of the gap. This radiation reaches a quartz-window photomultiplier with a dielectric filter transmitting at 2537 Å and the output voltage of the photomultiplier controls one beam of a cathode ray oscillograph to indicate ozone concentration in the chosen region. A single-shot time base is used which is triggered either by the discharge or a few milliseconds before discharge. The other beam indicates the ozonizer current; typical oscillograms are shown in Fig. 1. The first three half-cycles of a discharge are usually unsteady, and in deducing formation rates later half-cycles, usually from the sixth to the twelfth, were used. To find the formation rate in a half-cycle from the oscillogram, a correction for diffusion is made by using the slope in the quiescent part of the half-cycle. A correction is also

Table 1. Typical measurements in transition regime using aircraft hydraulic oil (AN-O-366)

Reynolds number	Vibration programme	△p (lb./in.² ft1 measured)	Δp calculated . for laminar flow	λ	Effect of vibration	Temp.	Mean pressure (lb./in.º above atmos.)	Kinematic viscosity* (ft.*/sec×104)	Unit weight (lb./ft.*)
1460	A	0.826	0.858	0.042	None	74	78	2.17	53-0
1530	.B	0.958	1.030	0.0406	None	70	91	2.38	53.1
1960	\boldsymbol{B}	1.390	1.400	0.0323	None	70	134	2.40	53.1
2120	\boldsymbol{B}	1.480	1.510	0.0294	None	70	150	2.40	53.1
1950	A	1.390	1.395	0.0326	None	70	135	2.40	53.1
2110	A	1.480	1.510	0.0297	None	70	148	2.40	53.1

^{*}Determined by Engler viscosimeter at atmospheric pressure and corrected to mean pressure by adding 1.5 per cent for each 100 lb./in.² above atmospheric pressure.

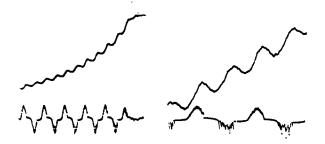


Fig. 1. Oscillograms of photomultiplier output (upper trace) against ozonizer current. Time increases from right to left. The oscillograms are for a distance of 0-20 mm from the glass wall of an aluminium-glass ozonizer using oxygen at 160 c/s. The first shows the first eleven half-cycles of a discharge, while the second shows only the seventh to the tenth half-cycles of another discharge.

necessary for the varying concentration of ozone across the ozonizer at the chosen half-cycle and when air is used a small correction is necessary for radiation from the discharge. The metal-glass ozonizer had an active area of 6.35 cm², the metal being aluminium and the dielectric soda glass 1.30 mm thick. Plates of the same thickness were used in the double-glass ozonizer. The frequency of the supply voltage was 160 c/s and the currents used were from 500 to 1,200 μamp for the metal-glass ozonizer and from 100 to 200 µamp for the other.

The results (Fig. 2) were unaffected by the intensity of the ultraviolet beam or the current density. Increasing the supply frequency to 300 c/s produced no changes and measurements at 50 c/s were consistent with those at 160 c/s when allowance was made for the much larger diffusion effects.

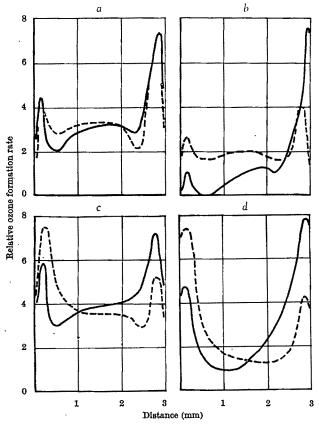


Fig. 2. Relative ozone formation rate against distance from the lower wall of the discharge gap. The full line refers to the upward half-cycle, when electrons and ions are moving away from the lower wall, which is the metal one for the aluminium-glass ozonizer. The broken line is for the downward half-cycle. The currents differ for the four pairs of curves. Aluminium-glass ozonizer: a, oxygen; b, air. Double-glass ozonizer: c, oxygen; d, air.

The results for both ozonizers and gases show clearly that the ozone formation rate varies greatly across the discharge gap, which is important when deducing from ozonizer characteristics the reactions leading to ozone formation^{2,3}. The accuracy of the relative formation rates is about ± 25 per cent near the main peak for all curves and elsewhere it is ± 0.4 for oxygen and ± 0.6 for air in the metal-glass ozonizer and ± 0.6 for oxygen and ± 0.8 for air in the double-glass ozonizer. A noteworthy feature is that, in a metal-glass ozonizer using air, 50 per cent more ozone is formed on the downward half-cycle compared with the upward. This was checked by illuminating the gap uniformly and detecting all the emergent radiation. The same charge passes in both half-cycles, for tests on the ozonizer current showed that no rectifier action occurs. Experiments with fused quartz as the dielectric, in the metal-glass ozonizer using oxygen, gave similar curves to those shown, but the main peak near the dielectric was 25 per cent stronger on the upward curve than on the downward. The use of borosilicate glass reduced the peaks near the metal to the same height as the central region, but the intervening minima were preserved and elsewhere the curves were the same as for soda glass.

The location of ozone formation has been discussed by various authors^{2,4,5}, but no detailed theory has been attempted and no direct results have been reported. The curves given are for formation rates averaged over a halfcycle. It seems reasonable to expect that the curves for the electrostatic field, averaged over the active part of the half-cycle, will bear a general resemblance to those for ozone formation and that large potential drops therefore occur at the sites of the large formation peaks. In the upward discharge of a metal-glass ozonizer, using oxygen, there are thus two regions of marked potential change corresponding to the cathode fall and anode fall in a lowpressure glow discharge. The anode fall of the former discharge indicates the presence of large space charges, and while these may be caused by the ready formation of negative ions in oxygen a more important agent may be the backscattering of ions and electrons at the dielectric surface. The differences in the air curve compared with the oxygen curve may then be explained as being caused by the stronger backscattering of electrons at the dielectric surface. The large anode fall in the upward discharge in air means that many of the electrons and ions are generated near the anode and that, while the same charge is passed as in the downward half-cycle, more energy is now given to the positive ions, which produce no ozone, and consequently much less ozone is produced in this half-cycle.

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Total Ionization Cross-section of some Tetra Alkyl Metal Compounds

One of the conclusions of a preceding paper was that the dissociation energy of the metal-carbon bonds decreases with the increasing mass number of the metal atom in the series (Alkyl)₄C, (Alkyl)₄Si, (Alkyl)₄Ge, (Alkyl) Sn and (Alkyl) Pb, where Alkyl is CH3 or C2H5. This we attributed to the increasing bond polarizabilities.

According to Lampe *et al.*², there is a linear relationship between the polarizability and the total ionization cross-section. This relationship can be rationalized theoretically as follows.

The static polarizability for an atom is, according to Slater³,

$$\alpha = \frac{2}{h} \sum_{a} \frac{|M_{a_0}|^2}{v_{a_0}} \tag{1}$$

(2b)

where $|M_{a_0}|$ is the a_0 'th element of the dipole moment matrix and v_{a_0} is the frequency corresponding to the transition between the states 0 (ground state) and a.

Using the Bethe-Born approximation, Mott and Massey⁴ derived an expression for the atomic ionization cross-section

$$Q^{i}_{nl} = \frac{2\pi e^{4}}{mv^{2}} \frac{c_{nl}}{|E_{nl}|} Z_{nl} \log \left(\frac{2mv^{2}}{c_{nl}}\right)$$
 (2a)

$$c_{nl} = (Z^2_{\text{eff}}/n^2 a_0^2) \int |X_{nl,K}|^2 dK$$

The integration in equation (2b) is carried out over the appropriate portion of the ionization continuum. The matrix element $|X_{nl,K}|$ will be proportional to the corresponding dipole moment matrix element $|M_{nl,K}|$, and to the extent that the variation in $\Sigma(M_{a0}^2/\nu_{a0})$ parallels

that in $c_{nl}/[E_{nl}]$ in going from one atom to another, a linear relationship between polarizability and cross-section should be observed.

Lampe et al. expect that analogous expressions should apply to molecules. In order to check the correlation of the bond polarizability with the behaviour of the metal organic compounds under electron impact, we measured the total ionization cross-sections of the compounds mentioned.

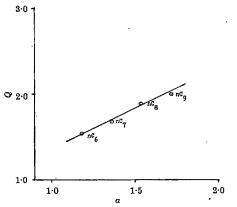


Fig. 1. Total ionization cross-section (Q) against polarizability (a) of some normal alkanes in arbitrary units.

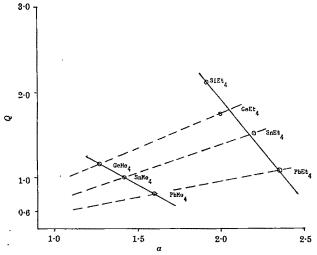


Fig. 2. Total ionization cross-section (Q) against polarizability (a) of some metal organic compounds in arbitrary units.

The cross-sections were measured on an AEI M.S.9 mass spectrometer under the following conditions: electron accelerating voltage 50 V; electron current $100~\mu A$; and temperature $230^{\circ} \pm 5^{\circ}$ C. Ion currents were read from the monitor when a known amount of sample has evaporated completely in the heated inlet system of the mass spectrometer. Cross-section values are normalized to the value for n-hexane.

In order to test the method, the cross-sections of some alkanes were measured and the relationship with the polarizability is shown in Fig. 1. The polarizabilities have been calculated from the molar refraction coefficients.

There appears to be a good linear relation between the total ionization cross-section and the polarizability, and these data fit in with the theory of Lampe *et al*.

In Fig. 2 the relative values of the total ionization cross-sections of the tetra alkyl metal compounds are plotted against the polarizabilities. Here the tendency is the opposite of that predicted by theory, although the relationship is again linear.

The cross-sections decrease with the increasing polarizabilities of the metal atoms, although they still increase with increasing polarizabilities of the alkyl groups.

We must conclude that the theory which was developed for atoms and which so far has seemed acceptable for dealing with molecules may only be applied for the comparison of molecules of closely related structures. Its limits have been reached with the introduction of metal atoms into organic molecules.

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Kinetics of Ligand Exchange in a Europium Complex

We have previously reported the synthesis of 4,4'-di-n-butyl-2,2'-bipyridyl (L) and its complexes with lanthanide nitrates. We now find that the complex $[\mathrm{Eu}(\mathrm{NO_3})_3L_2]$ in chloroform solution undergoes fairly rapid exchange with added free ligand

$$[\mathrm{Eu}(\mathrm{NO_3})_3 L_2] + L' \Longrightarrow [\mathrm{Eu}(\mathrm{NO_3})_5 LL'] + L$$

The reaction rate is independent of the concentration of added ligand and this is in accord with a dissociative mechanism

$$[\mathrm{Eu}(\mathrm{NO_3})_3 L_2] \xrightarrow{k_1} [\mathrm{Eu}(\mathrm{NO_3})_3 L] + L \tag{1}$$

$$[\mathrm{Eu(NO_3)_3}L] + L' \xrightarrow{k_2} [\mathrm{Eu(NO_3)_3}LL']$$
 (2)

The thermodynamic parameters of activation are as follows: $\Delta G^{\ddagger} = +15.0 \text{ kcal/mole}$; $\Delta H^{\ddagger} = +6.4 \text{ kcal/mole}$; $\Delta S^{\ddagger} = -28.9 \text{ cal/deg/mole}$ and $k_1 = 58 \text{ sec}^{-1}$ at 25°.

These data were obtained by a study of the 100 Mc/s ¹H nuclear magnetic resonance spectra of mixtures of the complex and ligand in deuterochloroform, in which measurements of the solvent vapour pressure showed no detectable association between complex and ligand. The complex [Eu(NO₃)₃L₂], where the Eu(III) ion is in rapid temperature-dependent equilibrium between the ⁷F₀ and ⁷F₁ states, gives the best resolved spectra of the complexes [$M(NO_3)_3L_2$], where M is a paramagnetic lanthanide. The aromatic and α -CH₂ proton resonances of the europium

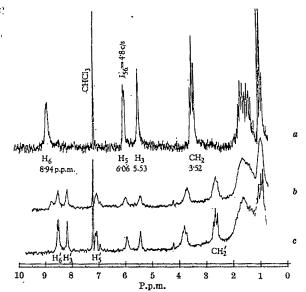


Fig. 1. 100 Mc/s 'H nuclear magnetic resonance spectra in deutero-chloroform: (a) $[\mathrm{Eu}(\mathrm{NO}_3)_2 L_2]$ at 50° C; (b) $[\mathrm{Eu}(\mathrm{NO}_3)_2 L_3]$ (28-0 g/l.) and $L(23\cdot1$ g/l.) at 20° C; and (c) mixture as in (b) but at 0° C. Unprimed peaks are those of the complex, primed peaks are those of the ligand. Assignments are by comparison with coupling constants of 2,2'-bipyridyl' and by comparison with $[\mathrm{Eu}(\mathrm{NO}_3)_2(5,5'-\mathrm{din-butyl-2},2'-\mathrm{bipyridyl})_2]$. δ -values are relative to an internal TMS standard.

complex show substantial shifts from the mutually rather similar values for the free ligand and the diamagnetic lanthanum, yttrium and lutetium complexes. On the addition of free ligand, the resonances are broadened (Fig. 1) but the spectra of the free ligand and of the complex remain distinct. The line width of the spectrum of the complex is constant at 0° C between complex to ligand molar ratios of 1:0.2 and 1:1.5, and the rate-determining process is thus independent of the added ligand. A typical plot of the line width parameter, in this case that of the 3-proton, against temperature (Fig. 2), shows the onset of exchange broadening at about 250° K and its subsequent dominance over the normal decrease in line width with increased temperature observed for a paramagnetic complex alone. The values already quoted for ΔH^{\ddagger} and ΔS^{\ddagger} were obtained by use of the relationship²

$$1/T_2 = 1/T_{2M} + 1/\tau_M$$

where T_2 is the observed transverse relaxation time, given by $1/T_2 = \pi w$ (w being the line width at half height in c/s), and T_{2M} is the transverse relaxation time in the absence of exchange (determined by the low temperature part of Fig. 2). τ_M is the average co-ordinated lifetime of any ligand molecule.

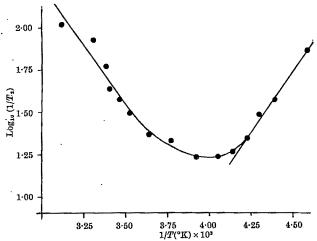


Fig. 2. Plot of temperature variation of line-width parameter for 3-proton of $[Eu(NO_3)_3L_2]$ for the solution corresponding with the spectra in Figs. 1(b) and (c).

The kinetics of reactions of lanthanide complexes have been little studied previously, but those studies which have been performed $^{3-5}$ when considered together with the general chemical properties of these compounds suggest that they are very labile. Thus dissociation in water of the mono-murexide complex of lanthanum into ligand and hydrated lanthanum ion has been shown by the temperature-jump method to have $k=3\cdot 2\times 10^4$ sec⁻¹ at 12° C while the reverse reaction has $k=8\cdot 6\times 10^7$ sec⁻¹.

Two further points are noteworthy. First, the contrast between the second-order kinetics of the exchange between $[MX_2(\mathrm{PPh_3})_2]$ and $\mathrm{PPh_3}$ $(M=\mathrm{Co,\ Ni;\ }X=\mathrm{Br,\ }I)$ in chloroform and our presently described first order reaction is in accordance with the simple proposition that the nickel or cobalt ion, being only 4 co-ordinated, might be susceptible to nucleophilic attack while the europium ion, which here is perhaps 10-co-ordinated, would be too well shielded for this mechanism to operate. Second, the small value of ΔH^{\ddagger} suggests that the transition state may be stabilized by co-ordination of the dipolar chloroform molecules with the europium ion.

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MOLECULAR STRUCTURE

Two Structurally Distinct Classes of Kappa-chains in Human Immunoglobulins

The general structural features of the various types of immunoglobulins are known and the details of the primary structure are gradually being filled in. A characteristic feature of the immune response, however, is its specificity and here the data have not been sufficient to reveal the structural basis of the specificity and even less the underlying genetic mechanism. The aim of the present investigation was to throw more light on this problem, and to this end we have determined the NH₂-terminal sequences of a large number of x-chains and made a comparison of their structures.

The sources of the x-chains were 18S macroglobulins, myeloma proteins and Bence-Jones proteins, all of immunological type K, obtained from patients suffering from macroglobulinaemia and myelomatosis, respectively. The serum proteins were isolated according to the procedures of Gelotte et al. and Fahey and Horbett². The purified IgM, IgG and IgA were partially reduced with mercaptoethanol, alkylated with iodoacetic acid and the light and heavy chains separated on 'Sephadex G-100' according to the procedure of Fleischman et al.³. The light chains of pooled human γG were prepared from the Cohn fraction II-1,2. The preparation was enriched in x-chain content by the procedure of Cohen and Gordon⁴. The urinary proteins were purified as described by Fahey and McLaughlin⁵.

Table 1. NH2-TERMINAL SEQUENCES OF x-CHAINS FROM MAN AND MOUSE

Speci-	Source		_			_	•	_					sition						40		00		20
men		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
70(M) 41(M)	BJ BJ																						- Ser - - Thr -
Cra(H) Pap(H) Lux(H) Mon(H) Con(H) Tra(H)	BJ IgM IgG IgM BJ IgG	Asp - Asp - Asp -	· Ile · Ile · Ile - Ile	- Glr - Glr - Glr - Glr	– Met – <i>Leu</i> – Met 1 – Met	- Thr - Thr - Thr - Thr	- Gln - - Gln - - Gln - - Gln	Ser Ser Ser Ser	- Pro - - Pro - - Pro - - Pro -	- Ser - - Ser - - Ser - - Ser -	Ser - Phe - Thr - Ser -	Leu Leu Leu Leu	- Ser - Ser - Ser - Ser	– Val – Ala – Ala – Ala	- Ser - Ser - Ser - Ser	- Val - Val - Val - Val	- Gly - Gly - Gly - Gly	– Asp – Asp – Asp – Asp	– Arg – – Arg – – Arg – – Arg –	Val- Val- Val- Val-	- Thr - Thr - Thr - Thr	- Ile - Ile - Ile - Ile	- Thr Ala Thr Thr Thr Thr Thr -
Nig(H) Win(H) Gra(H) Cas(H) Smi(H)	IgA BJ IgM BJ IgM	Asp - Glu - Glu -	- Ile - <i>Me</i> - Ile	- Va t - Va - Va	– Leu – <i>Met</i> – Leu	- Thr - Thr - Thr	- Gln - Gln - Gln	– Ser – – Ser – – Ser –	- Pro - - Pro - - Pro -	- Ala - - Ala - - Gly -	- Thr - - Thr - - Thr -	- Leu - Leu - Leu	- Ser - Ser - Ser	– Leu – <i>Met</i> – Leu	- Ser - Ser - Ser	– Pro – Pro – Pro	- Gly - Gly - Gly	– Glu – Glu – Asp	- Arg - - Arg - - Arg -	Ala - Ala - Ala -	- Thr - - Thr - - Thr -	- Leu - Leu - Leu	- Ser - - Ser - - Ser - - Ser -
Pool(H)	IgG	Asp Glu	Ile	Glr Va	Mei Leu	Thr	Gin	Ser	Pro	Ser Gly Ala Val Leu Pro	Ser Thr	Leu Val	Ser	Ala Val Leu	Ser Thr Val	Vai Pro Leu		Asp Glu	Arg				

The two mouse (M) sequences are taken from the work of Gray et al., and the human (H) sequences are all from the present work. Amino-acid symbols in bold face indicate major components in the pooled light chains of IgG.

Samples (approximately 7 mg) of the x-chain preparations were subjected to twenty-two degradation cycles in the protein sequenator using the technique already described. The results of these degradations are shown in Table 1, which also includes the NH2-terminal sequence of two mouse Bence-Jones proteins' for comparison.

A comparison between the structures reveals several The sequences consist of variant and nonvariant sections. The essentially non-variant positions are Nos. 2, 5-8, 11-12, 14, 16, 18 and 20. Exceptions do occur—for example, position 2 in Gra(H), position 12 in 70(M), position 16 in Cra(H), and position 20 in 41(M). In the variant positions the choice is usually between two amino-acids, for example, aspartic or glutamic acid in position 1, valine or glutamine in position 3, and leucine or methionine in position 4. Occasionally a third and even a fourth amino-acid is observed, though less frequently, for example, in position 13. The sequences are 'in phase", that is, without deletions or insertions. The amino-acids occupying the variable positions in the human x-chains vary in conjunction over the whole sequence. It is therefore possible to divide the human \varkappa -chains into two structurally distinct classes. If $\mathrm{Tra}(H)$ is chosen as the prototype of one class, it is found that Cra(H), Pap(H), Lux(H), Mon(H) and Con(H) will differ from the prototype in only six out of fifty-five variable positions. With Smi(H) as the prototype for the other class comprising Nig(H), Win(H), Gra(H) and Cas(H) also, the corresponding figures are seven out of forty-four. These observations seem to justify the division of the x-chains into two classes and we propose the provisional terms x_{Tra} and x_{Smi}. A division of the x-chains according to structure has in fact been surmised by Putnam et al.8 on the basis of limited sequence data.

The earlier non-recognition of the two classes of x-chains has probably led to an overestimation of the variability in the NH2-terminal half of the structure. The average number of amino-acid replacements in our material is 1.3 for each twenty-two positions. If this frequency is representative of the NH2-terminal half of the x-chains, it would mean an average of six to seven replacements in the variable part of an individual x-chain.

The amino-acid replacements observed, both in the variant and in the essentially non-variant positions, occur only singly. There are no replacements of stretches of amino-acid as one would expect if a genetic crossing-over mechanism were responsible for the replacements. Within the classes all replacements are in fact consistent with a single base mutation in the codon. On the other hand, a comparison between the two prototypes shows that in three positions, that is numbers 3, 13 and 15, two-base mutations are required for the replacements.

A comparison between the NH2-terminal sequences of two Bence-Jones proteins, Cra(H) and 41(M) originating one from man and the other from mouse, shows identity

in the first fifteen amino-acids. An extensive similarity of this kind could not be fortuitous. The simplest explanation would be that the genetic information for the manufacture of this sequence stems from a germ line common to both species. The wider generalization that the entire sequence of the individual x-chain is predetermined by the genetic information carried in the germ line seems plausible.

The sequential degradation of the pooled x-chains shows, as would be expected, an extensive heterogeneity. When the individual sequence positions, however, are analysed for their amino-acid composition, they are found to resemble closely an average of the amino-acids occupying the corresponding positions in the individual z-chains. This lends further support to the assumption that the serum proteins associated with macroglobulinaemia and myelomatosis are true representatives of the normal immunoglobulins. This does not exclude the possibility that certain of the amino-acid replacements observed in the pathological proteins are expressions of the pathological condition rather than of the normal variability of the immunoglobulins themselves. This could, for example, apply to the more radical replacements from the point of view of protein conformation, for example, glutamic acid-lysine in position 1 of Nig(H), or glycine-arginine in position 16 of Cra(H). At the present stage, however, this is no more than conjecture.

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Deuterium-Hydrogen Exchange of Collagen Like Synthetic Polypeptides

THE two models of collagen structure which have been developed since 1954 have been confirmed by X-ray diffraction studies of the collagen fibres. Both models are similar, the chief difference being in the number of

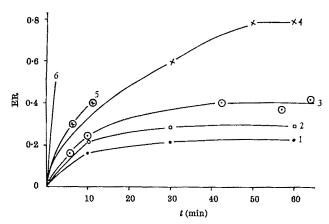


Fig. 1. The exchange rate (ER), which is a measure of the deuterium-hydrogen exchange, plotted against time. 1, Collagen; 2, poly-Dr-Ala; 3, poly-Pro-Ala-Gly, molecular weight, 2,500; 4, poly-Pro-Gly-Ala, molecular weight, 2,500; 5, poly-Gly-Ala-Pro, molecular weight, 2,100; 6, Pro-Ala-Gly-OMe.HCl.

hydrogen bonds. Rich and Crick¹ assumed that there was one hydrogen bond for each tripeptide unit (one bonded structure) while Ramachandran2 assumed two hydrogen bonds per unit (two bonded structure) in the structure he suggested. Thus differences in the sequence of the amino-acids and changes in the position of atoms are unavoidable. In the collagen structure II of Rich and Crick, position 1 of the tripeptide unit is always occupied by a glycine unit; the positions 2 and 3 may be occupied by any other amino-acid including proline and hydroxyproline. On the other hand, in the Ramachandran structure an imino-acid unit can only occupy position 3 if changes in the collagen structure are to be The results obtained by Harrington³ and Bensusan and Nielsen support the structure proposed by Ramachandran. Investigations into synthetic polypeptides composed of tripeptide units, such as gly-pro-hypro^{5,6} and pro-gly-pro⁷, have shown that only one hydrogen bond per tripeptide unit is sufficient for the formation of a collagen like structure in solution as well as in the solid state.

Until now, investigations into tripeptides containing three different amino-acids with an imino-acid in position 3, similar to the mentioned tripeptides, have not shown any tertiary structure. We have therefore synthesized a series of tripeptides corresponding to both the collagen structures proposed by Rich and Crick and the structure proposed by Ramachandran.

The synthesized tripeptides were polymerized using tetraethylpyrophosphites, and the resulting crude synthetic polypeptide polymers were recrystallized several times from alcohol/ether mixtures before being fractionated on 'Sephadex' columns. Fractions with similar molecular weights were then examined and the results compared. As the work on solutions is still in progress, we should like to report the results obtained with collagen films using the deuterium-hydrogen exchange technique. The exchange rate (ER) was measured by the method of Blout et al. using a Perkin-Elmer double beam spectro-photometer, model 221, equipped with a sodium chloride prism, and taking into consideration the increase in the amide II-band at 1,450 cm-1.

In Fig. 1, the quantity ER, as a measure of the deuterium-hydrogen exchange, is plotted against time. ER is derived from the following equation

$$\text{ER} = \left(\frac{A_2^{'}}{A_1}\right)_T - \left(\frac{A_2^{'}}{A_1}\right)_{T_0}$$

We have chosen the ratio of the optical density of the NDdeformation band (A'_{2}) to that of the amide I band (A_{1}) , for it does not change during deuteration. This ratio is independent of the concentration and the thickness of the sample. It is necessary to take the difference, because the intensity of the ND-deformation band is superposed by a CH-deformation band of constant intensity. Thus $(A'_2/A_1)_T$ is the ratio taken after a certain time and $(A'_2/A_1)_{T0}$ is the initial value before deuteration. Preparation of the samples for infrared investigations and the calculation of ER are described by Heidemann and Srinivasan¹⁰

Fig. 1 shows that the extent of exchange in the solid state in the sequence (gly-ala-pro), (5) and (pro-gly-ala), (4) is higher than in $(gly-pro-ala)_n$ (3), and therefore the structure of $(gly-pro-ala)_n$ is more stable than the other For comparison, the exchange rates (ER) sequences. of acid soluble collagen, poly-DL-alanine and L-prolyl-L-alanylglycin-methylester-hydrochloride are also included. All substances are soluble in water and this eliminates any hindrance of the exchange by hydrophobic effects.

The polypeptides containing the sequence (gly-ala-pro) (4 and 5), which corresponds to the Ramachandran twobonded collagen model, are seen to be less stable than polypeptides having the sequence (gly-pro-ala) (3), which are capable of forming a one-bonded structure as supposed by Rich and Crick. These findings also support earlier ones11.

The greater stability may also arise from the additive hydrogen bonds, which stabilize the quaternary structure favourably formed in the collagen model suggested by Rich and Crick.

These hydrogen bonds should also be considered in all cases where different investigations or measurements are carried out on collagen in the solid state.

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IMMUNOLOGY

Balanced Synthesis of Light and Heavy Chains of Immunoglobulin G

WE have already investigated the production of immunoglobulin G (IgG) by mouse plasma cell tumour 5563 and have shown that light chains are released autonomously from polyribosomes into a pool of free light chains. The time course of incorporation of radioactive amino-acids into free light chain and the myeloma protein indicated that the pool of free light chains is small and reaches maximum radioactivity after about 10 min of incubation. It behaves as a pool with rapid turnover from which light chains appear to be incorporated into G myeloma protein molecules^{1,2}. These findings led us to propose the possibility that the pool of free light chains controls the release of heavy chains from the polyribosomes.

In view of the neoplastic nature of the 5563 plasma cells, we have now turned our attention to the synthesis and assembly of IgG in lymph nodes from hyperimmune mice. From the results reported here, we conclude that in normal conditions IgG secreting cells produce equal numbers of light and heavy chains with a small pool of light chains serving as an intermediate in the assembly of IgG molecules. No excess secretion of either chain could be detected. This does not exclude the possibility that a very small amount of light chain from the pool can reach the extracellular spaces produced by cell death, or unbalanced synthesis of occasional cells.

The experiment was as follows. Popliteal lymph nodes from mice immunized with haemocyanin were incubated for from 5 to 100 min with ³H-amino-acids. Intracellular IgG or any free sub-units released with deoxycholate were fractionated by sucrose gradient centrifugation and their radioactivity across the gradient was determined by precipitation with antisera specific for determinants on light chain or on heavy chain. The extracellular culture medium was also analysed for the presence of secreted IgG and its sub-units.

C3H/He mice received, in each hind footpad, 25 µg of alum precipitated haemocyanin (Maia squinado) mixed with 2.5×10^8 B. pertussis (Burroughs Wellcome vaccine) and after 6-10 weeks a further 10 µg of haemocyanin in saline. Six days later about one-third of the total protein synthesized in vitro by the draining popliteal lymph nodes was precipitable with specific antiserum to IgG.

Popliteal lymph nodes cut into 0.5-1 mm pieces were incubated for 5-10 min in Falcon dishes $(35 \times 10 \text{ mm})$ in 0.15 ml. of Eagle's medium buffered with tris, pH 7.4, containing 10 µc. each of ³H-L-leucine and L-serine and · 20 μc. of ³H-DL-valine. The pieces of tissue were washed twice with cold medium; soluble intracellular proteins released with 0.5 per cent deoxycholate were prepared by centrifugation on a density gradient (8 ml. of 30 per cent, 1 ml. of 10 per cent and 1 ml. of 4 per cent (w/v) sucrose) at 100,000g for 80 min. The top 2 ml. was concentrated and dialysed for 1.5 h against saline buffered at pH 7.4. Light chain and IgG were fractionated on sucrose gradients (4-20 per cent w/v) by centrifuging for 16 h at 35,000 r.p.m. in a Spinco SW 39 rotor¹.

To determine the radioactivity of IgG and light chains, samples of each sucrose gradient fraction were treated with: (a) excess antibody to light chain plus carrier light chain; (b) excess antibody to Fc-fragment plus carrier myeloma protein; and (c) goat anti-rabbit IgG and rabbit IgG (at equivalence). The antibody/antigen precipitates were washed twice with saline, suspended in 5 per cent trichloroacetic acid and counted on 'Oxoid' membranes as previously described^{1,3}. Antisera were obtained from rabbits hyperimmunized with papain fragment Fc or light chains separated from G myeloma protein 5563 G myeloma protein 5563 has x type light (Table 1). chains, which appear to be the prevalent light chains in the mouse. The antiserum to light chain precipitates 75-80 per cent of mouse IgG labelled with iodine-131 (prepared from mice immunized with haemocyanin4) and the antiserum to Fc 85 per cent (Table 1). In addition Dr D. Dresser and Miss H. Brown tested the ability of these antisera to develop IgG plaque forming cells (P.F.c.) in

Table 1. CHARACTERIZATION OF ANTISERA

	Absorbed		Reaction		Percentage of 131I-mouse IgG
	with	$oldsymbol{L}$	H	M.P.	precipitated
Anti-Fo Anti-L	Fab Fc	<u>-</u>	+ -	÷ ±	85 75–80
			A	(high b/Ag ra	atio)

Fo and Fab are papain fragments, L and H light and heavy chains, all prepared from G myeloma protein 5563 (M.P.)³. Mouse IgG was trace labelled with iodine-131 by the method of Hunter and Greenwood⁴ to a specific activity of 3 μ c./ μ g. Precipitation with antiserum was tested in the presence of carrier myeloma protein or light chains in the conditions used for antiserum treatment of sucrose gradient fraction.

RELATIVE RADIOACTIVITY OF INTRACELLULAR MYELOMA PROTEIN AND FREE LIGHT CHAIN RELEASED WITH DEOXYCHOLATE

Time of incu	Ratio of radioactivity	
with	with	IgG
H-Leu, Val	cold Leu, Val	light chain
5		1.1
10		1.4
20		4
40		7
10	20	6

spleen from mice immunized with sheep red blood cells. Both antisera developed about 90 per cent of the P.F.C. detected with their antiserum to mouse IgG⁶. This validates the use of these antisera for the present study.

The pattern of labelling light chains and IgG in intracellular protein released with deoxycholate is illustrated in Fig. 1. After incubating lymph node pieces for 5 min with 3H-leucine and 3H-valine, the radioactivity incorporated into light chains and IgG is of similar order. After 10 min there is a relative increase in radioactivity of IgG and after 40 min the intracellular IgG is more highly labelled than the free light chains (Fig. 1C). The antibody to light chain has a high antibody: antigen ratio and does not precipitate all of the myeloma protein in the presence of carrier light chain. Approximate ratios of radioactivity found in completed IgG versus the free light chain were calculated from the peaks of radioactivity specifically precipitable by antisera specific for Fc and light chain, respectively. The ratio changes from about

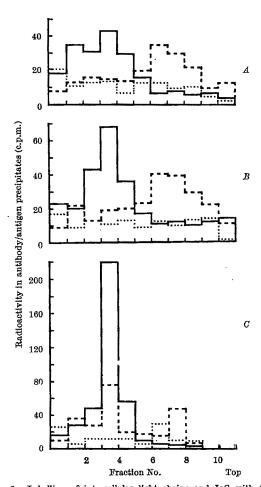


Fig. 1. Labelling of intracellular light chains and IgG with time. Lymph node pieces were incubated with ³H-L-valine, serine and leucine for (A) 5 min, (B) 10 min, and (C) 40 min. Intracellular proteins were fractionated on sucrose gradients (4-20 per cent w/v sucrose) for 16 h at 35,000 r.p.m. in a Spinco SW 39 rotor. Radioactivity of antibody/antigen precipitates in equivalent aliquots of the different gradient fractions using (——) antibody to Fc fragment, (———) antibody to light chain, and (.....) anti-rabbit IgG in the presence of rabbit IgC to serve as controls. The volumes loaded on to sucrose gradients were equal but represented varying proportions of the total soluble protein.

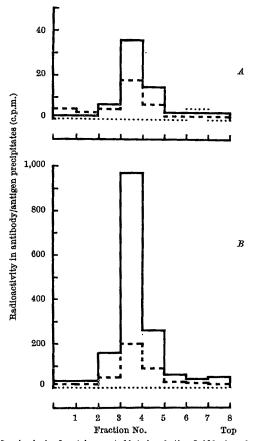


Fig. 2. Analysis of protein secreted into incubation fluid by lymph node. Sucrose gradient fractionation and treatment with antisera as in Fig. 1. Radioactivity in antibody/antigen precipitates using antiserum to Fc (——), antibody to light chain (———), and antibody to rabbit IgG (...). Extracellular fluid was collected after (A) 40 min and (B) 100 min of incubation at 37° with ³H-leucine and valine.

1 after 5 min to about 7 after 40 min of incubation (Table 2).

To ascertain that the change in the pattern of labelling of intracellular free light chain and IgG is not caused by more rapid secretion of light chain from the cell, compared with the myeloma protein, the incubation fluid was freed from cells by centrifugation for 10 min at 500g, and fractionated as described with added carrier G myeloma protein and light chain. Analysis of extracellular fluid obtained after 40 min of incubation of lymph node pieces (Fig. 2A) shows that only a little radioactive IgG and no radioactive light chain seem to be secreted from the cell during that time interval. After incubation for 100 min secreted myeloma protein is highly radioactive, but still no significant radioactivity was detectable in the light chain region with antiserum to light chain. It seems that the cells do not secrete significant amounts of light chain, and maintain the light chain pool within the cell.

Further evidence that we are dealing with a pool of free light chains which is rapidly turning over rather than with continuous excess synthesis of light chains comes from "pulse-chase" experiments. Two dishes with pieces of lymph node tissue were incubated for 10 min with ³H-leucine and ³H-valine. The incubation fluid was replaced with medium containing 0·1 molar non-radioactive L-leucine and L-valine for 5 min in the cold, and one dish was then incubated for a further 20 min at 37° C with fresh medium containing the high concentration of leucine and valine. The pattern of labelling of intracellular free light chains and IgG molecules released by deoxycholate was compared after 10 min of incubation with tritiated amino-acid (Fig. 3A) and, after the subsequent "chase", with non-radioactive amino-acids (Fig. 3B). The peak of radioactivity in free light chain is greatly reduced,

relative to that in IgG, during the chase incubation with non-radioactive amino-acids. The approximate ratio of radioactivity in IgG to light chain changed from 1.4 to 6 (Table 2). A higher proportion of the total soluble protein was loaded on to the gradient in Fig. 3B.

The present study using lymph node cells agrees well with our earlier findings with plasmacytoma 5563, which produces a G myeloma protein but no Bence-Jones protein. Like the 5563 tumour cells, lymph node cells present a pattern of balanced synthesis of light and heavy chains of IgG, but contain a small pool of free light chains rapidly turning over. The change in relative labelling of free light chains and IgG with time provides evidence for this turnover. After a short period of labelling (5 min), radioactivity is found to a similar extent in both free light chains and IgG. The relative labelling of light chains decreases with time so that after 40 min of incubation with radioactive amino-acids six or seven times as much radioactivity is found in IgG as in free light chain. The previous results with 5563 tumour cells were comparable except for a variation in the exact time relationships. An ascitic form of the plasma cell tumour 5563 was used which equilibrates much more rapidly with radioactive aminoacids than the small pieces of lymph node tissue incubated

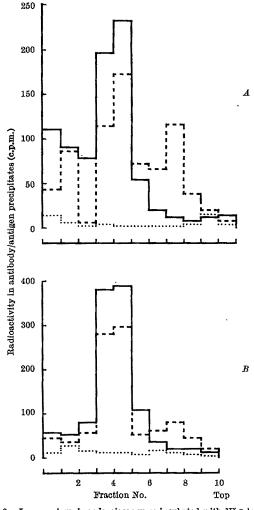


Fig. 3. Immune lymph node pieces were incubated with ³H-L-leucine and L-valine for (A) 10 min, and (B) 10 min and thereafter for 20 min with medium containing 0-1 molar L-leucine and 0-1 molar L-valine. The intracellular proteins, released with deoxycholate, were fractioned as in Fig. 1. Radioactivity in free light chain and IgG was determined by specific precipitation with (——) anti-Fc fragment, (———) antibody to light chain, and (...) anti-rabbit IgG. In this experiment a more hyperimmune anti-light chain serum was used which reacted more effectively with myeloma protein than with antiserum used for experiments illustrated in Figs. 1 and 2. (A) Two-thirds of the intracellular protein and (B) four-fifths were loaded on to the gradient, both in the same volume.

in this study, but the relative incorporation into light chains compared with IgG within each sample is highly

The pulse-chase experiment, in which the radioactivity in free light chains was greatly reduced relative to that in IgG after incubation with non-radioactive amino-acids adds weight to the idea that the pool of free light chains turns over as an intermediate in IgG assembly. We found no evidence for the secretion of excess free light chains into the extracellular fluid by the immune tissue.

Our results do not exclude the possibility that usually a very small amount of light chain may appear in extracellular fluids by cell death, shedding of the cytoplasm or unbalanced synthesis in occasional cells. In malignant plasma cells it is obvious that imbalance in synthesis of heavy and light chains occurs. In many plasmacytomas, in mouse and man, excess light chains are produced resulting in the appearance of Bence-Jones protein in the urine, or complete suppression of heavy chain synthesis or release occurs leading to Bence-Jones proteinuria with no detectable serum myeloma protein. În cells which continually synthesize excess light chains, the radioactivity of both light chains and myeloma protein increases with time7, whereas in the 5563 tumour the labelling of free light chains reaches a maximum after about 10 min of incubation2.

Our findings that in mouse lymph node cells there is no detectable excess synthesis of light chains accumulating in or being secreted by the cells contrast with the findings of Shapiro et al.8, using rabbit lymph nodes. ported that cell suspensions of lymph nodes from hyperimmunized rabbits synthesize and secrete into the culture medium an excess of free light chains when tested after I h of incubation. Labelled IgG and free light chains were identified in the intracellular and extracellular fractions, before and after reduction with mercaptoethanol in urea and sodium dodecylsulphate (SDS) by their position on acrylamide gel electrophoresis in the presence of SDS. This method separates molecules chiefly by size, and so the background of labelled proteins of similar size to light chain and reduction of other labelled proteins to sub-units of that size make the quantitation of radioactivity in light chain problematical.

Nezlin and Kulpina, purified in vitro labelled rabbit intracellular and extracellular immunoglobulins by absorption on a specific immune precipitate. On fractionation using 'Sephadex G-200' a "microglobulin" (smaller than ovalbumin) which is probably free light chain was found to contain only 5 per cent of the radioactivity of the immunoglobulins; this is consistent with our present results but would not agree with a large overproduction of light chains.

We conclude that in lymph nodes from immune mice there is an overall balanced synthesis of light and heavy chains of IgG. The lymph nodes contain also a small pool of free light chains which appears to turn over rapidly and to serve as an intermediate in IgG assembly. We could not detect secretion of free light chains from the cells.

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Effect of Neonatal Thymectomy on **Experimentally Produced** Immunological Cardiac Lesions

IMMUNOLOGICAL cardiac injury can be produced in experimental animals by the administration of: (a) homologous or heterologous heart extract with Freund's adjuvant; (b) passive transfer of humoral anticardiac antibodies; or (c) after passive transfer of cellular antibodies either through intravenous injections of immune cell suspension or by transplantation of immune spleens1.

Animals thymectomized at or shortly after birth are less able than unthymectomized animals to develop a normal immune response. This is demonstrated by an impaired ability to reject homograft and heterografts and lowering of the concentrations of circulating antibody to various antigens, both soluble and particulate 2-4. Immunological cardiac injury is caused both by circulating antibody and delayed hypersensitivity mechanisms, and so we decided to see whether it could be influenced

by neonatal thymectomy.

Albino rats 1-3 days old were thymectomized in the following way. Animals were placed in a glass container and chilled in a refrigerator at 4° C. Within 20 min deep hypothermic anaesthesia had been reached. The animal was pinned on a cork board and, in aseptic conditions, the chest was opened after a midline skin incision had been made. The thymus was freed from all attachments by blunt dissection and removed, usually intact. The wound was closed by interrupted sutures and the animal was warmed to room temperature. Control animals were treated similarly but without removing the thymus.

The animals were weaned when 3-4 weeks old and were fed on a balanced diet. They were sensitized at 15-16 weeks—the interval allowed for elimination of any preformed lymphocytes. Thymectomized and control animals were given six weekly subcutaneous injections of 10 per cent saline extract of mouse heart in 0.25 ml. doses mixed with an equal volume of Freund's adjuvant. The animals were killed I week after the last injection, and blood was collected for antibody estimation. The heart was removed carefully and preserved in 10 per cent formalin for histological study. Spleens of thymcctomized and control animals were removed and transplanted immediately into the omenta of normal adult rats. The spleen from one of the rats was transplanted into two animals. This technique was used to demonstrate cellular antibodies because of difficulty in demonstrating delayed hypersensitivity on the skin of sensitized rats. Recipient animals were killed 24 and 48 h after transplantation of the spleen, and the heart was examined for histological evidence of injury caused by cellular antibodies. Circulat ing antibodies were demonstrated by the tanned red cell

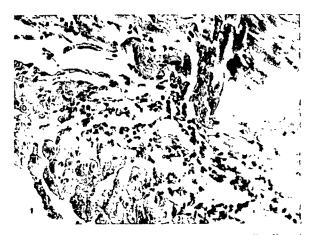


Fig. 1. Heart of rat given mice heart extract and Freund's adjuvant, showing damaged muscle fibres infiltrated by mononuclear and mesenchymal cells. (Stained with haematoxylin and cosin. × 150.)

agglutination (TRCA) technique in the sera of both groups of animals. All these methods have been reported in detail elsewhere. Only those animals which had no residual thymus were counted in the results.

Hearts of non-thymectomized control animals showed focal granulomatous cardiac lesions characterized by myofibral necrosis, mononuclear cell aggregates and mesenchymal reaction (Fig. 1). Circulating anticardiac anti-bodies in the sera as determined by the TRCA technique were found in varying titres in different animals; the highest was 2,560, the lowest 160 and the mean 720. The histological appearance of the hearts of recipients of spleen transplants was similar to those of control animals but the injury was more moderate in degree. The thymectomized animals, however, showed no cardiac damage after sensitization. The circulating antibody titre was considerably less than 10, and the cellular antibody could not be demonstrated by spleen transplantation in normal animals; the hearts of recipients of spleen transplants showed no evidence of injury (Table 1). Animals given normal spleen transplants showed no cardiac injury.

Table 1. EFFECT OF SPLEEN TRANSPLANTATION

Group	Circulating antibody titre (mean)	Pathological changes in heart	Pathological changes in the heart of recipient of spleen
Thymectomized	<10	0	0
Control	> 720	+++	++

Our results demonstrate that cellular as well as circulating antibodies produce cardiac damage in sensitized animals. In immunologically disturbed animals (thymectomized), cardiac antigens cannot provoke an immunological response and therefore no cardiac damage occurs. Our results also confirm the findings of Jankovic et al.5 that in neonatally thymectomized rats the production of circulating antibody and delayed hypersensitivity is depressed. Our results also agree with reports that allergic encephalomyelitis cannot be produced in neonatally thymectomized animals.

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Phylogenetic Differences among the **Gm Factors of Non-human Primates**

THE Gm factors are a group of genetically determined antigens located on the heavy chains of γG globulin¹. Their presence in non-human primates¹⁻⁷ has permitted a comparative immunological study giving information about the antiquity of the various Gm characters.

Sera of eleven different species of non-human primates were surveyed using seven Gm typing systems of established specificities^{8,9}. The factors tested for included $Gm(x)^{10}$, $Gm(y)^9$ and $Gm(g)^{11}$ not previously reported in primates, as well as Gm(a), (f), (b) and (x)*. The species were grouped according to the degree of similarity between their Gm antigens and those found in man (Table 1)

Table 1. COMPARISON OF GM ANTIGENS OF MAN AND NON-HUMAN PRIMATES

Family	Species	No. tested	γ((z)	l he	m fa eavy (f)	chai	n* y(33 (g)
Hominidae	Man Gorilla Chimpanzee	$\frac{2}{12}$	6 7 6	6 6 6	<u>8</u>	<u>5</u>	7 6 7	<u>5</u>
Pongidae (Great apes)	Gibbon† Orang-utan Baboon Drill Rheaus monkey Cynomolgous monkey	12 15 33 1 16 5	6 6 4 4 —				7 8 8 8 8 8	
Cercopithecoidea (Old World monkeys)	Black ape Patas monkey Vervet monkey	2 23 10	=	=	NT _		8 —	=

The reagents used for testing are given in ref. 9. All samples of each species studied gave the same result. The anti-Gm(h) system detected Gm (b3,4). The numbers used in this table which appear under the Gm factors refer to inhibitory units which are defined as the highest tube number of serial two-fold dilutions of a serum, initially diluted 1:20, which resulted in inhibition. The higher the number, the greater the inhibitory capacity of the Gm antigen. A difference of two units or more is considered significant. A line (—) indicates no Gm factor was found in a 1:20 dilution of the primate serum. Gm(x) was also tested for and was not present in any species. NT, Not tested.

* The Gm antigens are grouped under the sub-class of heavy chain with which they are associated. **

† The twelve gibbons were Gm(a—) using one genetic agglutinator and Gm(a+) using a second Gm reagent. Nine of the gibbons were Gm(b+) and three were Gm(b—) using the same agglutinator.

using as criteria both the presence and the comparative inhibitory capacity of the Gm antigens.

The order established in Table 1 agrees with known ajor taxonomic divisions^{12,13}. The increase in the major taxonomic divisions12,13. number of Gm antigens and in their inhibitory capacity parallels development within the primate order. Highest in the ranking were the gorilla and chimpanzee (Gm(z+, a+, b+)), an associated sequence of factors found in many humans. The presence of this human-like group of genetic characters at high inhibitory levels suggests that these two apes are phylogenetically close to man. Similar relationships among primates have been constructed from studies of the antigenic correspondence of serum albumin¹³ and comparative investigations of the amino-acid sequences of haemoglobin in animals¹⁴. Moor-Jankowski and Wiener15,16 tested the Rh-Hr reactions of primate red cells and concluded that the chimpanzee red cell antigens were closest to man, followed by the gibbon, gorilla and orang-utan, while a study by Masouredis et al. using isotopic measurements of the uptake of iodine-125 anti-RhO(D) by simian red cells placed the orang-utan nearer to man than any of the African apes17.

The taxonomic level at which each Gm character is first detected provides a clue to the antigens' antiquity. Gm(y), (g) and (x) appear to be the most recent while Gm(b) followed by Gm(z) and (a) are the more ancient (Table 1). Gm(f) occurs in several Old World monkeys at a small fraction of the inhibitory level seen in man, and this finding probably represents a weak cross reaction. If this reasoning is valid, then the primary structures responsible for the Gm antigens were established at quite different times during the development of the primates. The presence of each Gm antigen would be expected to be dependent on the previous development of the subclass of YG heavy chain 18,18 on which the factor is localized, and so the Gm antigens associated with the \(\gamma G1 \) and \(\gamma G3 \) sub-classes of heavy chains are considered separately. (The nomenclature used for the heavy chain sub-groups of YG globulin is that adopted by a sub-committee of WHO: γG1 (We or γ2b); γG2 (Ne or γ2a); γG3 (VI or γ2c); γG4 (Ge or γ2d).) Particularly striking is the complete absence of Gm(g)¹¹ despite the relatively long established presence of the allelic Gm(b) factors, suggesting that the former factor may be a recent mutation. During this study Gm polymorphism was detected only for Gm(b) in gibbons. Although polymorphism among non-human primates has been described for other Gm and Inv antigens^{1,3,6}, it is very limited when compared

^{*} WHO notations for the genetic factors are as follows: Gm(a) is Gm(1); $Gm(b^{a,4})$ are Gm(13,14); Gm(x) is Gm(2); Gm(f) is Gm(4); Gm(g) is Gm(21); Gm(z) is Gm(17); Gm(y) is Gm(22).

with that of man, indicating that much of it arose in the higher primates.

The present study suggests that the Gm factors were still developing and changing in higher primates. Gm characters are believed to involve only a small area of γG heavy chains, and so this finding may not reflect the trend of changes in the whole immunoglobulin protein. Furthermore, no relationship between the Gm characters and antibody specificity or efficiency has yet been established which would indicate a selective evolutionary pressure. Notwithstanding these limitations, the comparative study of the Gm antigens has provided a useful technique to supplement other approaches to the study of immunoglobulin development.

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MICROBIOLOGY

In vitro Increase in Virus Infectivity

Some of the many previous reports of an in vitro increase in virus or of infectivity of plant viruses have been reviewed1,2, but the phenomenon is not generally accepted3. I believe that the work reported here with cucumber mosaic virus (CMV) in beet is simpler, gives a greater increase and will be more easily confirmed than previous reports. The results are interpreted as caused by slow inactivation of inhibitors of infection, not by an actual increase in virus.

To prepare inoculum, 0.05-0.1 g of systemically infected leaf tissue was ground in 1 ml. of water, usually containing 0.01 g potassium sulphate, diluted to 10 ml. with water; any further supplements were added and the preparation was mixed. This mixture was wiped over the primary leaves of cowpea (Vigna sinensis (Torner) Savi variety Blackeye) immediately after grinding and up to 10 h later. This latter we called aged inoculum. 'Celite', added last to the inoculum, served as an abrasive. Infections appeared as necrotic lesions in 1 day and were counted after 3 days. If more lesions appeared from aged than from fresh inoculum, it is believed that increase in infectivity occurred.

Principal positive results are shown in Table 1. CMV gave greater in vitro increase in infectivity than the viruses of tobacco mosaic, tobacco necrosis, tomato ringspot, tomato spotted wilt, artichoke latent or citrus tatter With CMV, greatest in vitro increase resulted with inoculum from beet. Moderate in vitro increase resulted with inoculum from cucumber and no in vitro increase resulted with inoculum from tobacco. Virus inhibitors in these hosts increase in the sequence tobacco-cucumberbeet4, and so there is a correlation of in vitro increase and concentration of inhibitors. With CMV from cucumber, consistent in vitro increase has resulted only with inoculum from the upper leaves of systemically infected plants, which inoculum has a low level of initial infectivity. No supplement has brought about a clear in vitro increase in infectivity with inoculum from cucumber.

CMV from sugar beet, especially from young inner leaves of plants which have been systemically infected for 2 months or more, has a low level of infectivity in the absence of supplements. Bentonite greatly increased initial infectivity but did not bring about an increase in infectivity with time. Potassium sulphate is the only chemical tested which when used alone (but in the presence of abrasive) brought about a large increase in infectivity with time. When charcoal (C, Merck's activated charcoal has been superior to five other brands) was added after dilution the in vitro increase was much greater than with potassium sulphate and 'Celite' only (Table 1). C may function as an abrasive and as an inactivator of inhibitors, but in this situation with the abrasive 'Celite' present, the inactivation of inhibitors by C is considered dominant.

The greatest in vitro increase in infectivity in CMV from sugar beet yet observed has been with potassium sulphate added before grinding, and potassium phosphate, C and 'Celite', in this order, added after dilution. In the last seven trials, the infectivity of this inoculum (after ageing for 4 h at about 25° C) has averaged 548 times and reached a maximum of 1,800 times that of the fresh inoculum. Many variations of this inoculum, such as higher and lower potassium sulphate, potassium phosphate and C, have brought about similar but lower increases in infectivity. Potassium phosphate increased initial infectivity, but caused a rapid loss of infectivity with time except when used with both potassium sulphate and C.

In vitro increase in infectivity could be caused by an actual increase in virus, by cyclic changes in the susceptibility of the host or by inactivation of inhibitors. Increase in virus seems unlikely because even the highest infectivity of aged inoculum has been lower than the initial infectivity of the same concentration of virus with bentonite, because the rate of increase in infectivity has

Table 1. In vitro increase in infectivity of cmy

Donor tissue	Before grinding	Supplements After dilution	No. of trials	Average lesions/le Immediately	af for inoculations After 4 h
Cucumber	0·1% K ₂ SO ₂	3% 'Celite'	6	13	54
Cucumber		3% 'Celite'	4	52	4•5
Beet		3% 'Celite'	6	0	0
Beet	0-1% K ₂ SO ₂	1% K ₂ HPO ₄ , 3% 'Celite'	2	0·5	0
Beet		1% K ₂ HPO ₄ , 0·2% C, 3% 'Celite'	4	1·0	0·06
Beet		3% 'Celite'	5	5·9	61·0
Beet	0·1% K,80,	1% K,HPO, 3% 'Celite'	3	33.0	1·1
Beet	0·1% K,80,	0·2% C, 3% 'Celite'	8	3.6	182·0
Beet	0·1% K,80,	1% K,HPO, 0·2% C, 3% 'Celite'	7	1.0	548·0

been greater than any known for the increase of any plant virus, and because transfer of the aged inoculum to fresh substrate has not brought about any further increase in infectivity. Diurnal cyclic changes in the susceptibility of the indicator host seem an unlikely explanation because the phenomenon has been expressed at all times of the day. Decrease in activity of inhibitors is considered the correct interpretation.

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Influence of Host Age on Leukaemogenesis and Virus Proliferation after Infection with a Murine Leukaemia Virus

WITH few exceptions, the virus induced thymic lymphomas in mice are strongly dependent on age1-4. Early passages of virus were leukaemogenic only for mice less than 2 days old. After repeated passage of the virus, however, the age specificity diminished and leukaemia could be induced in older mice. Even in these systems, host susceptibility still decreases with advancing age as measured by the increasing latent period between virus inoculation and the development of leukaemia. Despite repeated reports of optimum susceptibility in young animals, there is at present little information available about the cause or mechanism of this phenomenon. The elucidation of its mechanism has been complicated by the fact that both susceptibility to virus infections (for example, ref. 5) and susceptibility to tumour cell transplantation 6-8 have been shown to be enhanced in younger animals. The virus induced thymic lymphomas are convenient for resolving this problem, for the development of viraemia in these systems is separated by some 40-70 days from the first appearance of tumour cells⁹⁻¹³. It was possible therefore, with this system, to determine the influence of host age on virus proliferation independently of the development of leukaemia.

A 20 per cent extract in physiological saline (w/v) of thymuses, spleen and lymph nodes was prepared from mice infected with a murine leukaemia virus (Rich), as Virus preparations were used immediately or stored at -70° C in sealed glass ampoules. Virus was diluted in physiological saline and injected intraperitoneally, in amounts proportional to body weight. Newborn (1-3 day), suckling (4-6 day) and adult mice received 0.1, 0.5 and 1.5 ml., respectively, of a 2×10^{-1} dilution of stock virus.

To determine the titre of infectious virus, organs were suspended in saline and a 20 per cent (w/v) extract prepared by gentle homogenization in a chilled Potter-Elvehjem homogenizer. Cell-free preparations were prepared as previously described¹⁴. Groups of newborn (about 2 day) mice were inoculated intraperitoneally Groups of newborn with 0·1 ml. of the appropriate dilution of extract. Animals were examined at regular intervals for lymph node, spleen and mediastinal enlargement. Diagnosis of lymphoma was confirmed by gross and microscopic pathology using criteria previously described15. Median latent period as

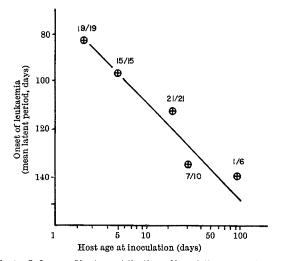


Fig. 1. Influence of host age at the time of inoculation on the development of virus induced leukaemia. Latent period represents the time interval between virus inoculation and the appearance of palpable tumour. Latency is inversely proportional to susceptibility. Numbers above each point represent the incidence of leukaemia (number leukaemic/number inoculated).

indicated in Table 1 represents the time interval between inoculation and the day when 50 per cent of the group inoculated are leukaemic. It is inversely proportional to the potency of the preparation being assayed 16-18.

To determine the influence of age on the susceptibility to virus induced leukaemia, we inoculated groups of random bred ICR/Ha Swiss mice ranging in age from 2 to 90 days with a dose of virus proportional to their body weight. It can be seen from Fig. 1, using the mean latent period for each group as a measure of relative susceptibility, that leukaemia development was strongly age dependent in this system. A latent period of 138 days (incidence: 1/6) for mice inoculated at 90 days old can be contrasted with a mean latent period of 83 days (incidence: 19/19) for animals inoculated at 2 days old14. Groups of mice at intermediate ages exhibited a latent period proportional to the age at which they were inoculated.

Table 1. EFFECT OF HOST AGE ON VIRUS PROLIFERATION AFTER INOCULATION OF A MURINE LEUKAEMIA VIRUS (RICH)

Age of host at inocula- tion	Organ assayed	Dilution (2×10*)	Virus assay No. leukaemic No. inoculated (%)		Median latent period (days)*
2 days	Thymus	-2	6/6	(100)	96
	****	-4	2/6	(33)	
70 days	Thymus	-2	8/8	(100)	79
		-4	4/5	(80)	113
2 days	Liver	-2	6/8	(75)	142
		-4	0/8	(0)	
70 days	Liver	-2	4/5 2/7	(80)	135
		_ 4	917	(98)	

*Time elapsed between inoculation and the appearance of palpable tumour in 50 per cent of the animals inoculated.

The thymuses and livers of mice inoculated at 2 day and 70 day old were assayed for virus content by inoculation of organ extracts into newborn mice.

To determine whether the diminished leukaemic response of older mice was caused by decreased competence to synthesize infectious virus, the following experiment was carried out. Groups of ten mice 2 and 70 days old were inoculated with a dose of virus proportional to their body weight. After intervals of 7 and 14 days the mice were killed. Thymuses and livers from each group were pooled, extracted and assayed for infectious virus.

The data for the groups assayed 7 days after virus inoculation are presented in Table 1. As shown by the incidence of leukaemia and latent period, the yield of infectious (leukaemogenic) virus from the thymuses of animals inoculated when 70 days old was somewhat greater than that obtained from mice inoculated when

2 days old. Similarly, with liver, a shorter latent period indicated higher virus yields from animals inoculated at 70 days. When liver extracts were diluted to 2×10^{-4} , activity was detected in the 70 day but not the 2 day age group. The results obtained with both age groups assayed 14 days after inoculation were essentially the same as those for 7 days and are not shown.

The increased viral titre observed in 70 day old as opposed to 2 day old mice probably resulted from the higher virus input dose employed19. Significant to this study, however, is the fact that the 70 day old mice, which were refractory to the development of leukaemia, were nevertheless completely competent to synthesize infectious

Thus the increased susceptibility of young mice to virus induced leukaemia is not a function of enhanced competence to synthesize infectious virus, but rather of some subsequent step in the leukaemogenic process. offers further support to the idea that viral proliferation and the subsequent steps in leukaemogenesis are separate although dependent processes3,26.

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Cytopathic Effects of the Parainfluenza Virus SV₅ in Vero Cells

SIMIAN virus $5 (SV_5)$ (ref. 1), a member of the parainfluenzamumps-Newcastle disease subgroup of myxoviruses2, is a common contaminant of primary cultures of monkey kidney cells. SV5 virus multiplies to high titre in monkey kidney cells, but causes only minimal cytopathic effects3. Haemadsorption is necessary to show the presence of the virus⁴. In contrast, in BHK-21 cells, it produces extensive fusion and disintegration, yielding relatively little virus.

This communication describes studies on the multiplication of SV₅ virus in a continuous line of African green monkey kidney cells (Vero). Vero cells not only support the growth of SV_5 , but also produce marked cytopathic effects. These changes are produced equally well by virus previously adapted to other cell lines. SV_5 also produced plaques in Vero monolayers under agar overlay. Furthermore, complement-fixing and haemagglutinin antigen yields are high.

The virus used in this study was a laboratory strain and was passed twice in primary, African green monkey kidney cells and twice in continuous, embryonic rhesus monkey kidney ($MA \cdot 104$) cells (report in preparation). Vero cells were obtained from Dr Y. Yasumura, Chiba University, Japan, in 1964, and have been maintained in Medium 199 supplemented with 5 per cent foetal bovine serum, as previously described. The 117th to the 135th passage levels of Vero cells were used in this study. Cell cultures of primary rhesus monkey kidney, MA-104, and BHK-21, were obtained from Microbiological Associates, Inc., and were maintained in Eagle's minimum essential medium in Earle's balanced salt solution containing 3 per cent foetal bovine serum. Two to four tubes were used for each ten-fold serial dilution tested. cultures were observed for 21 days following inoculation. and the fluids were changed every 5 to 7 days. The 50 per cent endpoint was calculated by the Reed-Muench formula7. Plaque assays in Vero cells were carried out as Haemadsorption⁸ and haemagdescribed elsewhere⁶. glutination titrations with guinea-pig erythrocytes were carried out at 4° C and 37° C, respectively. Complement fixation tests were done in the microtitre system as described by Sever³. Titres were recorded as the reciprocal of the highest dilution showing 3+ or 4+ fixation of 1-8 units of guinea-pig complement.

For comparison, the virus was titrated simultaneously in Vero, $B\hat{H}K$ -21, and primary rhesus monkey kidney cell cultures. In Vero cultures inoculated with SV_5 virus in low virus dilutions, early cytopathic changes were evident within 3 to 5 days. Focal formation of dark, granular, irregular syncytia was the most prominent feature of these The highest dilution of the virus produced changes. detectable cytopathic changes by the ninth day after The cytopathic effects progressed to an inoculation. extensive degeneration of the entire cell sheet after an additional several days. Examination of infected cells on Giemsa stained preparations revealed intracytoplasmic inclusions surrounded by white haloes as reported

previously⁵.

Table 1. SV_5 virus titres in primary rhesus monkey kidney. BHK-21

Infectivity titre Vero TCD50Rh. Mk. 5·7† 6·7 BHK-21History AGMK/2; MA-104/2 AGMK/2; MA-104/2; Vero/1

• Infectivity titre expressed as $\log_{10}TCD_{bo}/ml$.

• Haemadsorption titre with guinea-pig erythrocytes at 4° C.

— Not done.

At the end of a titration, 21 days after inoculation, CPE-negative Vero tubes were tested for haemadsorption and always found negative. Cytopathic effects were also observed in BHK-21 cells, but the virus titres were lower than those obtained in Vero cells (Table 1). No cytopathic effects were observed in primary rhesus monkey kidney cells's, and assays were completed by haemadsorption. Table 1 summarizes the results. Titres obtained in Vero cells were similar to or higher than those obtained in primary rhesus monkey kidney cells.

Virus titrations in Vero cells were incubated simultaneously in stationary and rotating positions at 37° C. No significant difference in endpoints was observed, but the extent of the cytopathic changes was strikingly

enhanced in those incubated in roller drums.

 $SV_{\mathfrak s}$ virus plaques were obtained in Vero bottle cultures by using a double overlay procedure. Small irregularly shaped plaques were visible as early as the ninth day after inoculation. They increased in diameter to 1-0-2-0 mm by the twenty-first day. There was little or no increase in number after the twenty-first day. Repeated endpoint titrations of the same virus stock in Vero cells indicated that titres by plaque assay were at least half a log lower than those done by the tube culture method.

Neutralization tests were performed in Vero cell cultures with hyperimmune rabbit serum which was obtained from Microbiological Associates, Inc.

volumes of ten-fold serial dilutions of virus and diluted inactivated serum (1:20) were mixed. Serum-virus mixtures and control virus preparations were held at room temperature for 1 h before inoculation of 0.2 ml. amounts into each of four cultures. The development of either cytopathic effects or plaques by SV_5 was inhibited by type-specific immune rabbit serum.

Cell packs (20 imes), prepared from infected Vero cells on the fourteenth day after inoculation, reacted in complement fixation tests at dilution greater than 1:32 versus $SV_{\mathfrak{d}}$ antiserum. Moreover, tissue culture fluids of infected Vero cells gave an haemadsorption titre of 1:128.

Extensive cytopathic effect and plaque formation in Vero cell cultures make possible an efficient and reproducible direct technique for the demonstration of $\overline{SV_5}$ virus and of specific neutralizing antibodies. In addition, growth of the virus to high titre in a continuous simian cell line provides a convenient source of virus and its antigens for other studies.

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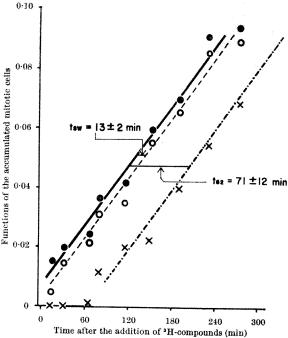
BIOCHEMISTRY

Determination of Switching-off and Switching-on Time of Overall Nuclear **RNA** Synthesis

There may be at least three modes of mRNA synthesis regulation in cultured mammalian cells: the repression of gene action as postulated in bacteria by Jacob and Monod¹; a mechanism in interphase nuclei $(G_1, S \text{ and } G_2)$ stages) involving mRNA synthesis only in the uncondensed chromatin and not in the condensed chromatin2,3; and the lack of or, at least, slowing down of mRNA synthesis in M stage cells. Inherent in the last mechanism is the tacit assumption that the overall mRNA synthesis is switched off about the beginning of M stage and on again about the end of the same stage4-13.

We have focused our attention on the third mode and have attempted to determine, by use of the life cycle analysis 14,15, the exact time in the life cycle when nuclear RNA synthesis is switched off and on. The mouse lymphoma cell line (L5178Y) (refs. 16 and 17) growing in suspension was used and, under our laboratory conditions, generation time was about 9-10 h. In these experiments, the uptake of uridine-5-3H into the nucleus of the cells, as seen in autoradiographs, was used to demonstrate nuclear RNA synthesis (possibly including mRNA synthesis). The silver grains in the autoradiographs of cells labelled with uridine-3H were removed on incubation with ribonuclease but not with deoxyribonuclease, indicating that most of the radioactivity was present as RNA labelled with uridine-3H.

To ascertain the switching-off time of nuclear RNA, two methods were used. The first was an application of



Time after the addition of ${}^3\text{H}\text{-compounds}$ (min)

Fig. 1. Estimation of the times of G_2 phase and of switching-off of over-all RNA synthesis by application of the method of Puck and Steffen (ref. 14). The horizontal distance between slopes of total mitotic cells (\blacksquare) and mitotic cells labelled with uridine- ${}^3\text{H}$ (\bigcirc) represents the time of switching-off of RNA synthesis (tsw). In this case, the time was 13 ± 2 min before mitosis. The horizontal distance between slopes of total mitotic cells and mitotic cells labelled with thymidine- ${}^3\text{H}$ (\times) represents the duration of the G_2 period (G_2). Functions of the accumulated mitotic index are: \blacksquare , $\log (1+m/n)$; \bigcirc , $\log ((1+m/n)/(1+m-m^*n))$; where n, total number of mitotic cells, m, total number of mitotic cells abelled with thymidine- ${}^3\text{H}$, and m^{**} , number of mitotic cells labelled with thymidine- ${}^3\text{H}$, and m^{**} , number of mitotic cells labelled with thymidine- ${}^3\text{H}$.

the method for determining the length of the G_2 period as described by Puck and Steffen¹⁴. To the exponentially growing cell populations, either colcemid (0.025 $\mu g/ml$.) and uridine-3H (1 µc./ml., 8 c./mmole) or colcemid and thymidine- 3 H (1 $\mu c./ml.$, 6 c./mmole) were added. Samples were removed from time to time and autoradiographs were prepared using Kodak NTB emulsion^{17,18}. Fig. 1 shows the results of one of two experiments. The exact time of switching off was calculated by using the equation derived previously14,18. In the two experiments, the switching-off time was 13 ± 2 min and 22 ± 11 min before mitosis. It is interesting that the time of switching off of DNA synthesis (at the end of the stage) occurred about 71 ± 12 min before the M stage.

In the second method for determining switching-off time, only uridine.3H was added to the exponentially growing cell population, and the ratio of the labelled mitotic cells to the total number of mitotic cells was estimated periodically. Fig. 2 shows one of three experiments. The switching-off time in the three experiments was found to be 9, 6 and 11 min. By summarizing the results of the two types of experiments, the average switching-off time can be placed at 12 ± 6 min before onset of the M stage.

The method for estimating switching-on time of nuclear RNA synthesis after mitosis required a synchronized cell population, which was achieved by the combined method of excess thymidine and colcemid previously described17. In the synchronized cell population, thymidine-3H was added during the last hour of the 5 h treatment with colcemid. Most S and G_2 stage cells were labelled by this procedure while most M stage cells were When the colcemid was washed out of the unlabelled. cell culture, uridine-3H (0.5 or 1.0 $\mu e./ml.$ and 8.0 e./ml.mmole) or thymidine-3H (0.5 or $1.0~\mu c./ml.$ and 6.0e./mmole) was added and samples were removed from time to time in order to estimate the percentage of labelled

Fig. 3 shows one of three experiments. Uridine-3H labelled cells appeared immediately after the anaphasetelophase counts reached a maximum, while thymidine-3H labelled cells appeared about 100 min after the appearance of uridine labelled cells. When thymidine-3H or uridine-3H was not added after colcemid treatment, the percentage

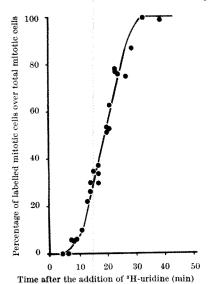


Fig. 2. Estimation of switching-off time of overall nuclear RNA synthesis by the rate of appearance of labelled mitotic cells. The switching-off time was estimated from the time $(t_{1/2})$ when 50 per cent of mitotic cells were labelled. The relationship of $t_{1/2}$ and the switching-off time is Switching-off time $= t_{1/2} + \frac{1}{4} \log \frac{1 + (m_0/2n_0)}{1 + (m_0/n_0)}$

where $a = \frac{\log 2}{\text{(generation time)}} = \frac{\log 2}{9.5 \text{ h}}$ and (m_0/n_0) is the fraction (0.035) of m stage cells in the exponentially growing cell population.

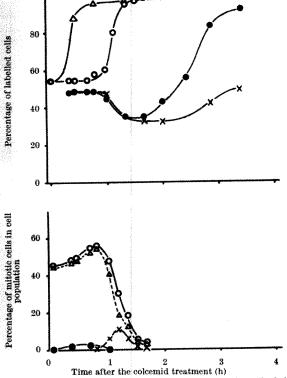


Fig. 3. Estimation of switching-on time of overall RNA synthesis by use of synchronized cell population. Upper figure: labelling of synchronized cells with thymidine. H(♠), with uridine. H(○), and with leucine. H(△). (×), Percentage of labelled cells in the synchronized cells after chasing with cold thymidine. Lower figure: percentage of mitotic cells in synchronized cell population. This represents the movement of unlabelled fraction of the synchronized population through mitosis. ○, Total mitotic cell; ♠, prophase cell; △, metaphase cell; ×, anaphase and telophase cells.

of labelled cells (crosses in Fig. 3) declined, indicating that the non-labelled portion of the synchronized cell population had divided, thereby decreasing the percentage of labelled cells. This decrease coincided in time with the increase of uridine-3H labelled cells. In other words, the cells started nuclear RNA synthesis around the end of mitosis. Close examination of dumbbell-shaped telophase cells revealed that the silver grains were present as soon as furrowing into two daughter cells occurred. This indicates that switching on time coincides with the end of

All previous observations of the switching off time of nuclear RNA synthesis in cultured mammalian cells were made on cells pulse-labelled with uridine-4H. It is interesting that the previous experiments implied two possibilities: the first that nuclear RNA synthesis does not occur in any stage of mitosis4-16; and the second that nuclear RNA synthesis occurs either in prophase only11,12 or throughout most of the mitotic cycle 15. In the latter case, the duration of pulse-labelling often lasted for 10 min or more 11,13 , enabling the G_2 cells to enter prophase; therefore such a result should be viewed with The present observations agree in principle caution. with the first and indicate that nuclear RNA synthesis does not occur during mitosis.

In conclusion, the overall nuclear RNA synthesis (uptake of uridine-3H into the acid-insoluble fraction of the nuclei), possibly into mRNA, was switched off completely about 70 min after the end of the S stage, or about 10 min before the M stage. The overall nuclear RNA synthesis began again at the end of cytokinesis of the Mstage, or approximately 100 min before the S stage of the next life cycle.

Finally, the interesting work of Johnson and Holland* and Buck et al. 19 suggests that M stage cells contain RNA polymerase and are able to synthesize RNA. If this is true, then mRNA synthesis must be switched off as a result of some mechanism, other than absence of enzyme. possibly a condensation of the chromosomes during the Mstage. Our present results are compatible with this idea.

In the present study, only the overall synthesis of nuclear RNA was determined and no information was obtained about whether mRNA for mitosis 20,23 was switched off at the same time as the overall RNA synthesis.

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Appearance of Specific Acid Phosphatase Isozymes in the Synovial Fluid of Patients with Rheumatoid Arthritis

ENZYMES may occur in multiple molecular forms or isozymes¹, often showing tissue specific variations¹⁻³. In different pathological conditions² and after certain types of medication^{4,5}, alterations in serum isozyme patterns have been observed. These findings suggest that isozymes may be valuable markers in clinical research, for example, for the identification of the tissue origin of enzymes occurring in pathological levels in various body fluids. High levels of acid phosphatase activity have been reported in the synovial fluid⁶ and synovial tissue⁷ of patients with rheumatoid arthritis. This report deals with the appearance of certain acid phosphatase isozymes in the knee-joint synovial fluid of patients with rheumatoid arthritis.

Samples of synovial fluid were obtained by puncture of the knee-joint from thirty-six patients. Out of these thirty-six patients, twenty had the clinical diagnosis rheumatoid arthritis (in most cases confirmed by immunological reactions) and sixteen had other diagnoses such as arthrosis deformans, chronic unspecific synovitis, chondromatosis, osteochondritis and various chronic post-traumatic conditions. Samples of synovial membranes were collected from thirteen patients and serum samples were obtained from nineteen patients.

The synovial membranes were homogenized in a mechanical homogenizer in one volume of physiological saline and afterwards centrifuged. The supernatant fluids and the samples of synovial fluid and serum were examined by means of starch gel electrophoresis using a modification (2/3 dilution) of the discontinuous buffer system by Ashton and Braden*. After the electrophoresis the starch gels were preincubated for 30 min in 0·2 molar acetate buffer pH 4·0 and afterwards stained for 2 h in 0·2 molar acetate buffer pH 5·2 using α-naphthyl phosphate as substrate and fast garnet GBC salt as a dye coupler.

In the synovial fluid of seventeen out of the twenty patients with rheumatoid arthritis two distinct acid phosphatase components were found (see Fig. 1). None of the sixteen patients with diagnoses other than rheumatoid arthritis showed this pattern. Four different electrophoretically distinct acid phosphatase components, called A, B, C and D in order of decreasing anodal mobility, have been described in man. The components A and B occurred in the synovial fluid of patients with rheumatoid arthritis. In Fig. 1, a tissue extract of placenta is used to indicate the electrophoretic mobilities of the four components. The A and B components were not found in the sera of the nineteen patients of whom nine had rheumatoid arthritis. A weak acid phosphatase component, with a mobility slightly faster than that of acid phosphatase D, is sometimes found in serum and synovial fluid.

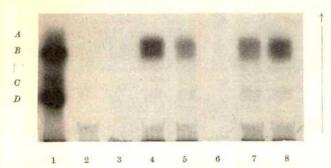


Fig. 1. Photograph of starch gel showing the electrophoretic acid phosphatase patterns in, 1, placental extract and 2–8, synovial fluids. Samples 4, 5, 7 and 8 are from patients with rheumatoid arthritis; 5 and 7 are repeated samples from the same patient. The arrow is indicating the direction of migration towards the anode.

In extracts of synovial membranes from thirteen patients there were variations in the acid phosphatase patterns. Six patients with rheumatoid arthritis (and a typical AB acid phosphatase pattern in the synovial fluid) had acid phosphatase components A, B and D in the synovial membrane. Out of seven patients without rheumatoid arthritis, five lacked acid phosphatase activity in the synovial membrane while two had weak acid phosphatases. Treatment with cortisone did not apparently affect the occurrence of acid phosphatase A and B in the synovial fluid. Three patients had an acid phosphatase component in their synovial fluid with a mobility faster than that of the A component. These patients had been given injections of cortisone into the knee-joint.

These preliminary results indicate that the occurrence of acid phosphatase components A and B in the synovial fluid is typical of rheumatoid arthritis. The acid phosphatase components are apparently derived from the pathological synovial membrane.

The high frequency of rheumatoid arthritis patients with the "acid phosphatase AB reaction" suggests that this synovial fluid alteration may be developed into a useful clinical test for rheumatoid arthritis.

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Dissociation of Urease by Glycol and Glycerol

The existence of a urease of low molecular weight has previously been noted¹⁻³ but in circumstances that prevented ready characterization. We have shown that highly purified preparations of jack bean urease can be reversibly and rapidly dissociated without appreciable change in enzyme activity. The preparative molecular weight of 485,000 (ref. 4) is halved in the process.

Urease can be dissolved in 90 per cent propanediol or glycerol in concentrations up to 1 per cent protein. At pH 9-2 (1 part 0.02 molar tris EDTA buffer plus 9 parts diol or glycerol), urease dissociates completely in a few minutes. In the initial experiments, the electrophoresis pattern produced in acrylamide gel showed only one band, and because the mobility was substantially greater than that of the preparative form, the band observed was termed α-urease. Electrophoretic mobilities in gels of varying concentration and the sedimentation velocity in the ultracentrifuge were consistent with a molecular weight about half that of the original protein. It is possible to free the protein of the propanediol if the sample is dialysed against 0.02 molar tris EDTA, pH 9.2. Such solutions were used to determine the molecular weight by the meniscus depletion sedimentation equilibrium method⁵. At concentrations of 1 mg/ml. of protein, the molecular weight is 236,000, assuming $\bar{v}^4 = 0.733$.

When the pH of a solution of α -urease is reduced from 9·2 to 7·0, in the absence of propanediol or glycerol, there

BD

Fig. 1. Electrophoretic patterns of urease, acrylamide gel, 7 per cent; 0-02 molar tris EDTA buffer, pH 9-2. a, A, urease, 62 mg/ml. in 0-02 molar tris EDTA buffer at 20° C; B, solution in A diluted 1:10 with propanediol, after 1 h; C, solution in B after 2 h; D, solution in A after 2 h; E, solution in B diluted 1:1 with 0-68 molar phosphate buffer, pH 7-0, after 1 h. b, A, B, urease, same as column A in a; C, same as column B in a, but propanediol then removed by dialysing for 72 h against 0-02 molar tris EDTA buffer, pH 9-2; D, control for C, diol not removed.

is a rapid reassociation which can be easily demonstrated by gel electrophoresis. Fig. 1a, column E, shows the pattern obtained with a reassociated preparation. The mobility is slightly less than the native urease, although the sedimentation velocity indicated reformation of the 485,000 molecular weight species. Small amounts of higher oligomers are also evident. Even at pH 9.2 (Fig. 1b, column C) there is a slow reassociation in the absence of diol or glycerol. The amounts of reassociated material are, however, small enough to cause no interference with the determination of molecular weight by the methods used.

The activity of a-urease was easily shown by equilibrating the acrylamide gels in a solution of 1 g of Na2EDTA and 0.5 g of cresol red/l. of water and then placing in a similar solution containing 15 g of urea/l. Areas of urease activity are visible as purple bands on an orange background. The gel can also be stained for protein subsequently with amido black.

The specific activity of α-urease is nearly that of the undissociated form and has similar stability. Urease was assayed as previously described⁶ and protein was estimated by measuring the absorption⁶ at 278 mμ. A control solution of urease, 5.95 mg/ml., in 0.02 molar tris EDTA, pH 9.2, at 20° C had a specific activity of 217 at 0.5 h and 183 after 20 h. α-Urease in 90 per cent propanediol, 6.25 mg/ml., gave specific activities of 212 and 175. Because assays are made at pH 7, it seemed possible that z-urease reassociated during the assay conditions but assay at pH 9 yielded nearly identical values for undissociated urease and α-urease.

On the basis of gel electrophoresis patterns obtained from samples of urease dissolved in buffers of varying ionic strength and pH, and exposed to propanediol or glycerol, it is clear that low ionic strength and high pH predispose to complete dissociation. Careful electrophoresis of highly purified urease preparations (Fig. 1b, columns A and B) shows that, even in the absence of diol, traces of α-urease were present.

These experiments suggest that the common use of glycerol as a "preservative" for urease may be based on its effectiveness in dissociating the enzyme and inhibiting the precipitation of aggregates.

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In vitro Hybridization of Normal and Variant Human Glucose-6-phosphate Dehydrogenase

NORMAL human glucose-6-phosphate dehydrogenase (Dglucose 6-phosphate: nicotinamide adenine dinucleotide phosphate oxidoreductase, EC 1.1.1.49) (G-6-PD) can be dissociated into smaller sub-units under certain conditions1-4, which suggests that normal and variant human G-6-PD may be hybridized in vitro. Studies of such hybridization may provide information on the polypeptide chains in which mutation occurred. Hybridization between human and rat G-6-PD has been observed, but despite the attempts made, no successful hybridization has so far been reported between the normal and variant human G-6-PD. We report here in vitro hybridization of the normal (B+), the common Negro variant with normal

red cell enzyme activity (A+) and the Seattle variant?.

G-6-PD was prepared from the blood of normal and variant individuals. The procedure used for the partial purification was the small scale modification of the method described previously4. Briefly, haemolysate (about 20 ml.) was placed on a 2×3 cm column of DEAE-cellulose buffered with 0.005 molar phosphate buffer pH 6.4 containing 10-3 molar mercaptoethanol, 10-3 molar EDTA and 10-5 molar NADP. The column was washed with about 10 ml. of the same buffer, and the G-6-PD which remained in the column was eluted with about 15 ml. of 0-1 molar phosphate buffer, pH 5-8, containing 0-5 molar sodium chloride. These procedures removed most of the haemoglobin and 6-phosphogluconate dehydrogenase from the haemolysate, and the recovery of G-6-PD activity was always better than 90 per cent. After adjusting the pH to 6.2 with 0.5 molar Na₂HPO₄ and adding disopropylfluorophosphate (10-4 molar) and NADP (5 × 10-5 molar), solid ammonium sulphate (35 g/100 ml.) was added to the eluent. After standing for several hours in the cold, the precipitates were collected by centrifugation. The enzyme could be stored in precipitated form for several months without significant loss of activity. Activities of the partially purified enzyme preparations were: 0.4-0.6 U/mg protein from B+ and A+ individuals and about 0.1-0.2 U/mg protein from the Seattle variant.

Dissociation and reactivation of G-6-PD were carried out in the following way. The enzyme precipitates (1-5 U) were dissolved in 2 ml. of 0.1 molar phosphate buffer (Na₂HPO₄-KH₂PO₄), pH 6·8, containing 10⁻² molar mercaptoethanol and 10-3 molar EDTA. An equal volume of diluted sulphuric acid containing 46 g of (NH4)2SO4/ 100 ml. was added to each enzyme solution. 0.2 molar H.SO, was used for B+ and A+ enzymes, while 0.1 molar H.SO, was used for Seattle G-6-PD. The samples were centrifuged for 10 min at 12,000g, the supernatants were discarded by capillary pipette, and the precipitates were resuspended in the same phosphate buffer. Precipitation

and resuspension were continued in the same manner until less than 20 per cent of the original activity remained. Four treatments when about 1 unit of enzyme was used or eight treatments when about 5 units of enzyme were used were usually required for the inactivation. All the treatments described were carried out at 0°-4° C. It has been shown that purified crystalline B+ enzyme dissociates into sub-units by this treatment, because after inactivation the molecular weight determined by the sedimentation equilibrium method was about one-half of the native active enzyme4.

One millilitre was taken from each sample for a reactivation control, and 1 ml. of each sample (containing approximately the same original activity) was taken to mix for hybridization. Each solution was adjusted to pH 6.8 by the addition of 0.5 molar Na₂HPO₄ and NADP was added to a final concentration of 10-4 molar. The solutions were incubated at 30°-35° C for 1 h and then kept at 4° C overnight. Siliconized tubes were used for both inactivation and reactivation.

The enzyme activity recovered was 60-100 per cent of the original activity. Attempts were made to inactivate and reactivate the enzyme (hybridization) by treatment with propanedithiol, which was used for hybridization of Drosophila 6-phosphogluconate dehydrogenase¹¹, by treatment with guanidine-HCl, which was used for hybridization of lactate dehydrogenases12, and by a modification of these methods. None of these procedures gave successful hybridization of human G-6-PD, but this does not exclude the possibility that suitable modification of these methods may hybridize human G-6-PD.

The mixture of inactivated B+ and A+ enzymes was reactivated as described and subjected to column chroma-Fig. 1A shows the elution diagram of the Compared with the diagram of the artificial sample. mixture of A+ and B+ enzymes, a new peak, indicating hybridization of the two enzymes, was recognized between the two original enzyme peaks.

The mobilities of A+ and B+ enzymes were not very different in starch gel electrophoresis, so the detection of

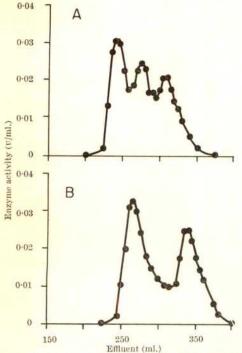


Fig. 1. Elution pattern of enzymes from a carboxymethyl-'Sephadex' column. The enzyme was placed on a carboxymethyl-'Sephadex' column (1-2×30 cm) buffered with 0-02 molar phosphate buffer, pH 5-7, and eluted with a linear gradient of sodium chloride with a concentration from 0-1 to 0-4 molar (mixing chamber 200 ml, each). Enzyme activity is expressed as the number of micromoles of NADP reduced per minute at 25°C. A. After hybridization treatment; B, mixture of A+ and B+ enzymes (without hybridization treatment).

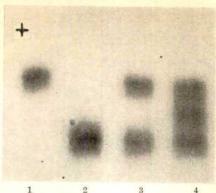


Fig. 2. Pattern of starch gel electrophoresis of G-6-PD. The conditions of electrophoresis (tris-boric acid. pH 8-5 (ref. 8)) and the development of the enzyme⁹ were slightly modified by addition of 10⁻⁹ molar NADP in the cathode buffer¹⁰. Starch was obtained from Electrostarch Co., Madison. 1, A+G-6-PD; 2, Seattle G-6-PD; 3, mixture of A+ and Seattle; 4, A+ and Seattle, after hybridization treatment.

hybrid enzymes, which should be located between A+ and B+, was not so easy as in the column chromato-

The hybridization between the Seattle variant and A+ enzyme could be demonstrated by starch gel electrophoresis (Fig. 2). Hybrid enzyme was detected between the two original enzymes used for the dissociation and co-reactivation. A similar result was obtained in the hybridization between the Seattle variant and B+ enzyme. It should be mentioned that each enzyme which was dissociated and reactivated separately showed identical electrophoretic mobility to the native enzyme.

These experiments revealed that dissociation and reactivation of any one type of G-6-PD gave only one enzyme which was apparently identical to the native enzyme, and that dissociation and co-reactivation of any two different types of G-6-PD produced one hybrid enzyme in addition to the two original enzymes.

The discovery that the hybrid G-6-PD is produced in vitro, and that, in contrast to autosomal 6-phosphogluconate dehydrogenase^{13,14}, no such hybrid enzyme has been detected in female heterozygotes, suggest that only one allele is functioning in any single cell of the heterozygous female. Since G-6-PD is sex-linked, this would imply the inactivation of one X chromosome in each female cell, as suggested by Lyon¹⁵.

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Effect of BP8 Ascites (Sarcoma) Tumours on Glycolipid Composition in Kidneys of Mice

A NUMBER of neutral glycolipids which contain ceramide have been isolated from a variety of mammalian tissues including brain1, liver2,3, serum2, spleen2,4, erythrocyte2,3,5-7, kidney⁸⁻¹², lung¹³ and aseites tumours^{14,15}. The composition of glycolipids in strain-specific ascites tumours differed in different strains of mice 15, which suggested that the composition of tumour glycolipids could reflect the specificity of either the tumour or the mouse strain. Further studies showed that there were significant differences in the glycolipid compositions of the kidneys between certain strains of normal mice. We have now compared the glycolipids of the kidneys of C3H mice carrying a BP8ascites tumour with those of the kidneys of normal C3H mice and with those of the BP8 tumour and the results with reference to the tumour-carrying mice are of considerable interest and are the subject of this communication.

Structural analysis of the carbohydrate portions of the glycolipids by the permethylation procedure 16 , methanolysis and gas chromatography 17 of the methyl ethers showed that the glycolipids in the kidneys of normal C3H mice were glucosyl- $(1\rightarrow 1)$ -ceramide, galactosyl- $(1\rightarrow 4)$ -glucosyl- $(1\rightarrow 4)$ -glactosyl- $(1\rightarrow 4)$ -glucosyl- $(1\rightarrow 4)$ -ceramide, which was the principal component. Only traces of sulphatides were present.

Thin-layer chromatography¹⁸ of samples of the glycolipids isolated from the kidneys of C3H mice carrying BP8 ascites tumours (intraperitoneal injection) indicated a

composition similar to the normal kidney glycolipids; that is, a mixture of ceramide monohexoside, -dihexoside, -trihexoside and aminoglycolipid. The results from the structural analyses of these glycolipids, however, did not confirm this composition. The only methylated hexoside detected by gas chromatography after permethylation and methanolysis of the assumed ceramide trihexoside $fraction \ \ was \ \ methyl \hbox{$^{-2,4,6-tri-O-methyl}$ galactoside}.$ component, considered to be aminoglycolipid from its thin-layer chromatographic properties, gave only methyl-2,4,6-tri-O-methyl galactoside and methyl-2,3,6-tri-Omethyl glucoside in equal proportions—the two components were not ceramide trihexoside and aminoglytolipid. The methylated derivatives that were obtained from the glycolipids and their chromatographic properties on thin-layer plates of silica gel suggested that they were sulphatides. Each of the two compounds was treated with 0.05 normal methanolic hydrochloric acid for 2 h at room temperature¹⁸. The lipids were recovered from the reaction mixtures and examined by thin-layer chromatography on silica gel H with chloroform–methanol–water (65 : 25 : 4by volumes) as solvent. The two principal components now showed the chromatographic characteristics of a ceramide monohexoside and a ceramide dihexoside and not a ceramide trihexoside and aminoglycolipid, only traces of which remained. These results were in agreement with the known ready elimination of the sulphate group18 from ceramide galactoside-3-sulphate in mild acid conditions and supported the identification of the two glycolipids as ceramide galactoside-3-sulphate and ceramide glucosylgalactoside-3-sulphate. Confirmation of the presence of a sulphate group was obtained from their infrared spectra¹⁹. Both glycolipids (as potassium chloride disks) showed a strong absorption band at 1,240 cm⁻¹ characteristic of a

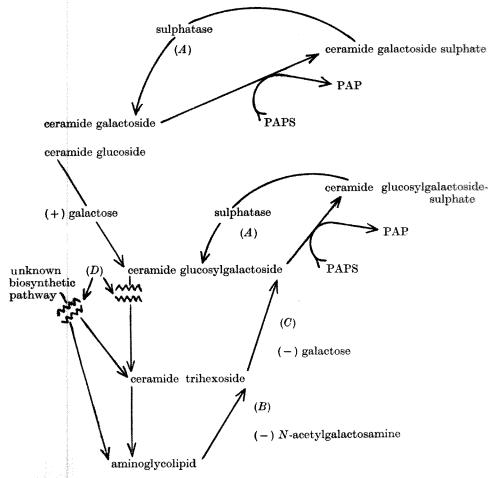


Fig. 1. Possible glycolipid interrelationships.

sugar sulphate. The intensity of the band shown by the ceramide monohexoside sulphate was much stronger than that shown by the ceramide dihexoside sulphate. Details of the glycolipid compositions of the kidneys from the C3H mice, with (a batch of 500) and without (a batch of 250) BP8 ascites tumours, and of the tumours, are summarized in Table 1. The fatty acid composition of the two sulphatides was typical of most mammalian glycolipids in containing a high proportion of lignocerie, nervonic and behenic acids. The distribution of these acids was similar to that in the sulphatides from human kidneys20,21.

The only apparent difference in the mice in the two batches studied was the presence of an ascites tumour, and in seeking an explanation for the considerable difference in their kidney glycolipids it seems reasonable to suggest that some product of the tumour cells is directly, or indirectly, capable of altering the metabolism and/or biosynthesis of one or more of the kidney glycolipids. This raises several interesting questions. Is the observed change in the glycclipids the result of the action of a single substance (for example, a tumour metabolite) produced by the tumour cells and released into the peritoneal fluid, from where it reaches the kidney through the systemic circulation, or the result of a number of biochemical and physiological disturbances arising from the presence and growth of the tumour cells in the host animal? Are the glycolipids in other tissues such as liver, spleen and lungs affected? Do other ascites tumours in other strains of mice produce similar effects? Other unpublished work of Dod and Gray has suggested that the glycolipids may occur exclusively in the plasma membranes of mammalian cells and therefore the question also arises of the possible effect of the change in glycolipid composition on membrane permeability and consequently on renal function.

The results from further comparative studies will provide answers to some of the questions, but it is difficult to relate the presence of the ascites tumour cells to some abnormality in the function of certain tissues of the host animal. A possible approach to the problem is through a study of the metabolism and biosynthesis of the glycolipids in the kidneys of the tumour-bearing animals. The biosynthesis of ceramide monohexoside sulphate probably involves the direct transfer of a sulphate group from 3'phosphoadenosine-5'-phosphosulphate²² (PAPS) to an acceptor substance (Fig. 1) which is ceramide galactoside23 or a ceramide galactoside-protein complex24. In the presence of a microsomal enzyme, galactocerebroside sulphokinase, esterification with the sulphate group occurs at the C-3 hydroxyl of galactose. The formation of ceramide lactosyl sulphate probably involves a similar mechanism. Ceramide glucoside is not a suitable substrate in this transfer system²³ and ceramide glucoside sulphate has not yet been detected in any mammalian tissue. A hydrolytic reaction causing a reversal of sulphatide synthesis has been demonstrated in normal brain and kidney tissue25, and Mehl and Jatzkewitz26 have shown that ceramide galactoside-3-sulphate is degraded by a lysosomelocated sulphatase in pig kidney. This particular sulphatase system contained at least two components, one of which is heat labile and appears to be identical with arylsulphatase type A. A deficiency or absence of this enzyme could allow an accumulation of sulphatide in the tissue and such a situation has been observed in the case of metachromatic leucodystrophy²⁶⁻²⁸. The increased concentration of sulphatides in the BP8/C3H mouse kidneys bears some similarity to the increased depositions of sulphatides in the kidneys in cases of metachromatic leucodystrophy. Martensson et al.²⁹, however, found that in this disease there was no significant change in concentration or relative distribution of the neutral glycolipids although the amounts of sulphatides were markedly increased, whereas in the BP8/C3H mouse kidneys the increase in sulphatides appears to be at the expense of two of the neutral glycolipids. It seems that the particular glycolipid composition

Table 1			
Glycolipid	Glycolipid composition (% of total glycolipid)		
· ·	C3H mouse kidneys Mouse		BP8 ascites
	Normal mouse	carrying BP8 ascit tumour	tumour
Glucosyl-(1→1)-ceramide Galactosyl-(1→1)-ceramide	$\frac{19.5}{3.5}$ 23	${28 \atop 5}$ 33	55
Galactosyl- $(1\rightarrow 4)$ -glucosyl- $(1\rightarrow 1)$ -ceramide Galactosyl- $(1\rightarrow 4)$ -galactosyl- $(1\rightarrow 4)$ -glucosyl-	19	21	21*
$(1\rightarrow 1)$ -ceramide N-acetylgalactosaminyl- $(1\rightarrow 3)$ -galactosyl- $(1\rightarrow 4)$ -galactosyl- $(1\rightarrow 4)$ -glucosyl- $(1\rightarrow 1)$ -	25	Trace	9
ceramide (aminoglycolipid)	33	Trace	15
Galactosyl (3-sulphate)-(1→1)-ceramide Galactosyl (3-sulphate)-(1→4)-glucosyl-	Trace	31	
(1→1)-ceramide	Trace	15	

Includes some galactosyl-(1→4)-galactosyl-(1→1)-ceramide.

found in the BP8/C3H mouse kidney might arise from a deficiency or inhibition of sulphatase (Fig. 1A) together with a block (Fig. 1D) in the biosynthetic pathways to the ceramide trihexoside and aminoglycolipid. Alternatively an inhibition of sulphatase (A) coupled with increased activation of the hydrolytic enzymes (B) (ref. 30) and (C) (refs. 30, 31) would also produce a similar composition.

A detailed study of glycolipid metabolism in the kidneys of tumour bearing mice might provide evidence to confirm that there is a definite relationship between the presence of a tumour metabolite and the composition of the glycolipids.

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Nomenclature of the Pepsins

The Commission on Enzymes of the International Union of Biochemistry¹ recommends that the highly active pepsin prepared from pig gastric mucosa² which is presumed quantitatively to be the principal pepsin should retain the name originally given to it, pepsin A; the other pig pepsins³.⁴, initially called parapepsins, should be named B. There are three of these minor pepsins, and so Ryle⁵ has subsequently used the name B for the former parapepsin I, C for parapepsin II and D for the third. But this alphabetical system is now inadequate, for B has been found to have two components⁵. Fortunately, none of the groups has used the A, B, C, D nomenclature, so that for the time being these initials, which are also given to the porcine pepsins.

The first sub-division of the human pepsins was into fundic and pyloric enzymes^{6,7}. This differentiation continues to have a physiological validity, but has proved inadequate now that more than two human pepsins are Unfortunately each of the groups which has worked on human material has adopted its own nomenclature so that, for example, the pepsin I of Seijffers et al.8 is evidently the pyloric enzyme described earlier6,7 and its pepsinogen is the PG II of Hanley et al. but probably the P III of Kushner et al. 10. This chaotic situation clearly should be resolved. One of us has worked during the past 17 yr with all the separative techniques that have been used by other workers in this field, and has found that agar gel electrophoresis 10,11 provides the quickest method of scanning an activated gastric mucosal extract for pepsins. It can be the most easily standardized, it gives the sharpest separation of the individual enzymes, it recognizes more pepsins than any other method and, being exquisitely sensitive, it can be carried out with only a small amount

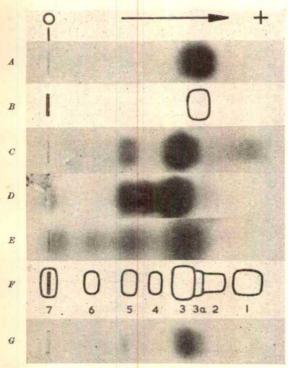


Fig. 1. Agar gel electrophoresis at pH 5·0 of the pepsins. The zymograms illustrate the seven bands that may be seen in normal gastric juice. A, Crystalline swine pepsin; the pepsin is analogous to, but electrophoretically different from, human pepsin 3. B is a drawing of A. C, A human gastric juice showing pepsins 1, 3, 3a and 5. D shows pepsins 2, 3, 3a, 4, 5 and 7. E shows pepsins 3, 5, 6 and 7. F is a drawing illustrating the position of the above zones of proteolytic activity. G, A gastric juice which illustrates more clearly than in C and D that pepsin 3 may sometimes contain a more rapidly moving anodal fraction, numbered 3a.

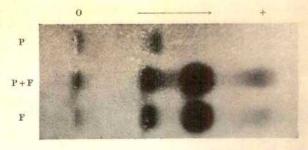


Fig. 2. The pepsins of fundic and pyloric mucosa from a patient with peptic ulcer, displayed after agar gel electrophoresis at pH 5-0. P, Pyloric mucosal extract, showing pyloric pepsins 5 and 7. F. Fundic mucosal extract, showing pepsins 1, 3, 3a, 5 and 7. P+F, A mixture of the two extracts which indicates that the pyloric 5 and fundic 5 enzymes have different mobilities.

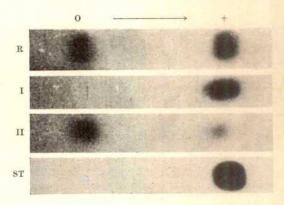


Fig. 3. The proteolytic enzymes of a commercial rennet solution. R, Commercial rennet, showing enzymes in the positions 3 and 7; ST, crystalline swine pepsin, as a standard; I, a chromatographic fraction of rennet containing only calf pepsin 3; II, a chromatographic fraction containing chiefly rennin, which is in position 7.

of enzyme material. It seems only reasonable, therefore, to base a nomenclature on this technique. Anyone working with a gastric fraction can check the behaviour of its pepsins at any stage in a manipulative procedure by agar gel electrophoresis.

With such a nomenclature in mind, more than 200 gastric juice and mucosal extracts from normal subjects and from patients with diseases affecting the gastric mucosa were analysed by agar gel electrophoresis. The variety of patterns displayed reveals that seven proteolytic bands may arise (Fig. 1). Accordingly, it is suggested that these are numbered 1 to 7, in order of decreasing mobility. Fig. 2 shows that there is more than one pyloric enzyme, and that pyloric pepsin 5 is not quite identical with the correspondingly placed band in fundic extracts. Consequently, the number should be prefixed by the initial of the material used: F, fundic; P, pyloric; D, duodenal; M, whole gastric mucosal; J, gastric juice. Fig. 1 shows further that human pepsin 3 and pig pepsin A are not chemically identical, although they appear to be in other respects analogous7,12, so that a further prefix, denoting the animal, could be used: H, human; P, pig; D, dog. Thus HF3 would indicate the principal human fundic pepsin. From published data 10,13, swine pepsin A would on this nomenclature become PF3, and pepsin

Not all these proteolytic bands may indicate an active enzyme, but this is no disadvantage. Thus the 4 band, which we find to be labile, may perhaps be a pepsin inhibitor complex^{12,14}, but it can clearly be demonstrated in gastric juice as a definite biochemical compound and it may well have a physiological significance in this form. This nomenclature would not only locate it accurately for all laboratories, but would describe its transformation into, say, band 3, with economy and precision if the letter I is used for inhibitor; for example, HJ4I→HJ3+I. Future investigation may show that

some of the seven bands have more than one component, and there is evidence in Figs. 1 and 2 that sometimes the 3 band exhibits a slightly faster moving 3a. This, too, may

have a physiological significance.

Until such a nomenclature is widely adopted, it is necessary to record those correlations between the various present nomenclatures that have been established. Thus pepsin C (Ryle) is analogous to pyloric pepsin (Taylor), which is equivalent to pepsin I (Seijffers et al.) and to P III (Kushner et al.). Pepsin B (Ryle) is probably gelatinase (Northrop¹⁵). Pepsin A (Herriott, Desreux and Northrop) is analogous to the fundic pepsin (Taylor), to pepsin II or III (Seijffers et al.) and to P II (Kushner et al.). By the nomenclature suggested here pepsin A would be 3, and pepsin C, 5. The position of pepsin B is not known. As Fig. 3 shows, rennin is 7.

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Electrophoretic Separation of the Soluble Proteins of Brucei Sub-group **Trypanosomes**

The brucei sub-group trypanosomes, now assigned to the sub-genus Trypanozoon by Hoare1, have antigens which continually undergo variation and which are apparently soluble proteins localized in the cytoplasm^{2,3}. The nature of the variation is not yet clear and the antigens have not been well characterized, probably because of the lack of a satisfactory method of separation. Chang et al.4 have drawn attention to the advantage to physiology and genetics of efficient separation of protein fractions, and the immunology of trypanosomes would also benefit by such a method, especially with regard to the correlation of their antigenic and enzyme properties.

Attempts have been made for several years in this laboratory to resolve these proteins by zone electrophoresis-using paper, cellulose acetate membrane, polyacrylamide and starch gels, among other media—with a

varying degree of success.

A Trypanosoma brucei Plimmer and Bradford subgroup⁵ strain (a derivative of stabilate⁶ EATRO 691, originally isolated from Glossina pallidipes Austen at Lugala, south-east Uganda7) was passaged with a syringe through Swiss white mice from the closed colony maintained at the East African Trypanosomiasis Research Organization (EATRO) and stabilized at -80° C by the method of Cunningham et al.8. T. rhodesiense strain SS 518, originally isolated from a patient at EATRO, was kept in rats by syringe passage, with intermediate stabilization at -80° C. The two strains were each inoculated intraperitoneally into groups of ten rats. At peak parasitaemia the rats were bled by cardiac puncture;

heparin at a concentration of 4 IU/ml. of blood was used as an anticoagulant. The blood from each group of rats was pooled, agitated with glass beads? for several minutes to remove platelets10 and then decanted. It was then diluted with five times its volume of a solution of 1 per cent glucose in phosphate buffered salt solution (BS-1) (refs. 11 and 12) at 0° C. The trypanosomes were separated from the blood components using the differential centrifugation method of Moulder13 as modified by Simmons et al.14 by the use of cattle serum (which has been found to agglutinate rat blood14,15) instead of rabbit anti-rat serum, until the parasites were microscopically pure.

The trypanosomes were lysed with a third of their volume of water and broken by freezing (ethanol and dry ice) and thawing (ice water) five times, followed by disintegration for 15 sec using the MSE (60 W) ultrasonic disintegrator and freezing and thawing a further five times. The resulting suspension was spun for 15 min at 21,000 r.p.m. (34,000g) with an MSE 'Magnum' refrigerated centrifuge high speed attachment at $2^{\circ}-4^{\circ}$ C and the supernatant was collected. Some of the supernatant was either used immediately for electrophoresis or stored in capillary tubes at -20° C. The remainder was either freeze dried or concentrated overnight at 4° C, over phosphorus pentoxide or using 'Carbowax 20M' (Union Carbide)16, to a protein concentration of 90-120 mg/ml. as determined by the modified biuret method of Ellman¹⁷, and then used for electrophoresis.

The first trials on paper (Knight, R. H., and Simmons, V., unpublished results) did not give any improved resolution over that of Williamson and Dezowitz¹⁸ of four bands and so were discontinued. Encouraging results, however, were obtained using cellulose-acetate

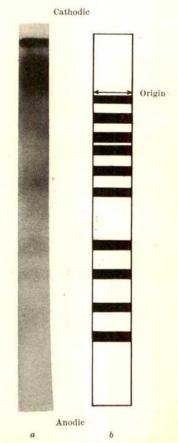


Fig. 1. Cellulose membrane electrophoretic pattern of brucei sub-group trypanosome proteins stained in 1 per cent amido black 10B in methanol/water/acetic acid (50/50/10, y/v). $0\cdot1$ molar tris buffer, pH $8\cdot9$. (a) Photograph of the stained electropherogram. (b) Line drawing of (a).

membranes. Using high resolution tris buffer (LKB Produkter, AB, Stockholm), pH 8·9, a resolution of at least nine bands was obtained (Fig. 1a and b). Although this method was more successful it was discontinued because of the increased resolution which was obtained by gel electrophoresis. Of the gel methods used, disc electrophoresis of Ornestein and Davis^{4,19,20} gave a resolution of eleven components (Fig. 2). Although the method used less material compared with the others and had a better definition, it was discontinued in favour of the starch-gel electrophoresis method of Smithies²¹ which gave us a greatly increased resolution. The starchgel method had an additional advantage in that it

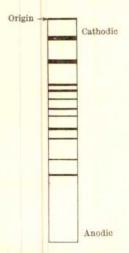


Fig. 2. Line drawing of polyacrylamide disc electropherogram of brucei sub-group trypanosome proteins, stained in 1 per cent amido black 10B in 7 per cent acetic acid. 2.5 per cent large pore gel and 7 per cent small pore gel. See ref. 20 for buffers.

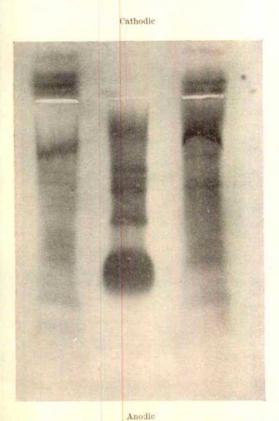


Fig. 3. Photograph of a normal starch-gel electrophoretic protein separation pattern of brucei sub-group tryjanosomes EATRO 691 and T. rhodesiense, using normal rat serum (centre) as control. See Fig. 4 for further explanation.

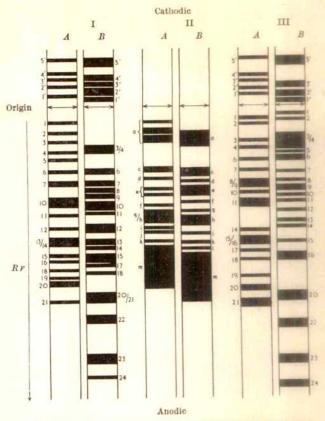


Fig. 4. Scale drawing of starch-gel electrophoretic pattern of bruces sub-group trypanosome proteins from two different preparations A and B. Separation in 0-05 molar glycine/sodium hydroxide buffer, pH 8-9 and staining as in Fig. 1. B is drawn from the same preparation as Fig. 3.

I, EATRO 691; II, normal rat serum; III, T. rhodesiense.

could be sliced into several slabs (four in our case) for staining for different enzymes.

The improved resolution on starch-gel came to our attention through the work of Williamson and Brown³, who had obtained a resolution of eleven anodic components. Our trials were at first unsuccessful because, as we later discovered, the success of the run depended on, among other things, low operating temperatures. We routinely performed the electrophoresis in the refrigerator (and later, in a cold room) at 4° C for 2.5 h at a volt gradient of 8 V/cm. The gel was prepared from hydrolysed starch in the discontinuous glycine–sodium hydroxide–borate buffer system of Fahey and Askonas 22 , but using pH 8-58 instead of 8-9 for the borate buffer. The protein solution was applied to strips of Whatman No. 3 filter paper which were then inserted into the gel. A normal rat serum control was run at the same time and bromophenol blue was applied, as a tracking dye, to a 1 mm hole on one side of the gel. After the run the gel was sliced horizontally, one slice stained for proteins (amido black 10B) and the remainder for enzymes. We found that freeze-drying denatured the proteins, and concentration, although it improved the definition, introduced "necking" and streaking of the proteins. In addition, some of the cathodic and fast moving components (5', 22, 23, 24) disappeared on concentration with 'Carbowax 20M'. We therefore used unconcentrated material, usually freshly prepared but occasionally after storage for 24 h at -20° C, which did not affect the separation pattern.

Fig. 3 shows the resolution of a normal preparation in which at least twenty-two components can be detected. Using the R_F concept, introduced by Chang et al. and defined as the mobility of each band expressed as a percentage of the mobility of the tracking dye, it is possible to compare the reproducibility of the runs. Fig. 4 shows a scale drawing of the resolved components of Fig. 3

(Fig. 4B) and another preparation (Fig. 4A). Each R_F is ±0.5 of the true value to allow for errors in measurement for an error of 0.5 mm introduces an error of 0.5 in the R_F value in a 10 cm run (the usual distance travelled by the dye during the run). The resolution is reproducible to within $1 R_F$ value. Not all components appear on each separation, and inspection of Fig. 4 suggests that there are at least twenty-nine components.

The five cathodic components and three or four anodic components which have a mobility greater than that of rat albumin are particularly interesting. They do not seem to have been reported before, although we have found them in all our preparations (although not all components on each preparation). Some of these components have the same mobility as the rat serum components (the notation on rat serum proteins is that of Beaton et al.23), but two observations suggest that they are not contaminants from the serum. First, trypanosomes separated by the method we used are about 99 per cent pure14 and the concentration of the serum proteins is so low that they are unlikely to be detected by amido black 10B. Second, staining for various enzyme activities (our unpublished work) shows that bands of the same mobility do not correspond in enzyme activities. Although the separation is high, some of the bands still remain relatively complex. Staining for dehydrogenases, for example, shows that some of the bands have multiple enzyme function which implies that they consist of several enzymes of the same mobility. With this improved resolution, however, it will probably now be possible to correlate enzyme and antigenic properties of some of these components.

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New Aminoquinoline with Schistosomicidal Activity

DURING routine testing of compounds, we have discovered schistosomicidal activity in a series of 5-aminoquinolines. Many compounds with this general formula were made and tested and some were found to have activity, but one compound was outstanding. This was 6-chloro-5-βdiethylaminoethylamino-8-methylquinoline.

$$\begin{array}{c} \text{NHCH}_2\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2 \\ \\ \text{Cl} \\ \\ \text{CH}_3 \end{array}$$

The compounds were tested against a strain of Schistosoma mansoni maintained in Australorbis glabratus, originally obtained from Dr O. D. Standen of the Wellcome Institute. All routine tests were carried out in mice. The animals were infected by subcutaneous injection of an estimated 100 cercariae/mouse, because this method was found to be quicker and to give a higher rate of recovery of adult worms than percutaneous infection. Dosing was started on the fifty-sixth day after infection. The mice were given one oral dose daily for 4 consecutive days, starting at 500 mg/kg if the acute LD_{50} was over 1,000 mg/kg, and one-third to one-quarter of the LD_{50} if this was below 1,000 mg/kg. Further testing was carried out at reduced levels when schistosomicidal activity was found, to determine the lower limit of activity. Finally, comparative tests were carried out, using fractions of the subchronic LD_{50} (that is, the dose that killed 50 per cent of the mice given daily doses for 4 successive days) to establish the most active compounds and therapeutic ratios. The mice were killed 3 weeks after the completion of treatment and the numbers of dead and living worms recorded. This time interval was selected because lucanthone, which was used as a reference drug in the comparative tests, did not kill worms before this time, although the aminoquinoline killed them in 7 days.

6-Chloro-5-β-diethylaminoethylamino-8-methylquinoline was found to be active at 30 mg/kg. When the dose was expressed as a fraction of the subchronic LD_{50} we found that the compound killed 90 per cent of the worms at one-eighth of the subchronic LD_{50} (300 mg/kg) while lucanthone killed 70 per cent or less at its subchronie LD_{50} (200 mg/kg).

Acute toxicity studies have shown that the compound has little toxicity in mice, rats, guinea-pigs, rabbits and monkeys. In cats and dogs, however, the compound produced thoracic lesions consisting of peribronehial and pulmonary oedema, a gelatinous exudate around the pericardium and oesophagus, and submucosal oedema and inflammation in the muscle of the bladder. The marked species difference in susceptibility is indicated by the fact that the oral LD_{50} in the cat is 10 mg/kg, while in the rat it is 900 mg/kg.

Chronic administration to mice and rats produced reversible enlargement of the bladder and liver, without any histopathological changes related to treatment at a dose to mice of 200 mg/kg a day for 30 days, but in rats this regimen produced some bladder change. Cats given 4 mg/kg once or twice daily on successive days died within 4 days, but when given two doses on 1 day only there were survivors. The thoracic lesions could be detected radiographically 48 h after dosing, and in the survivors they were shown to be completely resolved 8 Bladder damage was, however, more perdays later. sistent.

Dogs were less susceptible than cats. Mongrels tolerated 80 mg/kg daily for 30 days, and showed only some bladder damage. In beagles, however, 100 mg/kg once or 20 mg/kg twice a day was fatal to some. In these dogs the lesion was oedema of the lungs, with little or no bladder damage.

Monkeys tolerated 120 mg/kg daily for 5 days without showing any gross or histopathological lesions.

No explanation has yet been found for these differences in species susceptibility, and it is impossible to forecast how man would respond to the compound. Unless metabolic studies help to explain these species differences it is likely that this compound will be regarded as too toxic for clinical trials in man, although it could have high activity in human schistosomiasis.

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PATHOLOGY

Antigen Induced Release of Histamine and SRS-A from Human Lung passively sensitized with Reaginic Serum

REAGINS are the skin sensitizing antibodies believed to be responsible for the clinical symptoms of hay fever and allergic asthma in man. They differ from precipitating antibodies in that they bind only to tissues of primate species1, are heat labile and do not form precipitate or fix complement on mixing with specific allergen in vitro2. As a consequence of these properties, the classical method of Prausnitz and Küstner³ (the PK test) remains the only reliable technique for measuring reagin activity. In this test allergic serum is injected into the skin of a nonsensitive human and a weal and flare reaction develops after challenge with antigen. The danger of transmitting infective hepatitis virus to the normal recipient in the PK test has led to many attempts, usually unsuccessful, to develop alternative in vitro procedures. Van Arsdel and Sells4, however, with human leucocytes, and Goodfriend et al.5 with monkey lung tissue, have demonstrated satisfactory passive sensitization after incubation with human allergic serum by measuring histamine release after addition of antigen. This communication describes an in vitro technique involving the passive sensitization of human lung tissue with serum from allergic individuals. The release of histamine and "slow reacting substance of anaphylaxis" (SRS-A) after exposure to specific antigen(s) has been used to measure the antigen-antibody reaction.

Fresh specimens of macroscopically normal human lung were obtained after surgery for carcinoma of the lung. The specimens were transported to the laboratory in ice-cold oxygenated Tyrode solution. The primary bronchi and blood vessels were dissected out and about 12 g of lung parenchyma was chopped finely with sharp seissors in a loz. 'Universal' bottle. The chopped tissue was mixed in about 200 ml. of cold Tyrode solution, filtered through gauze, washed thoroughly and drained of excess fluid. Portions (400 mg) of the chopped washed tissue were suspended in 4 ml. volumes of sera from allergic patients and diluted 1:10 with Tyrode solution or in Tyrode solution alone. Chlortetracycline (0.01 mg/ml.) was added to each tube as a bacteriostat. All tubes were incubated overnight at room temperature, followed by 1 h at 37° C. The samples were then washed thoroughly, to remove excess antibody before incubation at 37° C for 15 min, with 4 ml. of Tyrode solution alone or Tyrode solution containing an extract of cocksfoot pollen antigen(s). The extraction procedure was as follows. 1 g of dry cocksfoot pollen was mixed for 1 min at room temperature with 10 ml. of glass distilled water. suspension was immediately filtered through 'Celite', the filtrate discarded, and the pollen further extracted for

1 h with 10 ml. of Coca's solution from which the phenol was omitted. The suspension produced was filtered through 'Celite' and the filtrate was sterilized by membrane filtration. By an aseptic technique the sterile extract was bottled in 5 ml. portions and stored at -20° C. Before use the pollen extract was diluted to 10^{-2} with Tyrode solution.

After incubation of the lung samples with antigen the supernatants were removed, placed in ice, and assayed for histamine and SRS-A on the same day using the isolated guinea-pig ileum bathed with Tyrode solution containing atropine sulphate (10-6 molar). When assaying SRS-A, mepyramine maleate (10-6 molar) was also added to the bathing fluid to prevent interference from the histamine in the samples. Histamine content was expressed as histamine base and SRS-A in arbitrary units with reference to 0.05 ml. of a standard sample of freeze-dried perfusate. The total histamine content of the lung tissue was determined as follows: duplicate lung samples were treated as experimental samples except that after washing to remove excess antibody they were heated in 10 ml, of Tyrode solution for 5 min in a boiling water bath and then left for 10 min at room temperature before the supernatants were removed for assay.

Table 1. HISTAMINE AND SES-A RELEASED FROM HUMAN CHOPPED LUNG

Lung tre Sensitized with	Challenged	No. of amples	(ng	amine released y/g of lung) Range of mean (P = 0.95)		(U/g Mean	released of lung) Range of mean $(P = 0.95)$
Tyrode only	Antigen	18	287	208-366	1.4	31	20-42
Allergic serum	Tyrode only	20	247	176-318	1.2	41	26-56
Allergic serum	Antigen	36	3,024	2,174-3,874	14.7	298	238-358
Allergic serum	Boiled for total hist- amine content	20	20,572	13,230-27,914		ser terri	en e

Sera from twelve patients exhibiting hay fever and allergic asthma provoked by mixed grass pollen have been studied. Sera were obtained using standard aseptic techniques and were stored in 2.5 ml. portions at -70° C without preservative. Small amounts of histamine and SRS-A were found in the supernatants of the two types of control tubes containing (a) sensitized lung tissue "challenged" with Tyrode solution only and (b) unsensitized tissue "challenged" with antigen (Table 1). Consequently, we have adopted the following criteria to denote sensitization of lung tissue by the serum, that is, that in at least two experiments with different lung tissue the antigen induced histamine and SRS-A release should be at least 2.5 times the control values. By these criteria three of the sera caused sensitization and a further three produced excellent sensitization with histamine and SRS-A release from five to twenty-five times the control values. One of the latter sera (from patient P.St.) has been used extensively in our experiments (Table 1). The clinical data give no obvious reason for the differences in the sensitizing activity of serum from P.St. compared with the inactive serum of, for example, R.A. Both patients had severe hay fever and moderately severe asthma confirmed by aerosol provocation as well as skin tests and had not been desensitized. It is interesting that our colleagues have found that both sera were able passively to sensitize "reactive" leucocytes for histamine release on antigen challenge. Similarly leucocytes from both sera released histamine on direct challenge with cocksfoot antigen. Levy and Oslers found that only one in every five non-allergic individuals provided suspensions of leucocytes suitable for passive sensitization with reaginic serum. Active sera from different individuals, however, could sensitize the "reactive" leucocytes from this small

proportion of donors. Our results imply the opposite, that is, that only certain sera will passively sensitize human lung, but that active serum P.St. passively sensitized all but three samples of lung tissue from thirty patients. There is a marked specificity in a reaginic antibody-human tissue reaction the basis of which is unknown.

Results of ten experiments with different lung tissue but the same serum (P.St.) at a dilution of 1:40 are shown in Table 1. Release of histamine from sensitized lung after antigen challenge was 11.3 times the mean control values and the antigen induced SRS-A release was 8.3 times the mean control values. These increases were very significantly different from the controls (P < 0.001)according to Student's t test.

Brocklehurst⁷ studied the release of these same pharmacological mediators of anaphylaxis during perfusion of an actively sensitized human lung segment (200 g) removed from a woman allergic to birch tree and timothy grass pollen. After challenge with these antigens, totals of 1,425 ng of histamine/g lung and 135 U of SRS-A/g of lung were found in the perfusate after 78 min. These amounts were about half those from passively sensitized lung during 15 min in the experiments reported here (3,024 ng of histamine/g of lung and 298 U of SRS-A/g of lung).

In an attempt to verify the role of reaginic antibody in these experiments, equal volumes of the human allergic serum from patient P.St. were heated at 56° C for varying The heated sera were then incubated with portions of human lung tissue as already described.

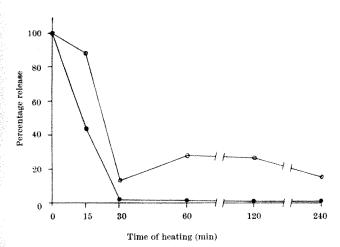


Fig. 1. Effect of subjecting serum to a temperature of 56° C for varying times and the ability of the serum passively to sensitize human lung.

◆. Histamine; ○, SRS-A.

Results are shown in Fig. 1. Releases of histamine and SRS-A are shown as percentages of the release obtained after sensitization with unheated serum and have been corrected for the small spontaneous "leakage" of histamine and SRS-A in control samples. Each point on the graph is the mean of triplicate samples. Heating the serum for 30 min at 56° C substantially destroyed the capacity of the serum passively to sensitize human lung. This suggests that the sensitizing antibodies were reagins.

In summary, a technique for passive sensitization of human lung with homologous reaginic serum is described and its efficacy demonstrated. Experiments with heated serum provided evidence that the sensitizing antibodies were reagins. Three out of the twelve sera from allergic individuals were particularly potent and a further three gave satisfactory sensitization. Thus not all sera from allergic patients would sensitize human lung, although clinical data gave no obvious reason for the differences in the sensitizing activities of the sera. The technique described is not therefore an acceptable substitute for the PK test. With suitably potent reaginic sera, however, this technique can be used as an in vitro model of an immediate hypersensitivity reaction in the lung.

We thank Mr Gordon Jack for specimens of human lung, Dr Geoffrey Taylor for some serum samples, Drs J. S. G. Cox, R. E. C. Altounyan and W. E. Brocklehurst for encouragement and advice, and Mrs S. Twiss and Mrs P. M. Bryson for technical assistance.

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CYTOLOGY

In vitro Production of Meiosis Inducing Substance by Nerve Extract in Ovary of Starfish

It is well known that spawning can be induced by injecting a water extract of radial nerves into the coelomic cavity of mature male or female starfish1-4. The coelomic fluid of the starfish, Asterias amurensis, at the time of natural spawning shows gamete shedding activity similar to that of nerve extracts, and so the active substance seems to be liberated from nerve into the coelomic cavity, where the gonads are suspended. This gamete shedding substance is a polypeptide^{3,6} and seems to be a neurosecretory substance^{3,7}. As well as inducing spawning, it may act directly on the ovary and induce occyte maturation7,8, for in A. amurensis local application of nerve extract to an isolated ovary induces maturation of oocytes located only in the treated portion, and isolated oocytes in calcium free sea water containing nerve extract undergo meiosis*. Oocytes of A. amurensis usually undergo meiosis when isolated in normal sea water but not in calcium free sea

In the case of Asterina pectinifera most of the oocytes isolated in sea water fail to undergo meiosis, and addition of nerve extract has little effect in inducing maturation. We found, however, that these oocytes do undergo meiosis when transferred to sea water containing nerve extract in which ovarian fragments have spawned heavily 1-3 h previously (Fig. 1). This suggests that some active substance is liberated into the medium from the ovary. Breakdown of follicles around the oocytes was clearly observed when the isolated oocytes were transferred into the active medium.

These active media were subjected to gel filtration on a column of 'Sephadex' in an attempt to separate a meiosis inducing substance from the gamete shedding substance of nerves which had been introduced into the media. Asterina ovaries were weighed and placed in artificial sea water containing nerve extract (200 mg of wet ovary/ ml. of sea water containing 100 µg of lyophilized nerve). The nerve extract was prepared by a method described before⁵. After 2.5 h these mixtures of ovary and nerve extract were centrifuged at 3,500 r.p.m. for 10 min. The supernatants induced meiosis even at ten- to twenty-fold dilution. Samples (3 ml.) of each supernatant were applied to a column of 'Sephadex G-15' (1.4 × 42 cm) equilibrated with artificial sea water (pH 8·2-8·3), which was also used as eluant. The fraction size was 5 ml. and the flow rate was 30 ml./h. Samples in small Petri dishes (1.5 ml. each of test solution) containing either isolated oocytes or ovarian fragments were used to assay for maturation and spawning. Observations were made after 1 h. Fig. 2A shows a representative result. After gel filtration the meiosis inducing substance did not appear in the same fractions (fraction No. 21-23) as the gamete shedding substance (fraction No. 5-7). It was interesting that the fractions which induced meiosis also induced spawning (fraction No. 21-23).

For comparison, 3 ml. of sea water containing nerve extract (100 µg/ml.) and 3 ml. of the supernatant (centrifuged at 31,000g for 1 h) of ovary homogenate (200 mg of wet ovary/ml. of sea water) were also gel filtered on the same column of 'Sephadex G-15' and respective fractions were assayed in the same manner. No fractions which induced meiosis, however, were obtained by gel filtration of either nerve extract or ovary extract (Fig. 2B and C).

These facts suggest that the gamete shedding substance of neural origin acts on the ovary and induces the production of a new substance which is, in a true sense, a meiosis inducing factor and probably also a spawning inducing factor. In other words, a polypeptide, probably a neurosecretory substance, derived from nerves seems to act on the ovary, the target organ, and there to produce a second active substance(s) which is responsible for maturation and spawning. We propose, to avoid confusion, that the second substance of ovarian origin is called "meiosis inducing substance" (MIS), for the active principle of neural origin has been called "gamete shedding substance" (GSS).

Some investigations of the nature of MIS gave the following results. First, heating in boiling water for at

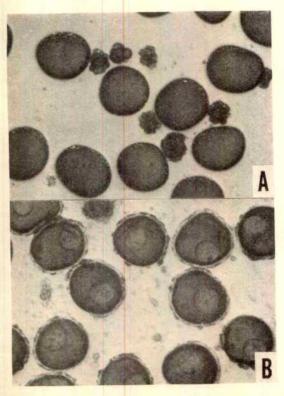


Fig. 1. Induction of meiosis of isolated Asterina occytes, A, Oocytes in the supernatant of mixture of ovary and nerve extract. B, Control in sea water. Observations were made after 1 h. Note breakdown of germinal vesicles and follicles in A.

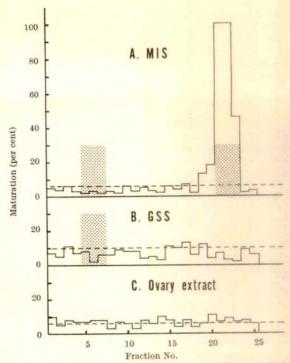


Fig. 2. Meiosis inducing activity of fractions obtained by gel filtration of supernatant of (A) mixture of ovary and nerve extract (MIS), (B) nerve extract (GSS), and (C) ovary extract on 'Sephadex G-15'. Broken lines represent percentages of maturation in sea water (control). Dotted area represents the fractions having gamete shedding activity.

least 30 min had no effect on its maturation and spawning inducing activities. Second, after successively shaking the active supernatant with ether, benzene and petroleum ether, all these activities remained in the aqueous phase. Third, treatment with trypsin (Sigma, 10,000 U/mg), chymotrypsin (Tokyokasei, 22,000 U/mg), pepsin (Sigma, 2,800 U/mg) for 2 h at 25° C and finally pronase P (Kakenkagaku, 45,000 PUK/g) for 5 h at 37° C had little effect on its meiosis and spawning inducing activities, whereas the spawning inducing activity of the nerve extract was completely removed by the same treatments with these proteolytic enzymes (Table 1). The final concentration of the enzymes was 0.01 per cent and the pH of the reaction mixtures was 2.0 for pepsin and 7.9 for the other enzymes. These results indicate that MIS is heat stable, water soluble, not a breakdown product of the gamete shedding substance, and probably not a peptide.

Table 1. EFFECT OF PROTEOLYTIC ENZYMES ON INDUCTION OF MEIOSIS AND SPAWNING BY MEIOSIS INDUCING SUBSTANCE AND GAMETE SHEDDING SUBSTANCE IN Asterina pectinifera

Enzymes		inducing ce (MIS)	Gamete substan	Sea water (control)	
24-0-	Spawning	Maturation (per cent)	Spawning	Maturation (per cent)	(per cent)
Trypsin	+	98	-	6	4
Pepsin	+	99	_	9	. 9
Chymotrypsin	+	98	_	11	12
Pronase P	+	100	_	10	4

Production of meiosis inducing substance in the isolated ovarian fragments under the influence of nerve extract was also found in other starfish species, such as Coscinasterias acutispina and Asterias amurensis. Although the nerve extract of A. amurensis failed to induce spawning in A. pectinifera, the meiosis inducing substance of A. amurensis induced maturation of isolated oocytes and spawning of A. pectinifera. In other words, although there are some species differences among the gamete shedding substances of nerves, this is not observed among the meiosis inducing substances of ovaries. Indeed, the meiosis inducing substance of one species acts equally well on the isolated oocytes of other starfish species without exception as so far tested.

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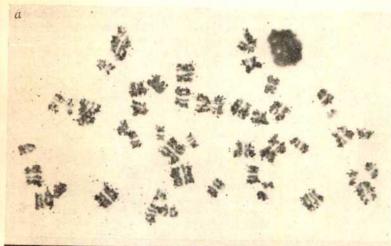
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Spatial Distribution of Old and New Chromatid Sub-units and Frequency of Chromatid Exchanges in Induced **Human Lymphocyte** Endoreduplications

In Vicia faba, chromatids are formed by two functional sub-units that replicate during interphase and segregate at anaphase together with their replicas1. This has been confirmed in other organisms2-6 and interpreted on the basis of a uninemic chromosome model and semiconservative DNA replication6.

Old and newly synthesized chromatid sub-units show a well defined spatial relationship in spontaneous and induced endoreduplications7,8. We have therefore sought further data on the spatial distribution of newly synthesized chromatid sub-units in diplochromosomes and quadruplochromosomes of endoreduplications induced by desacetylmethylcolchicine ('Colcemid', Ciba)'. have also sought to estimate the types and frequency of sister chromatid exchanges in human material.



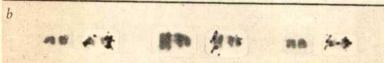


Fig. 1. a, Autoradiograph of an endoreduplication labelled with ³H-thymidine during ES_1 . Most diplochromosomes have the label chiefly in the external chromatids. b. Acentric fragments and their autoradiographs, showing the same labelling pattern of the normal chromosomes of the endoreduplication shown in a.

1. Position of Labelled Chromatids in diplochromosomes after uptake of tritiated thymidine during the first S period

Chromo- some group	0.00	exchanges Outer/inner		Diplochromo- somes with exchanges	Total
A	47	3		69	119
B	25 240		1	27	53
D	162	8		195	439
E	122	2	_	60 41	231 165
G	88	1	_	20	109
Total	141	9	-	11	157
Lotal	825	23	2	423	1,273

Blood was obtained from two normal male donors and cultures were set up by a modification of the technique of Moorhead et al.10. On the third day of incubation, 3H-thymidine (specific activity 5 c./mmole) was added to the cultures at a concentration of 1 µc./ml. of culture medium. After 6 h, 'Colcemid' was added to a final concentration of 0.5 µg/ml., and 2 h later the radioactive medium was removed and the cells were resuspended in fresh medium containing 0.02 ml. of phytohaemagglutinin and 0.1 µg of 'Colcemid'/ml. and carrier thymidine at a concentration 100 times greater than that of the 3H-thymidine in the discarded medium. The cultures were incubated for a further 48 or 72 h and chromosome preparations were made by the air drying technique.

The slides were stained with acetic orcein for the cells to be photographed before autoradiography. Cultures collected 48 h after the change of medium showed only single endoreduplications (about 4 per cent of all the metaphases), while those collected after 72 h contained both single (8 per cent) and double endoreduplications (0.6 per cent). The slides were then coated with Kodak AR-10 autoradiographic stripping film, exposed for 6 or 10 days and developed in Kodak D-19b for 5 or 2 min,

respectively.

Ninety per cent of the endoreduplications were labelled and almost all of these showed diplo- or quadruplochromosomes with only two labelled chromatids. This indicated that they had taken up 3H-thymidine during the first DNA synthetic period of the process of endoreduplication (ES₁)^{7,11}. One hundred and thirty single and ten double endoreduplications showing fairly uniform chromatid labelling were analysed as follows. A schematic drawing was made of all diplo- or quadruplo-chromosomes

showing well spread and overlap-free chromatids from examination of the unlabelled photographs. The autoradiographs of these chromosomes were analysed and the labelling patterns were marked in the drawings. We studied 1,273 diplochromosomes and sixty quadruplochromosomes.

Of the 1,273 diplochromosomes analysed, 850 gave information on the distribution of old and new chromatid sub-units. As shown in Fig. 1a and indicated in Table 1, most of these diplochromosomes were labelled over the two external chromatids (outer/outer labelling pattern), while only a few had one external and one internal chromatid labelled (outer/inner pattern), and even fewer had both the internal chromatids labelled (inner/inner pattern). The only labelled DNA was that of chromatid the two sub-units synthesized during ES1, and so a random spatial arrangement of old and new sub-units after the second replication in diplochromosomes should result in a 1:2:1 ratio of outer/outer, outer/inner and inner/inner patterns of labelling. Consequently, the observed frequencies of these patterns (see Table 1) show clearly, in agreement with previous reports7,8, that the old and new chromatid sub-units are not distributed at random. The few instances of outer/inner and inner/

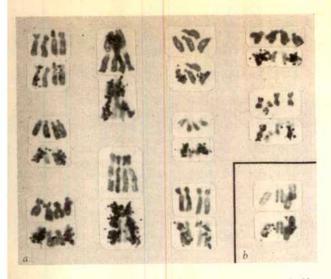


Fig. 2. a, Autoradiographs of quadruplechromosomes from double endoreduplications labelled during ES_1 with *H-thymidine. The label is chiefly in the internal chromatids of the two outermost chromosomes. b, Acentric fragment showing the same labelling pattern of the normal chromosomes shown in a.

inner patterns are probably explained by technical artefacts.

The 423 diplochromosomes showing chromatid exchanges could not give clear information on the distribution of old and new chromatid sub-units, but on the average even in these diplochromosomes the external chromatids showed larger labelled areas than the internal ones.

Our findings in double endoreduplications provide additional evidence for the non-random spatial arrangement of old and new chromatid sub-units. The quadruplo-chromosomes labelled during ES_1 showed only two

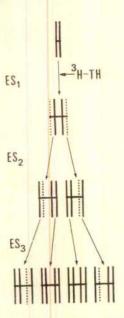


Fig. 3. Schematic representation of the spatial relationship of old and new chromatid sub-units during successive replication cycles leading to single endoreduplication (after ES_s) and double endoreduplication (after ES_s). Each chromatid is represented by two sub-units which are drawn as solid or broken lines to indicate whether they are unlabelled or labelled. The centromere is represented by a transverse line. ES_1 , ES_s and ES_s are respectively the first, second and third DNA synthetic periods. "H-thymidine ('H-TH) was present only during ES_1 , and so only the sub-units synthesized during this period are labelled. The figure shows that if at each replication the new sub-units are synthesized on the outside of the old ones, the labelled sub-units will be part of the external chromatids of the diplochromosomes (after ES_s) and of the internal chromatids of the outermost chromosomes of the quadruplochromosomes (after ES_s).

Table 2. CHROMATID EXCHANGES IN SINGLE ENDOREDUPLICATIONS

Chromo- some group	No. of chromo- somes	No. of ex Twins	xchanges* Singles	Ratio† twins/singles	frequency/ chromosome and cell cycle†
A B C D E F	238 106 878 462 330 218 314	18 (12) 7 (3) 63 (28) 27 (4) 7 (5) 3 (2) 3 (1)	62 (30) 20 (15) 131 (88) 46 (18) 15 (21) 4 (12) 10 (7)	1:3·4 (3) 1:2·8 (3·5) 1:2 (2·4) 1:1·7 (2) 1:2·1 (3) 1:1·3 (3·2) 1:3·3 (4·2)	0·205 (0·319) 0·160 (0·259) 0·146 (0·228) 0·107 (0·136) 0·043 (0·090) 0·022 (0·059) 0·025 (0·039)
Total	2,546	128 (55)	288 (191)	1:2.2 (2.6)	-

The numbers of exchanges at the centromere are in brackets.
 † The calculations which include the exchanges at the centromere are in

brackets.

labelled chromatids. These were the inner ones of the two outermost chromosomes in all the quadruplo-chromosomes, which did not show chromatid exchanges (Fig. 2a), but even when exchanges had occurred the two inner chromatids of the outermost chromosomes generally showed the largest labelled areas. A preferential pattern of labelling was therefore also evident in quadruplo-chromosomes where two cycles of DNA replication had

taken place after labelling in ES1.

As previously pointed out 7.8, the labelling pattern observed in the diplochromosomes could be explained if at each replication the new DNA sub-units of each chromatid are synthesized on the outside of the old ones. Fig. 3 shows that this explanation fits also the described quadruplochromosomes labelling pattern. Walen has suggested that the centromere could prevent replication between the old sub-units and thus be responsible for the peripheral location of those newly synthesized. We have found, however, in a few endoreduplications, acentric fragments showing the same distribution of labelling found in normal chromosomes (for example, Figs. 1b and 2b). This suggests that some other feature of the chromosome structure is responsible for this phenomenon.

Sister chromatid exchanges in human mitotic chromosomes have been observed 2,5,11 , but an estimate of their types and relative frequencies has not been given. In the diplochromosomes labelled during ES_1 it is possible to score twin and single exchanges, that is first and second cycle exchanges after labelling (compare ref. 3). Our findings in 1,273 diplochromosomes (2,546 single chromosomes) are summarized in Table 2. This shows that the frequency of exchanges per chromosome and cell cycle decreases from groups A to G suggesting that this frequency is related to the mean chromosome length.

Several exchanges appear to have occurred at the centromere region. These have been listed separately in Table 2 because they could be mimicked by a morphologically non-perceptible twist at the centromere. Marin and Prescott¹², however, have shown that in Chinese hamster cells true centromere exchanges are very frequent, and this might be true also in our material.

Finally, Table 2 shows that the ratio of twin to single exchanges is quite close to 1:2, in keeping with Taylor's

findings in plant material4,6.

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PHYSIOLOGY

Surface versus Intracellular Polarization of Cortical Cells

Although it is almost 100 yr since the original report of Fritsch and Hitzig1, many of the effects of direct current stimulation (polarization) of the surface of motor cortex on cellular activity remain unclear. Electrophysiological research has shown that surface anodal polarization increases while surface cathodal polarization decreases the rate of discharge of cortical cells2-5. These results have been interpreted in terms of a model developed to explain the effects of direct current applied extracellularly on isolated nerve terminals^{6,7}. In its simplest form, increases and decreases in discharge rate have been related to polarization induced displacements in membrane potential of cells subjected to a longitudinal potential gradient 8-12. Presumably some fraction of diffuse extracellular applied current enters areas of superficial membrane close to the focal polarizing electrode. The chief effect of this current. however, is exerted as it leaves from distant parts of the excitable membrane. Accordingly, in the cortex, a surface anode provides inward current as it enters superficial dendritic regions of vertically oriented cells which subtend several cortical layers. At the same time the anode generates an outward depolarizing current as it passes out through deep-lying areas of excitable membrane in the soma or axon-hillock region of the cell. The sequence of events is presumably reversed when a surface cathode is used.

No cell in the cortex, however, can be assumed to exist in true isolation, and certainly the prototype cell for the hypothesis outlined here—the pyramidal type cell-

forms only a small segment of a synaptically connected neural assembly comprised of multiple vertical links¹³. Changes that occur in cell behaviour during polarization as a result of secondary synaptic effects have been considered 12,14,15, but never demonstrated. The purpose of this communication is to show that some of the changes observed in cell activity during polarization result from indirect effects mediated by presynaptic sources.

The results are based on intracellular responses obtained from identified pyramidal tract cells (PT) and unidentified cells in the motor cortex of the cat. Recordings were made from unanaesthetized preparations 6-12 h after transection of the midbrain 16. Polarizing currents were applied to the cortex through a silversilver chloride electrode connected to the pial surface through a pool of 0.9 per cent sodium chloride and a 0.5 mm diameter opening in the base of a plastic cup used to stabilize the cortex. The path for the return of the current was provided by a diffuse electrode in the mouth. Recording micropipettes (3 molar potassium chloride) were inserted through the opening used apply polarizing currents. Intracellular stimulation was accomplished with a modified Wheatstone bridge¹⁷.

The effects of different intensities of anodal polarization are illustrated in Fig. 1A, 1-4. As in the case of most cells that were affected by polarization, the average rate of cell discharge increased as a function of stimulus intensity. In many cells, however, there was a pause after the initial discharge evoked at the onset of polariza-This cessation of activity was succeeded by a resumption of spike discharge that continued at an increased rate until the current was turned off.

To exclude the possibility that the cessation of cell discharge was elicited by a direct effect of current on the postsynaptic membrane, different intensities of transmembrane polarization were tested (Fig. 1C, 1-4). At each intensity, intracellular stimulation evoked rhythmic cell firing without any indication of a pause in cell activity comparable with that seen during surface polarization.

Results of transmembrane stimulation indicate that the pause in cell discharge during surface polarization originates either from a direct but remote effect of extracellularly applied current on the postsynaptic membrane, or from an indirect effect mediated by presynaptic elements. A close examination of the pause in cell discharge revealed a perceptible downward displacement in the polarization potential induced by polarization immediately after the initial cell discharge (see Fig. 1A, 2-4). Often small prepotentials were detectable during this period, suggesting that there was an active inhibition of the process of spike generation. The hyperpolarizing displacement in the potential induced by polarization was not apparent in extracellular records. Rather, the potential recorded from near the cell after the onset of surface polarization showed a smooth exponential rise to the final steady state (compare Fig. 1D with 1A, 4).

To determine whether the inhibitory pause was dependent on the level of the postsynaptic membrane potential, surface polarization was applied during intracellular hyperpolarization of the cell membrane which produced two important changes in the discharge pattern evoked by anodal polarization. The rate of discharge was reduced. Conversely, a greater number of discharges appeared during the period usually occupied by the inhibitory pause (compare Fig. 1A, 4 with 1B). This seemingly paradoxical effect would be expected if the inhibitory pause results from an underlying inhibitory

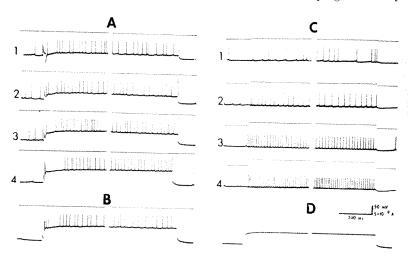


Fig. 1. Comparison of surface and intracellular polarization effects on a cell in the motor cortex. All responses are from the same cell. A, Different intensities of surface anodal polarization: 1, 25 μamp; 2, 50 μamp; 3, 75 μamp; and 4, 100 μamp. B, Effect of surface anodal polarization (100 μamp) during intracellular membrane hyperpolarization. C, Different intensities of intracellular polarization. Current trace shown in top trace, cell response to depolarizing currents shown in bottom trace. D, Extracellular response during surface anodal polarization (100 μamp) recorded after withdrawal of electrode from cell. Approximately 8 sec of surface or intracellular polarization deleted from each response. Deleted portion signified by separation in continuous trace. Cell depth 1-53 mm, action potential 69 mV and average resting potential 60 mV. Voltage and time calibration refers to all records; current calibration refers to records shown in C.

postsynaptic potential (IPSP). Intracellular hyperpolarization reduces the efficiency of the IPSP by driving it closer to its equilibrium potential. Membrane hyperpolarization only partially restricts the excitatory drive on the cell induced by polarization, and so more discharges can occur during the inhibitory pause.

The cell shown in Fig. 1 was one of a group, not from the pyramidal tract, which exhibited complex synaptic patterns of activity following stimulation of nucleus ventralis lateralis (VL) or the peduncle (PED) (Fig. 2A). To determine whether synaptic activity could modify the effects elicited by a surface anode on cell discharge, VL or PED stimulation was applied during surface polarization (Fig. 2B). Both excitatory and inhibitory potentials were apparent during polarization, and while the total duration of the IPSP was shortened its amplitude was unchanged. Spike discharges induced by surface polarization were completely abolished during the initial period of the IPSP.

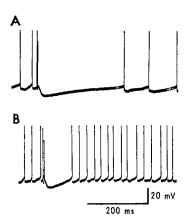


Fig. 2. Effects of surface anodal polarization on inhibitory post-synaptic responses. A, EPSP-IPSP sequence evoked by stimulation of the pyramidal tract (PED). B, Same sequence during surface anodal polarization (100 μ amp). Responses from the cell shown in Fig. 1.

A comparison of the results of intracellular and surface polarization indicates that the inhibitory pause probably does not result from a direct effect of extracellularly applied current on the postsynaptic membrane. Instead, the evidence suggests that the inhibitory pause represents a synaptically induced event. The inhibitory pause is characterized by a movement of the membrane potential in a hyperpolarizing direction. Prepotentials without associated spike discharge often occur during this period. The effects of intracellular hyperpolarization indicate that the inhibitory pause is dependent on the postsynaptic membrane potential in a manner analogous to the dependence of identified IPSPs on membrane potential. Finally, cell discharge elicited by surface anodal polarization can be inhibited by IPSPs evoked by VL or PED stimulation.

These results are an example of an effect of surface polarization that cannot be attributed to the direct effect of extracellularly applied current on cortical cells. Recognition of this example was simplified by the fact that excitatory rather than inhibitory events have traditionally been associated with the action of surface anodal polarization. Both excitatory and inhibitory potentials, however, occur in cortical cells after stimulation of the cortex with short duration pulses 18-22. Often the threshold for surface induced synaptic stimulation is lower than that for direct stimulation of cortical cells22. In all instances where the inhibitory input has been carefully studied, it has been necessary to postulate a disynaptic or polysynaptic intra-cortical pathway²³. The cells or fibre system which produce the inhibitory pause may belong to this general intracortical inhibitory system.

The immediate consequence of these results is to reopen the question as to what part of the other effects of surface polarization attributed to a direct effect on cell behaviour is caused instead by indirect or synaptic effects. The results also suggest that the interpretation of all effects of polarization in terms of a potential difference between dendrite and axon is based on an oversimplified model which neglects important secondary synaptic effects in an interconnecting system of cells.

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Adrenergic Transmission in Small Arteries

Transmission of excitation from nerve to vascular smooth muscle is poorly understood. Bevan and his co-workers1,2 were unable to detect any change in membrane potential in pulmonary arterial muscle after sympathetic nerve stimulation, although contraction occurred. Steedman³ found that sympathetic nerve stimulation increased the firing of action potentials in rat mesenteric arterial muscle, as did nerve stimulation of rabbit portal vein muscle4. Only in the arterial muscle of one species—the guinea-pig -has a discrete depolarization, reminiscent of the excitatory junction potential (EJP) recorded from other smooth muscle, been reported. The following data show that EJPs can be elicited from muscle cells in several arteries of the rabbit.

Arterial segments were removed from rabbits, placed in a horizontal organ bath and perfused with Krebs solution (at 36°-38° C) using the method of de la Lande and Rand⁶. Three arteries were used: the ear artery;

Table 1. EXCITATORY JUNCTION POTENTIALS OF RABBIT ARTERIAL MUSCLE IN RESPONSE TO PERIARTERIAL NERVE STIMULATION

	Max. membrane	Excitatory	unction potential
Artery	potential $(\mathbf{mV} \pm \mathbf{S}. \mathbf{D}.)$	Delay	Max. size*
Branch of	(mv ± 5.D.)	(msec)	$(mV \pm S.D.)$
posterior	54.1 ± 4.2	16-40	4.0 ± 0.8
mesenteric Ear	(n=9) $62\cdot 4 + 4\cdot 4$	12~24	(n=8) 6·1 ± 4·9
	(n=9)		(n=8)

* For fully facilitated EJPs. Two cells were lost before facilitation was

the first side branch leaving the posterior mesenteric artery; and a caecal artery. Membrane potentials were recording intracellularly using glass microelectrodes filled with 3 molar potassium chloride (resistance > 20 m Ω) and suspended in a way previously described, a Nikon Kohden preamplifier (grid current $< 10^{-11}$ amp) and a Tektronix $502\hat{A}$ oscilloscope. The periarterial nerves were stimulated by means of a pair of platinum electrodes partially embedded in the wall of a hole in an 'Araldite' block through which the proximal part of the artery was inserted.

EJPs to periarterial nerve stimulation were recorded from muscle cells in five mesenteric arteries, three ear arteries and one caecal artery. The mean maximum membrane potentials (E_{max}) were high (Table 1) and stable compared with the values reported for other vascular smooth muscles. With the exception of the rabbit pulmonary artery^{1,2} the mean E_{max} for rabbit, rat and guinea-pig arteries and veins^{3-5,7,8} varied between 33 and 39 mV and the membrane potential fluctuated.

Stimulation of the periarterial nerves with single . square pulses of maximum or near-maximum duration (0.5 or 1 msec) and maximum strength produced a depolarization of the cell membrane lasting 0.5 to 1 secthe EJP (Fig. 1a and b). Marked facilitation of the EJPs occurred in response to repetitive periarterial nerve stimulation. With rates of stimulation of 2/sec or faster there was summation of EJPs because of incomplete repolarization of the preceding EJPs (Figs. 1 and 2). The delay between stimulus artefact and onset of the EJP could be as brief as 12 msec (Table 1, Fig. 1b), a result which indicates a rapid access of the transmitter to the receptors and their prompt activation by the transmitter. Rise times of the EJP varied not only between preparations but also within a series of EJPs produced by repetitive stimulation (Fig. 1a).

The nature of the link between depolarization and contraction remains to be established. An action potential initiated by an EJP is necessary for nerve stimulation to produce a contraction of a number of smooth muscles. With arterial muscle, however, the necessity of an action potential for a contraction is controversial^{1,8}. In the present experiments, no EJPs were seen to trigger an action potential, although previously they could be

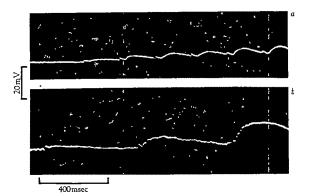


Fig. 1. Excitatory junction potentials to periarterial nerve stimulation recorded intracellularly from rabbit arterial muscle cells (arteries perfused with Krebs solution in vitro). a, The first side branch leaving the posterior mesenteric artery. Nerve stimulation at 5/sec with square pulses of 1/msec duration and 50 V. Maximum resting potential was 60 mV. b, Rabbit car artery. Nerve stimulation at 2/sec, 0.5 msec and 50 V. Maximum resting potential was 60 mV.

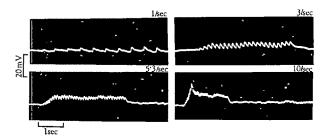


Fig. 2. Excitatory junction potentials to periarterial nerve stimulation at different stimulus frequencies. All records are from the same muscle cell in the first side branch of the rabbit posterior mesenteric artery. Repetitive nerve stimulation with pulses of 1 masc duration and 50 V.

Maximum resting potential was 60 mV.

demonstrated when guinea-pig mesenteric arteries with an intact circulation were used. Instead, high rates of stimulation produced a sustained depolarization which showed a peak before falling to a lower level of depolarization (Fig. 2). These rates of stimulation were sufficient to cause a marked contraction. The stimulus frequencycontraction curve for the mesenteric artery has a threshold of about 2/sec and reaches a maximum between 10 and 20 pulses/sec (Speden, unpublished).

The risk of damage to the small cells on insertion of the microelectrode necessitates caution in the interpretation of negative results. Insertion of an electrode into a cell was accompanied by an abrupt fall in potential to negative values of 49-69 mV, which were high for vascular muscle, and the potential could remain relatively stable at these values for 3-4 min. These signs of satisfactory penetration were consistent with observations that action potentials of up to 25 mV could readily be recorded from the spontaneously active anterior mesenteric vein of the rabbit. The failure to elicit action potentials in the arterial muscles may therefore be an outcome of the high and stable membrane potential in vitro compared with in vivo3,5. Removal of the arteries from their natural site and their perfusion at the low perfusion pressures used (15-35 mm of mercury) must greatly reduce the excitatory influences acting on the muscle. Consequently, the muscle may be expected to be less easily excited, a conclusion consistent with the smaller EJPs observed, with one exception (Fig. 1b), in vitro as compared with in vivo. The maximum size of EJPs recorded from all other cells in the ear artery varied from 3.6 to 5.8 mV.

The EJPs which were recorded from the outermost muscle cells in the wall of the artery were similar in shape to those reported for the guinea-pig vas deferens¹⁰⁻¹². The smaller amplitude of the EJPs, compared with those of the vas deferens, may have resulted from a lesser density of innervation, a smaller release of transmitter from an equally dense innervation, a more rapid removal of released transmitter or a lower sensitivity of the cells to transmitter.

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Transmission of Excitation from the Parasympathetic Nerve to the Smooth Muscle

Transmission of excitation from the parasympathetic (cholinergic) nerves to the smooth muscle has been investigated on the colon of the rabbit^{1,2}. Bennett³ has described the excitatory junction potential in response to stimulation of the intramural nerves of the taenia There is, however, much less coli of the guinea-pig. information about parasympathetic neuromuscular transmission than there is about transmission from sympathetic nerve to smooth muscle^{4,5}. This seems to be because there is no suitable parasympathetic nerve-smooth muscle preparation like the hypogastric nerve-vas deferens of the guinea-pig. Most smooth muscle cells previously used have both excitatory and inhibitory innervation and when stimulated transmurally the excitatory response may be modified by the inhibitory one. A more suitable preparation was therefore very necessary

We have used longitudinal muscles from the upper oesophagus, as far as the crop, from the chicken. The preparations have the advantage of receiving only cholinergic excitatory innervation. Excitatory junction potentials can be elicited by stimulating the intramural The junction potentials are comparable with those in the vas deferens of the guinea-pig, except for the much longer delay between nerve stimulation and the

onset of the responses.

Strips from the longitudinal layer of the upper oesophagus were mounted in a sucrose-gap apparatus. A pair of electrodes for transmural stimulation were embedded in the vertical tube of the apparatus through which flowed Krebs solution, so that stimuli could be given across part of the strip 3 mm from the recording electrode. Changes in membrane potential were recorded with the tension during transmural stimulation with supramaximal single square pulses lasting 0.3 msec or less.

The resting potential of the muscle was 39-53 mV (mean, $47 \pm 2.5 \text{ mV}$; n=14). Some preparations discharged spontaneous action potentials, which varied in amplitude from less than 5 mV to 25 mV, and also in shape. The most frequently observed shape of discharge consisted of complex spikes, two or more spikes occurring before the repolarization of the preceding spike was complete. This probably results from an asynchronous spike discharge from a number of cells in contact with the recording electrode. The intervals between discharges also varied.

The preparations with little spontaneous activity permitted observation of the response to the transmural

stimulation. Single pulses at intervals of 5 sec (Fig. 1a) gave a depolarizing response which initiated an action potential, thus resulting in a contraction. Usually the action potential arose from a slow depolarization (Fig. 1b).

The responses to transmural stimulation seem to result from stimulation of postganglionic cholinergic nerves, for they are completely blocked by atropine (10-7 g/ml. or less) and tetrodotoxin $(5 \times 10^{-8} \text{ g/ml.})$, which abolishes excitability of nerve and striated muscle but does not affect that of smooth muscle^{8,9}, and they are unaffected by hexamethonium (10⁻⁴ g/ml.). This is also supported by the fact that the chronaxie of about 0.1 msec is incompatible with the direct stimulation of the smooth muscle.

The muscular layer of the oesophagus in some species contains both striated and smooth muscle, and so we used d-tubocuraring and flaxedil to see if skeletal muscle

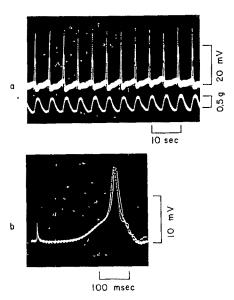


Fig. 1. Action potentials and contractions recorded from smooth muscle strips of the upper oesophagus of the chicken during stimulation of the intramural nerves. (a) Action potentials and contractions produced by stimuli with square pulses of 0·1 msec duration and 25 V at a frequency of 0·2/sec; (b) superimposed record, at faster speed than (a), of action potentials produced by five stimuli in another strip.

participated in the responses. The responses, however. were unchanged in the presence of these drugs in a concentration large enough to block the neuromuscular junction in skeletal muscles. Okamoto, Sugimura and Nonomura (personal communication) have also observed histologically that this part of the oesophagus in the chicken does not contain striated muscle. It is thus concluded that the slow depolarization in response to transmural stimulation is a junction potential of smooth muscle. The preparations, even after treatment with atropine, did not give a hyperpolarizing response, and therefore the preparations probably receive only the cholinergic excitatory innervation.

We could not record only junction potentials when the stimulus strength was gradually decreased with a constant pulse duration. A possible explanation for this result is that the depolarization in response to the stimulation reaches easily a critical level to fire a spike because of the lower membrane potential of the smooth muscle cells.

In Krebs solution containing more magnesium ions (10 mmolar), however, the spike generation of the smooth muscle cells was blocked, but the junction potentials persisted. The amplitude of depclarization of the junction

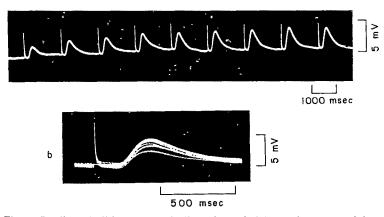


Fig. 2. Junction potentials in response to stimulation of the intramural nerves recorded from a smooth muscle strip of the upper oesophagus of the chicken in Krebs solution containing 10 mmolar magnesium ions. (a) Junction potentials produced by nine stimuli of 0.3 msec duration and 20 V at a frequency of 0.8/sec; (b) superimposed record, at faster speed, of junction potentials produced by five successive stimuli.

potentials increased with successive stimuli, and reached its maximum after several stimuli (Fig. 2, a and b). This facilitation occurred even when the interval between stimuli exceeded 1,000 msec. The junction potential took from 150 to 250 msec to reach its maximum height and it lasted from 700 to 950 msec. Sometimes the junction potential triggered a spike even in solutions containing high concentrations of magnesium ions.

The delay between nerve stimulation and the onset of the excitatory junction potential was from 90 to 160 msec (mean, 130 ± 22 msec; n = 15). This delay might be caused by conduction in the intramural post-ganglionic fibres and by transmission from the nerve to the smooth muscle.

In a few experiments, the stimulating electrodes were moved to various distances from the recording electrode to measure the conduction velocity of the intramural nerve fibres. Changes in the delay were observed which suggested that impulses might conduct more slowly in the intramural nerve fibres than in C fibres.

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Effects of Presynaptic and Postsynaptic Inhibition on the Variability of the Monosynaptic Reflex

Successive monosynaptic reflexes evoked by essentially constant afferent volleys usually show considerable fluctuations in size1,2. It has been suggested1-3 that the chief cause of such variability would be background activity of interneurones impinging on to motoneurones. Monosynaptic transmission from afferent fibres to motoneurones may be controlled at the postsynaptic as well as the presynaptic level4, and so the question arises: which internuncial system, terminating either pre- or postsynaptically, is actually contributing to the mono-synaptic reflex fluctuations? For this reason we have compared the effects of afferent volleys leading to preand postsynaptic inhibition on fluctuations of the monosynaptic reflex.

Twenty-one cats were initially anaesthetized with ether and the spinal cord was transected at the first cervical segment, after which circulation in the head was occluded. All animals were immobilized with gallamine triethiodide ('Flaxedil') and maintained by artificial respiration. Monosynaptic reflexes were produced by graded stimulation, at a constant frequency (0.6-0.8 c.p.s.), of group I afferents of the medial and lateral gastrocnemius. The reflex responses were monophasically recorded from the central ends of the ipsilateral cut L7 or S1 ventral roots. The afferent volley was recorded monopolarly from the

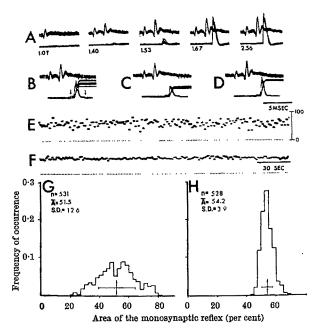


Fig. 1. A, Monosynaptic reflexes produced by graded stimulation of medial and lateral gastroenemius afferent nerves. Upper traces, afferent volleys recorded from the dorsal root entry zone. A subthreshold constant stimulus (1·12T) preceded the test shock by 2 msec to obtain a large reflex response. Lower traces, reflex discharges from the L7 ventral root. Strength of test shock given at the bottom. B, Control test responses produced by a constant stimulus (1·57T). Upper trace, afferent volley; lower trace, monosynaptic responses and its corresponding areas obtained with an integrator. The response superimposed on a small square wave (see arrows) showing the interval of integration. C, Same as B, but test response preceded 30 msec before by three shocks at 300 c.p.s. to the deep peroneal afferents (1·5T). The monosynaptic reflex is depressed and D shows the result of increasing the test stimulus (1·8T) to obtain the same mean response. Note the decrease of area fluctuations between B and D. E and F, Continuous records of the areas of B and D respectively, stimulating at 0·6 c.p.s. The height of the coarse dots above the baseline (fine dots) gives the area of each reflex. G, Histogram for 531 successive reflexes partly illustrated in B and E. Arrow points to mean area (A) and horizontal segment gives two standard deviations (S.D.). Area of maximum response set at 100. H, Histogram of 528 reflexes under deep peroneal inhibition as in D and F. Note mean area is within 5 per cent of that in G, but the S.D. is three times smaller.

dorsal root entry zone. An analogue computer was usually used to calculate continuously the average area of the group I afferent volley (Ai), average area (Ao) and variance (σ_0^2) of the monosynaptic reflex. The computation will be described elsewhere (see also ref. 5). Computer measurements were often verified by hand calculation and agreed within 15 per cent. The temperature of the exposed cord was kept at 37°-38° C with radiant heat.

The monosynaptic reflexes produced by stimulation, with increasing strengths, of group Ia afferents of gastro-cnemius are shown in Fig. 1A. Although the afferent volley was nearly constant, the area of the monosynaptic reflex showed considerable fluctuation (Fig. 1B). From a continuous run of 531 reflexes, partly illustrated in Fig. 1, B and E, the histogram G was prepared. The distribution of areas was approximately normal with a mean of 51.5 (maximal response set to 100) and a standard deviation of 12.6. The variation coefficient, that is, the ratio of standard deviation to mean response, was 0.245 for this control series.

The monosynaptic reflex was reduced to about 60 per cent of its original size when preceded 30 msec before by a conditioning volley 1.5T (1.5 times the threshold of the most excitable fibres) applied to the deep peroneal afferent fibres (Fig. 1C). In these conditions depression of the monosynaptic reflex would be essentially the result of presynaptic inhibition⁴. To compare variabilities for the same mean response the strength of the test stimulus was increased until the monosynaptic reflex regained its original size (Fig. 1D). With conditioning volleys from the deep peroneal nerve there was a striking reduction in fluctuations of the monosynaptic reflex (Fig. F1 and H). The standard deviation decreased to 3.9 and the variation coefficient to 0.072.

Fig. 2A, B and C shows the data obtained from the same animal where the action of pre- and postsynaptic inhibition was compared throughout the range of the monosynaptic reflex input-output curves. When the average area of the monosynaptic reflex (Ao) was increased (by increasing the afferent volley), the variance (σ_2^2) also increased up to a maximum and then declined (Fig. 2A, open squares and circles). The variation coefficient decreased monotonically as the mean monosynaptic reflex was increased (Fig. 2C).

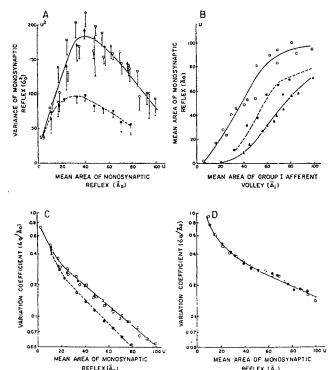


Fig. 2. A and B, Variance versus mean area and corresponding inputoutput curves for the monosynaptic reflex. Each point is the average
of the raw data obtained by point-plotting the computed values every
7-5 sec for 5 min at a fixed stimulus strength. Open circles, control
measurements without any inhibition. Closed circles, during presynaptic inhibition (three shocks to deep peroneal at 300 c.p.s., 1-35T,
35 mscc before test stimulus). Control and conditioned points were
obtained alternately. Closed triangles, during postsynaptic inhibition
(one shock to PL-FDHL, 1-31T, 2 msec before test stimulus). Open
squares are the corresponding control values. The vertical bars in A
give the standard deviation of the raw data obtained from the same
cat over a period of 4 h. C, Varlation coefficient versus mean area
curves constructed from the data shown in A. Stimulus frequency
0-8 c.p.s. D, Varlation coefficient versus mean area for another animal.
Open circles, control curve. Closed triangles, conditioned curve obtained when the test monosynaptic reflex, recorded from a small L7
ventral rootlet was preceded by a constant supramaximal pulse applied
10 msec earlier to the other part of the root. Note logarithmic scales
in the ordinates of C and D. In both experiments, monosynaptic
reflexes were produced by a single afferent volley to gastrocnemius.

When the test stimulus was preceded by a constant train (1.35T) applied to the deep peroneal afferents 35 msec earlier, so as to produce presynaptic inhibition, the input-output curve was displaced to the right (Fig. 2B, closed circles). In addition the variance curve was displaced below the control curve for most of the explored range (Fig. 2A, closed circles). This made the variation coefficient curve much steeper (Fig. 2C, closed circles). A similar reduction in variability was observed when the conditioning train was applied to the posterior bicepssemitendinosus (PBST, 1.2-2T) or to the sural (1.5-2T)afferent fibres, 30-50 msec before the test stimulus. The time course of reduction of variability closely resembled that of the depression of the monosynaptic reflex by pre-

synaptic inhibition4: onset at 5-10 msec, peak at 20-40 msec and a gradual decay up to 100 msec. Picrotoxin (1-2 mg/kg), which depresses presynaptic inhibition4, also reduced the effects of deep peroneal and PBST afferent volleys on the variability of the monosynaptic reflex.

Contrary to the actions produced by presynaptic inhibition, the variability curves remained essentially the same when an afferent volley to the plantaris flexor digitorum and the hallucis longus (PL-FDHL) was timed so as to reduce the monosynaptic reflex by postsynaptic inhibition^{4,6}. For example, in Fig. 2A, B and C the closed triangles show measurements obtained when the test stimulus was preceded, 2 msec before, by a single volley to Ia and Ib afferents (1.31T). Although the inputoutput curve was displaced to the right, indicating inhibition (Fig. 2B), the variance-output and variation coefficient—output curves remained unaffected (Fig. 2A and C). Similar negative results were obtained when the afferent volley was applied to the deep peroneal afferents $(1\cdot2-1\cdot5T, 2-5 \text{ msec earlier})$ or to a neighbouring ventral root, which also depresses the monosynaptic reflex by way of inhibitory synapses with motoneurones' (Fig. 2D).

The results show that the variability of the mono-synaptic reflex can be reduced by presynaptic inhibition but not by postsynaptic inhibition. This suggests that in the non-anaesthetized spinal cat the variability of the monosynaptic reflex is chiefly introduced at the presynaptic level. Intermittent activity along the paths leading to primary afferent depolarization (PAD)4 would produce fluctuations of the membrane potential of the $\tilde{\mathbf{I}}a$ nerve terminals. This would in turn affect the amount of transmitter substance released by each presynaptic terminals or the number of fine Ia terminals invaded by the presynaptic impulse. Variability reduction produced by the presynaptic conditioning volley can be explained by assuming that the potential fluctuations of the Ia terminals are reduced either at the terminal itself or along the paths leading to PAD. In the first case, PAD produced by the conditioning volley could reduce the potential fluctuations by decreasing the input resistance of the terminals. In the second case, the activity along the paths leading to PAD would be decreased by refractoriness after the conditioning volley or by active inhibition. Evidence for the latter is given by the reduction in variability produced by the Sural low-threshold afferents which are known to inhibit transmission along the paths leading to depolarization of the Ia fibres10. The role of variability reduction by presynaptic inhibition is still uncertain because we do not know whether the same effect would be obtained by natural, asynchronous activation of peripheral receptors¹¹.

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Spontaneous Activity recorded from the Central Cut End of the Carotid Sinus Nerve of the Cat

THE receptor in the carotid body for changes in blood gas tensions has for many years been assumed to be the glomus cell^{1,2} or Type I cell³. Lever, Lewis and Boyd⁴ showed by electron microscopy that the nerve endings adjacent to these cells contain microvesicles, of the same dimension as synaptic vesicles, and small mitochondria, and this observation was confirmed by Biscoe and Stehbens^{5,6}, who described the structure of the endings in greater detail and showed that they are similar to those of an efferent type in other parts of the nervous system, and share a dense junctional region with the Type I cell membrane. Biscoe and Stehbens's later found that the endings degenerated after the carotid sinus nerve was cut, and it seemed probable, in accordance with the evidence of De Castro^{1,2}, that they were afferent nerve endings. It is also possible, however, that such endings arise from efferent nerve fibres coursing in the carotid sinus nerve to the carotid body. The purpose of the present experiments was to seek evidence for such efferent fibres.

Six cats were used in the experiments. anaesthetized with 'Dial-Urethane' (Ciba), 0.6 ml./kg given intraperitoneally, a tracheal cannula was inserted, and the carotid sinus region was exposed on the medial The end-tidal carbon dioxide was continuously monitored with an infrared analyser (Beckman LBI). A femoral artery was cannulated to record arterial pressure with a Statham strain gauge, and arterial blood samples were taken through this cannula for blood gas analyses. In some experiments the femoral artery and vein on the opposite side were also cannulated and were joined through a cuvette which housed a Beckman oxygen macro-electrode to monitor arterial oxygen tension (paO₂). The variables were monitored continuously and recorded on a polygraph, in parallel with the output of a ratemeter which was used to monitor the discharge rate of nerve impulses. The carotid sinus nerve was sectioned close to the carotid body, and nerve impulses in fine strands (5-25μ diameter) from the central cut end of the sinus nerve were recorded through platinum wire electrodes and displayed on an oscilloscope. They were also photographed on moving film from another oscilloscope. In some experiments, gallamine triethiodide was administered intravenously, and the cats were artificially ventilated.

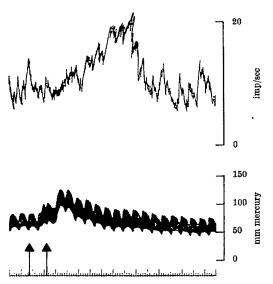


Fig. 2. Effects of adrenaline on the discharge of fibres recorded from the central cut end of the sinus nerve. The fibres were of the type that remain discharging after the ipsilateral sympathetic nerve is sectioned pre-ganglionically (see Fig. 1C). The upper tracing represents the output of the ratemeter; the lower tracing is arterial blood pressure. At the first arrow, adrenaline (20 µg/kg) was given intravenously, and at the second arrow it was washed in. The time scale is in seconds.

In all preparations two types of spontaneous activity were found in the central end of the sinus nerve. The first, and the easiest to record, often showed a pattern of discharge in phase with respiration, and in some cases a cardiac rhythm was also apparent. The nerve activity increased when the arterial blood pressure fell, decreased when the arterial pressure rose after the intravascular administration of adrenaline, and it was abolished by section of either the pre-ganglionic cervical sympathetic nerve or the post-ganglionic internal carotid branches of the superior cervical ganglion. Fig. 1 shows one example of this; in A is the resting discharge in a multi-fibre strand and in B, at the arrow, the ipsilateral pre-ganglionic sympathetic nerve was cut, and all activity, except for a single action potential shown in C, was abolished. This potential could be identified on the original film record before the nerve was cut, and it showed a delayed increase in rate of discharge after the intravenous injection of adrenaline, at a time when the activity of the other nerve potentials was depressed. Finally, stimulation of the

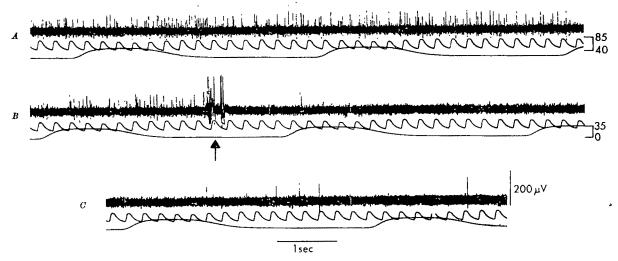


Fig. 1. Continuous record of nerve action potentials recorded from the central cut end of the sinus nerve. In each record the upper tracing is the nerve potential, the middle tracing is arterial blood pressure, and the lower tracing is end-tidal carbon dioxide. Calibration in A represents blood pressure (mm of mercury), in B it represents end-tidal carbon dioxide (mm of mercury), and in C it represents action potential amplitude. Calibrations and time scale apply to all records. A, Resting discharge in a multi-fibre strand. B, The pre-ganglionic cervical sympathetic nerve was cut at the point marked by the arrow, where there is also a movement artefact. C shows a single nerve potential which remains after nerve section. The arterial pressure and end-tidal carbon dioxide were unchanged throughout.

pre-ganglionic cervical sympathetic nerve on the recording side evoked a compound action potential in the central end of the sinus nerve.

The second type of activity was more difficult to find and the action potentials as recorded by this method were generally smaller than those of the first type in any one preparation. The activity did not seem to occur in bursts synchronized with either respiration or the heart beat. In addition, it was not abolished by cutting the pre-ganglionic cervical sympathetic nerve ipsilaterally, nor did stimulation of the cervical sympathetic nerve evoke a compound action potential in strands which contained this type of nerve activity. The rate of discharge of fibres of this type increased when the paO2 was lowered from 250 to 50 mm of mercury, or when the arterial carbon dioxide and hydrogen ion concentrations were raised by altering the end-tidal carbon dioxide in the range 20 to 120 mm of mercury. This increase in discharge was roughly linear with increasing carbon dioxide and approximately hyperbolic with decreasing oxygen tension. The response curves were not as smooth as those for the carotid body chemoreceptors, however, because the discharge in the sinus efferents tends to occur irregularly in bursts. These may be seen in the ratemeter record of Fig. 2. The changes in discharge rate with alterations in blood gas tensions occurred even after the contralateral sinus nerve, both aortic nerves, both vagus nerves and both pre-ganglionic cervical sympathetic nerves were cut. The rate of discharge also increased when the arterial pressure was raised by the intravascular injection of adrenaline, although there was usually a long latent period before this rise in activity. Sometimes the rate did not increase until the arterial pressure had started to fall, as long as 20-30 sec after the injection (Fig. 2). The increase in rate of response to adrenaline persisted even after the contralateral sinus nerve, both vagus nerves and both pre-ganglionic cervical sympathetic nerves had

We conclude from these experiments that the first type of activity is sympathetic in origin, and that the second type arises intracranially, perhaps from neurones which are directly excited by adrenaline. In studies of the central connexions of the sinus nerve, such neurones would be excited antidromically by electrical stimulation of the sinus nerve, and their identification would be of great interest.

The destination of these two groups of nerve fibres is unknown. With reference to the first group, it has previously been shown that nerve fibres from the superior cervical ganglion terminate adjacent to the blood vessels in the carotid body^{8,10}, but no signs of degeneration of endings in this position were seen after the sinus nerve had been cut. If the fibres of the second group terminate in the carotid body, they may be efferent to the glomus or Type I cells. Such a possibility can be substantiated only by degeneration studies with the electron microscope. If these fibres do provide the source of the nerve endings on the Type I cells, however, it is legitimate to ask, where are the chemoreceptor nerve endings?

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Effect of Arterial Oxygen on Mammalian Brain Oxygen Tension

EXPOSURE to one atmosphere of oxygen has been found to cause changes similar to retrolental fibroplasia in the cerebral cortex of newborn mice1, and exposure to oxygen at pressure greater than 2.5 atm. can often cause a grand mal type of convulsion in mature mammals2. The nature of such oxygen toxicity is unknown, and studies of the effect of this gas on various tissues have been hampered by the inability to monitor directly the oxygen tension in a tissue with accuracy.

One of the most useful techniques so far used to estimate the tissue oxygen tension (pO₂) makes use of a metal polarographic electrode³. Such an electrode, however, tends to consume oxygen faster than tissue diffusion can replace it; the bare metal may react with many of the substances present in biological systems resulting in artefactual pO2 values and these electrodes are usually too large for intracellular measurements. These difficulties can be overcome by using electrodes covered by a polyethylene membrane, which is impermeable to most biologically important molecules other than oxygen4; and by using the intermittent pulse method of Naylor and Evans^{5,6} which greatly reduces the amount of oxygen consumed.

Intermittent polarographic membrane electrodes (IPM electrodes) were used for oxygen determinations in the cerebral cortex of four healthy rhesus monkeys. The rhesus monkeys (weighing 2·4-3·4 kg) were anaesthetized with 30 mg/kg of 'Nembutal' and then an endotracheal tube was inserted. This tube was attached to a respirator pump which was run at 10 strokes/min (100 ml./stroke). Normal ventilations reported for non-anaesthetized monkeys are 1,410 and 863 ml./m in 3.08 and 2.68 kg monkeys, respectively7. Body temperature was maintained by a thermostated pad and a Charlton flexible pO2 microelectrode was inserted into one of the femoral arteries8. The other femoral artery was catheterized for continuous blood pressure measurements.

The scalp was reflected from the right parietal area and a hole 1 cm in diameter was made in the skull by trephination. The dura was dissected away and an IPM electrode (tip diameter 1 mm) was gently positioned at a depth of about 1 mm below the surface in the grey matter of the frontal lobe, 1 cm to the right of the midline and 1 cm anterior to the sulcus centralis. The IPM electrode was supported in a pivoting counterbalanced clamp allowing it to move with the pulsations of the brain. Although the IPM electrode was placed in relatively avascular sites in the tissue, its surface area was such that the readings obtained must have been representative of average tissue oxygen tension. Only when the electrode was located directly over an arteriole were larger values obtained. Furthermore, the size of the tip was such that there was some damage to tissue during its insertion.

The IPM electrodes used had platinum cathodes 0.003 in. in diameter which were polarized at -0.7 V every 1.6 sec for 30 msec to assure minimal oxygen consumption. They had an accuracy of ±5 mm of mercury. Intermittent arterial samples were taken in heparinized

		Room air			High oxygen•				
		Arteria	l pO ₂	Brain p	003	Arteria	l pO ₂	Brain 1	0,
Animal	Cycles observed	Range	Average	Range in areas examined †	Average	Range	Average	Range in areas examined †	Average
	0.000	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg
1	5	102-114	109	4-57	18	634-660	645	0-18	6
.2	5	110-126	115	4-37	19	580-640	619	6-17	11
3	5	95-124	112	13-37	25	577-635	606	6-22	16
4‡	3	60 90	80	19-76	63	360-540	406	27-58	39

Table 1. SUMMARY OF EXPERIMENTAL DATA OBSERVED IN THE RHESUS MONKEY

- * 100 per cent or 95 per cent oxygen and 5 per cent carbon dioxide gas mixture.
- † Each value given represents the mean of at least five readings.
- ‡ This animal had an average arterial pCO₂ 22 mm of mercury greater than the other animals in this study.

syringes for determination of the partial pressure of carbon dioxide (pCO₂) using a pCO₂ electrode. All electrodes were calibrated using solutions tonometered with analysed gas mixtures accurate to $\pm\,0.03$ per cent.

Each monkey was monitored until it reached a steady state on room air (no change in arterial gas tension for 5 min or longer), then the gas intake was switched to a high oxygen atmosphere at ambient pressure until a steady state was again achieved. Finally, the animal was returned to room air. This gas mixture cycle was usually repeated at least three times with 100 per cent oxygen and at least once with 95 per cent oxygen and 5 per cent carbon dioxide. The IPM electrode was often moved to an adjacent site for each cycle. These experiments usually lasted 2–3 h.

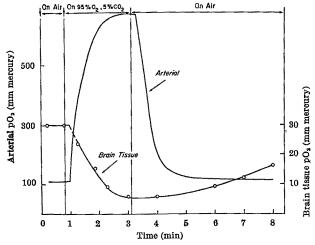


Fig. 1. Oxygen tension in monkey brain during changes in arterial oxygen tension. This study represents one of five similar cycles of events during 2 h. The continuous curve represents pO_n in femoral artery, redrawn from continuous recordings. The discontinuous dotted line represents brain pO_a . Each point represents the mean of at least five readings. The time-course was not corrected for response rates of the electrodes which were: arterial pO_a electrodes, 21 sec to 95 per cent full scale; brain IPM electrode, 6 sec to 95 per cent full scale.

Table 1 shows both the range of and the average pO₂ in the arterial blood and in eighteen areas in the cortical grey matter: five adjacent areas were monitored in the cerebral cortex in each of three monkeys and three adjacent areas in the fourth. The range of pO₂ observed in each monkey brain was rather large; however, in any particular area monitored the pO₂ was relatively constant during the steady state while breathing either air or an atmosphere rich in oxygen. The average arterial pO₂ was 104 mm of mercury, while the average brain pO₂ was 31 mm of mercury on room air. When these monkeys were switched to 100 per cent oxygen the average arterial pO₂ rose to 582 mm of mercury, while the average brain pO₂ dropped to 18 mm of mercury. Blood pCO₂ remained relatively constant at about 20 mm of mercury on air and 100 per cent oxygen in three monkeys and 42 mm of

mercury in the last monkey. (A pCO2 of 20 mm of mercury seems to be low when compared with normal pCO2s found in man (about 40 mm of mercury); however, a mean arterial pCO₂ of 28 mm of mercury (range 34-20 mm) was obtained from samples taken anaerobically from a catheter in the femoral artery of five non-anaesthetized rhesus monkeys in restraining chairs. taken to prevent excitement of the monkeys and subsequent hyperventilation during these studies.) A similar result was obtained with 5 per cent carbon dioxide and 95 per cent oxygen, with an increased arterial pCO₂ (about 36 mm of mercury) shown in Fig. 1. This decrease in brain pO2, in response to increased arterial oxygen, occurred in seventeen of the eighteen cycles monitored. In one cycle, no change in brain oxygen tension was observed. There was no significant change in either brain temperature or arterial blood pressure during the course of these experiments.

We have carried out similar experiments with rats which suggest that they may have a less well developed defence mechanism towards oxygen toxicity than the monkey, and the observation of Bean and Siegfried that the rats subjected to brief repeated exposures to oxygen at high pressures often became permanently paralysed supports this speculation. In our experiments, however, the rise in the average brain pO₂ from 50 mm to 88 mm of mercury when the rats were taken from room air to 100 per cent oxygen may be the result of experimental artefacts caused by an inability to control the rat's arterial gas tension.

A depressed cortical oxygen tension, observed in the monkey cortex in the presence of an elevated arterial pO2 and constant arterial carbon dioxide tension, could be caused by varying cortical tissue diffusion parameters, a change in capillary permeability, arteriovenous shunts, an increased cortical metabolic rate and/or a decreased The first two possibilities have not been amenable to extensive experimental examination. The studies which have been performed on rates of oxygen exchange between blood and tissues have not, however, revealed such changes10. The third possibility is contradicted by a number of anatomical studies which have failed to demonstrate the existence of arteriovenous shunts in the normal brain11,12. As for an increased metabolic rate, normal young men¹³ showed no change in cerebral oxygen consumption during inhalation of high mixtures containing 85-100 per cent oxygen. In the same study, however, a 13 per cent decrease in mean cerebral blood flow (from 52 to 45 ml./100 g/min) was noted with a moderate increase in cerebrovascular resistance. If the decrease in cortical tissue pO2 was the result of a vasoconstrictive effect of high arterial oxygen tension, it is surprising that the 5 per cent carbon dioxide in the inspired air is unable to overcome this effect, because carbon dioxide is one of the most powerful vasodilators in the central nervous system.

Whether the decrease of cortical tissue oxygen tension observed in the monkey in these experiments is caused by a regional vasoconstriction or some other mechanisms, such as increased cortical metabolic rate or a change in diffusion of oxygen, could not be ascertained in these experiments.

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Effect of Anabolic Steroids on Plasma Glycoproteins

WE have previously reported an increase in α_2 - and β -globulins and an increase in β -lipoprotein after administration of the anabolic steroid, 17-ethyl-19-nortestosterone (norethandrolone) (Fig. 1A) to human subjects¹, and our subsequent work (unpublished) disclosed that this increase in serum protein fractions is associated with increases in protein bound carbohydrates (Fig. 2). We have recently had the opportunity to study the effects of an anabolic steroid with a unique chemical configuration, oxandrolone (Fig. 1B), in eleven patients with disorders of lipid metabolism. This compound was found to cause a marked increase in α_2 -globulin (P < 0.001) but no change in β-globulin. Associated with this, there was a highly significant increase in α_2 -glycoprotein (P < 0.01, > 0.001) in all the patients studied (Fig. 3). The increases in α_2 -globulin and glycoprotein were definitive in all the eleven patients 5 weeks after the start of oxandrolone therapy. Thus it seems that both these anabolic steroids significantly enhance the concentrations of plasma glycoprotein.

Testosterone may lower the fasting blood sugar and reduce glycosuria and insulin requirements in some diabetic patients2, and anabolic steroids have recently been found to have a similar action in the diabetic state and

Fig. 1. Structural formulae for norethandrolone (A) and exandrolone

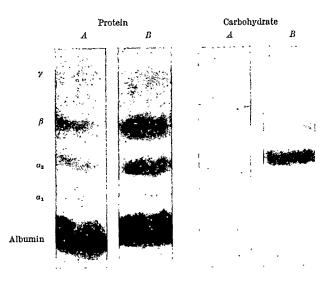


Fig. 2. Serum proteins and glycoproteins before (A) and during (B) administration of norethandrolone. Note the increases in α_2 - and β -globulin and glycoprotein during administration of the steroid. The electrophoretic strips were stained for protein with naphthalene black 12B 200 (ref. 5) and for carbohydrate by the periodic acid-Schiff reaction (ref. 6).

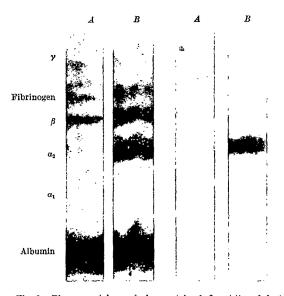


Fig. 3. Plasma proteins and glycoproteins before (A) and during (B) administration of oxandrolone. Note the increase in α_2 -globulin and glycoprotein during administration of the steroid. The protein strips are on the left as in Fig. 2.

also to restore reactivity in the patient who is resistant to insulin^{3,4}. In view of the increased glycoprotein synthesis and the improvement in diabetes with these anabolic steroids, hypotheses correlating the two effects may be put forward. It is possible that these compounds divert glucose to glycoprotein synthesis by: (a) enhancement of the hexokinase pathway in the liver which is not dependent on insulin, resulting in shunting glucose to this intermediate metabolic pathway; (b) a primary enzyme induction of glycoprotein synthesis; or (c) inhibition of gluconeogenesis, diverting amino-acids from synthesis of carbohydrate precursors to glycoprotein. One or a combination of these mechanisms could result both in an increase in glycoproteins and an improvement in carbohydrate metabolism in the diabetic. It will be necessary to study further the effect of this increase in plasma glycoprotein on basement membrane or on small blood vessel disease of the diabetic.

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BIOLOGY

Possible New Approach to the Chemical Control of Plant Feeding Insects

THE chemical control of insects has been hampered by the widespread development of strains which are resistant to Resistance mechanisms often involve detoxication processes2, and many detoxication mechanisms in resistant strains have two features in common. First, they are not peculiar to the resistant insects. Susceptible strains often degrade the insecticide by similar metabolic pathways, but much less extensively, and the biochemical novelty which confers resistance is thus often quantitative rather than qualitative. Moreover, comparable detoxication pathways establish the natural tolerance of species which lack resistant strains2,3. Second, resistance involving detoxication can usually be overcome or reduced if the insecticide is applied with a non-toxic dose of synergist². The synergists appear to inhibit enzymes involved in detoxication. The best known are methylenedioxyphenyl compounds such as piperonyl butoxide and sesamex which reduce the resistance of insects to DDT^{2,4,5}, pyrethrins⁶, organophosphates⁵, carbamates⁷ and dieldrin⁸, and the tolerance of susceptible strains to these and other insecticides, including several derived from plants, as discussed in refs. 9 and 10.

Whereas resistance to man made insecticides has evolved in a few decades, relationships between insects and plants have evolved since long before the evolution of man himself. Plants have evolved minor constituents which have been called secondary plant substances and include glucosides, alkaloids, etc., which are often specific to particular plant taxons. The function of many of these compounds is unknown, but Fraenkel considered that the host-specificity of plant-feeding insects was based on their presence or absence¹¹. This interpretation may not always be applicable 12; nevertheless, it is obvious that a successful pest must be able to tolerate any substance present in its host plant. Some secondary plant substances are highly active pharmacological substances, or are mammalicidal. Some—for example, pyrethrum and nicotine—are insecticidal¹³, and it has been suggested that others -for example, cyanogenic glucosides14, alkaloids15 and mustard oils16-protect plants from the attack of insects or other pathogens.

Although investigations of plant-host relationships have involved studies of the repellency, attractiveness or toxicity of secondary plant substances, little seems to be known about the mechanisms by which potentially toxic substances in the normal host plant are tolerated. seven species of insect feeding on tobacco plants, however, three are known to detoxify nicotine 17.

It seems probable that some other plant feeding insects tolerate poisonous substances in their plant hosts because they have evolved detoxification mechanisms. If so, it should be possible to interfere with these mechanisms by

the application of a synergist. Such compounds would represent a new type of chemical control agent which need not be toxic because they would act by enhancing the effectiveness of naturally occurring plant protectant substances. They would be unlikely to affect predatory and pollinating insects and would probably be most useful in plants containing potentially toxic substances chiefly in those parts not eaten by man-for example, solanin in the leaves of potato plants. Such compounds might also be effective against other plant pathogens such as fungi.

In view of the long period during which the interrelationships between plants and insects have evolved, it is possible that some secondary plant substances have evolved as synergists to enhance the action of other naturally occurring protective substances. In this respect, it is of interest that the synergistic properties of the methylenedioxyphenyl compounds were discovered when sesamin was found to be the active principle responsible for the synergism of pyrethrins by sesame oil. More than three hundred compounds containing the methylenedioxyphenyl group, including alkaloids, flavones, benzophenones and lignan compounds, are known to occur naturally in plants18, and several besides sesamin have been shown to synergiza insecticides 19-21.

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Nature's Time-scale: Degenerative Disease in Man

McCormick¹ recently formulated the following hypothesis: "All naturally occurring phenomena proceed according to exponential functions of time and each process in Nature conforms to its own unique time-scale". He finds that the empirical equation

$$Y = \exp(-kt^{-c}) \tag{1}$$

describes a variety of different processes and that the values of the constants k and c are characteristic of the particular process. Fremlin² points out that for any natural phenomenon the value of t must be subjected to an arbitrary cut-off at some finite value and that this kind of law is usually restricted to processes which occur under controlled conditions. In replying to Fremlin's² objection that equation (1) is incapable of describing periodic phenomena, McCormick³ concedes that in such situations a series of exponential growth and decay functions are needed, each valid within certain limits of t.

The age distribution of disease in man is of considerable interest in the context of this discussion. Clinicians have long been aware that the sex and age patterns of many diseases are more or less invariant both with respect to secular time and geography. It has been found4-10 that the sex-specific and age-specific prevalence, P, of many diseases of either autoimmune or unknown (but possibly autoimmune) aetiology can be described, often with impressive accuracy, by the following general equation¹¹

$$P = S\{1 - \exp(-kt^r)\}^n$$
 (2)

and that sex-specific and age-specific initiation rates (dP/dt) are often remarkably consistent with the differentiated form of the equation

 $dP/dt = nrkSt^{(r-1)} \exp(-kt^r) \{1 - \exp(-kt^r)\}^{(n-1)}$ where S is the proportion of the population (specific for sex where necessary) predisposed to the disease at birth, kis a constant from birth (or earlier) to all t of interest and t is age, corrected for latent period—the average interval between the initiation of the disease process and the onset of symptoms or signs. For most diseases4-11, it is sufficiently accurate to measure t from birth; r is a positive integer and $n \ge 1$. When r is unity, equations (2) and (3) reduce to the homogeneous birth or Yule process, and when n is unity, they reduce to a restricted form of the Weibull renewal process. For the great majority of diseases I have investigated $^{4-10}$ in this way, either n or r are equal to unity. For any specific disease conforming to (2) or (3) or both, the value of k is either the same in the two sexes or it is twice as high in XX females as in similarly predisposed XY males⁴⁻¹⁰. I have not yet encountered any clear-cut violation of this rule.

Equations (2) and (3) are derived from a simple biological model4-11 in which Burnet's12-14 "forbidden-clone" theory of disturbed-tolerance autoimmunity is combined with Burwell's15 concept that growth is regulated by lymphoid elements. In this unified view, the stochastic equations describe the probability of occurrence of spontaneous gene mutations in central, growth-control stem cells. These random events are supposed to initiate the growth of either one or n phenotypically distinctive and independent forbidden-clones, which synthesize cellular or humoral autoantibodies. At the end of the latent period, the attack of the autoantibodies on target tissue bearing complementary antigenic determinants results in the symptoms with or without signs of disease. Burnet's forbidden-clone concept12-14 explains how one, or a few, spontaneous somatic gene mutations can give rise to a disease in which numerous cells, in many anatomical situations, are simultaneously affected.

If there are at least n sets of cells at mutational risk, and if each set contains L cells, then when r = 1, k is defined as Lm. Here m is the average number of pathogenic mutations, per cell at risk, per unit time. When $r \ge 2$, k is defined as $Lm_1 \cdot m_2 \cdot ... \cdot m_r$. Equations (2) and (3) require, among other things⁴⁻¹¹, that m_1t , m_2t , ... etc. should be appreciably less than unity at all t of interest. When $k_F = 2k_M$, it is suggested that the mutation of an X-linked gene is implicated4-11.

If the spontaneous mutation of a specific gene in a specific set of stem cells is regarded as the elementary process in the initiation of many diseases in man, then this process is described by the familiar equations

$$dL = - mL dt (4)$$

$$L = L_0 e^{-mt} (5$$

Because m appears to be independent both of postnatal age and ordinary environments4-10, the mutational event may involve a unimolecular reaction process. Novick and Szilard¹⁶ studied the spontaneous mutation to resistance to bacteriophage T5 in E. coli (strain B/1) and they found, somewhat unexpectedly, that their results were consistent with a unimolecular reaction mechanism. The mutation rate m at 37° C, which was independent of the (controlled) generation time of bacteria, was found¹⁶ to be 1.1×10^{-4} yr⁻¹. I estimate that the value of m in man is

typically 10^{-3} yr⁻¹, although wide differences between one locus and another should be anticipated.

The parallel between the form of "biological decay" described by (4) and (5) and the law of radioactive decay is exact and, viewed in this way, many idiopathic human diseases may be said to exemplify McCormick's hypothesis (in its non-mathematical presentation). On the other hand, if the initiation of the disease itself is regarded as the basic process, then complications in the mathematical form of the age-distributions arise (when n and/or $r \ge 2$) because many diseases would appear to be initiated by multiple random events. A different kind of complication, analogous to one of those mentioned by Fremlin2, will manifest itself when, for example, S is a changing function of time. Nevertheless, it is intriguing to find that the age patterns of so many diseases in man should conform to such simple time scales.

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Relationship of Sexual Receptivity to Menstrual Cycles in Adult Rhesus Monkeys

THE control of sexual reproduction in female rhesus monkeys involves not only an internal secretory rhythm, but also behavioural responses which must be in concert for the successful union of gametes. Grooming and mounting behaviour has been shown recently to be related to stages of the menstrual cycle1, and presentation to the male has been shown to relate to ovulation². The purpose of this study was to determine the extent to which receptivity of female monkeys to cannulation of the vaginal canal varies with the stages of the menstrual cycle in both brain lesioned and intact animals.

The female rhesus monkeys used had become accustomed to vaginal smear and cannulation procedures, and included a bilaterally temporal lobectomized animal. two which had sustained bilateral amygdalectomy and two animals with frontal lobe lesions. The animals were studied in two periods: the first period was during June-July 1966, in the presumed non-fertile season during which twenty menstrual cycles were recorded, two of which proved ovulatory; the second period was during October-November 1966, the presumed fertile season.

Physiological changes in the female reproductive tract were simultaneously gauged by assessment of vaginal smears taken daily by a procedure previously described. Ovulatory menstrual cycles were established by direct visualization of the ovaries during laparotomy. The animals were lavaged daily to recover all exfoliate materials available from the vaginal tract (mostly epithelial cells). This material sedimented out in 24 h when the

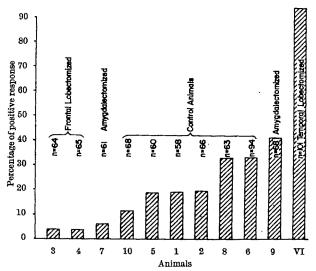


Fig. 1. Positive vaginal responses on cannulation in adult rhesus monkeys. n, No. of units of trials.

quantity was measured, thus permitting a determination of the vaginal exfoliation rate (VER), that is, c.c. of cells/24 h.

In the test procedure, the glass cannula was a blunt-ended eyedropper which was inserted and partially withdrawn in a penile-like action. One unit of trial, performed daily, consisted of up to twelve pulsatile movements followed by complete withdrawal repeated seven to ten times. The attempts of the animals to escape handling, or their co-operation, were tested by timing the interval they remained stationary after completion of the cannulations—"completion delay timings". This test was alternated with a procedure of counting the number of withdrawals from presentation made by the animals during periods of 30 sec taken at random during the cannulation trials.

Often during cannulation a tightening of the vaginal tract on the cannula occurred, which led to an airtight seal of the cannula in the vagina. When this occurred, it was scored as a positive vaginal response. Each animal was tested for this condition of muscular contraction and/or vasocongestion daily during and after cannula trials. Although the animals by and large did not interfere with the cannulations, there were two exceptions. On withdrawal of the cannula, one animal (VI) twisted back on the investigator and attempted—with a number of successes—to nip the hand of the investigator without

breaking the skin. She readily presented again after turning, and cannulation could be continued. A second animal (IX) manually sought out the cannula and the hand of the investigator without interrupting cannulation.

In control animals, there were positive vaginal responses in 11-33 per cent of the tests (Fig. 1). These occurred least in the frontal lobectomized animals, while one temporal lobectomized animal responded in 94 per cent of the trials, an extreme variation. The results in two amygdalectomized animals differed from one another, and further observations are necessary to see whether these results differ significantly from those from control animals.

In relation to the menstrual cycle, the test was usually sporadically positive in the intact animals and, when it could be related to stages of the menstrual cycle, it often occurred during the premenstruum (Fig. 2). In some instances, this response, as well as increased co-operativeness as judged by interval timings, could be related to the period of probable ovulation.

In Fig. 2, behavioural changes during both an ovular (July) and anovular (October-November) menstrual cycle have been correlated with daily vaginal exfoliation rates. The period of marked oestrogen effect in vaginal smears which occurred on the twelfth to fifteenth day in the July menstrual cycle is not indicated in Fig. 2. This period of probable ovulation corresponded to the period of marked proclivity on the part of the animal for the test procedure. While at other times in the menstrual cycle the period in which the animal waited for cannulation averaged 36.5 sec, during this period waitings were approximately six times more protracted, averaging 5 min 18 sec. Vaginal responses were positive on 3 days of this 4 day period.

During the anovular cycle no increased behavioural receptivity during the fertile period occurred; instead, the animal maintained presentation with the least number of postural changes in the premenstruum; in this period positive vaginal responses also occurred. This was a general finding in the anovular menstrual cycles of control animals.

Considering the animals as a whole, the results do not show any conclusive differences between the two periods of study. While four animals ovulated in the autumn, the seasonal variation differences which occurred in these four animals were believed to reflect differences in endocrine status.

While the test procedure used in this study can only be said to simulate male sexual activity, it has an advantage in that the response was measured from a constant, standardized stimulus.

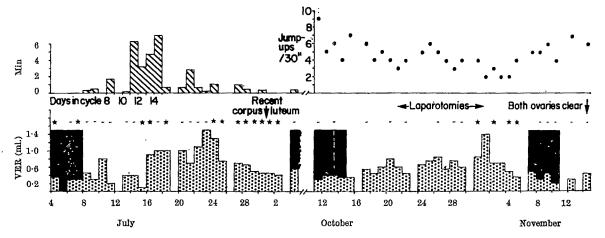


Fig. 2. Tests of sexual receptivity (♠) and vaginal responses (*) in relation to ovulation and stages of the menstrual cycle, together with vaginal exfoliation rates in rhesus monkey VI. Black bars denote menstrual bleeding.

Field studies4 indicate that the copulative behaviour of rhesus monkeys in the wild is more apt to occur in a 9 day period, presumably during the intermenstruum, and may not necessarily occur as an oestrous type of behaviour. Neither presenting—an invitational type of behaviour -nor mounting (including willingness to be mounted) are specific of oestrous behaviour. In the view of Carpenter⁴, the most discretely specific reaction of oestrous behaviour in the wild is when a female accepts a series of mountings with intromission.

The purpose of this study has been to explore this capacity for repeated intromission within a short interval of time, and the number of test trials were meant to induce a degree of satiation. If there was an oestrous type of receptivity, we measured how steadfastly the animals remained in the presentation position (completion delay and withdrawal timings) or persisted in the copulative

The vaginal muscular and/or vasocongestion test proved to be discriminative of the animal presumed to be hypersexuals, and permitted ranking of the less responsive animals. The condition of positive vaginal response could also be shown to occur during the probable period of ovulation, although the test did not prove specific for this period.

The finding of a premenstrual increase in vaginal response was unexpected because mounting and presentation behaviour at this time was not observed by Michael¹ or by Ball and Hartman². In the human, an increase in sexual desire in this period has been described6,7, as well as contractility or vasocongestion of the external third of the human vaginal tracts.

These present observations indicate that sexual excitability, as judged by vaginal contraction and/or vasocongestion, and receptivity, and also by completion delay timings, typically increases at the probable period of ovulation, and also occurs at other times during the menstrual cycle. This is consistent with what is known, namely, that female primates differ from lower animals in accepting the male in copulation throughout the menstrual cycle instead of for only a short period of time at ovulation.

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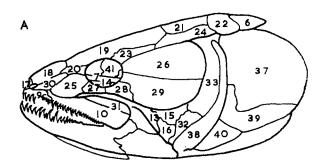
Numerical Homology

THERE have recently been many objections to the orthodox definitions of biological homology in terms of common ancestry on the grounds that such definitions are circular¹⁻⁴. Parts of two organisms are defined as homologous if they are represented by a single part in a common ancestor. But homology itself is invoked in making this identification of parts of organisms with a part in a common ancestor. Various authors have advocated a return to Owen's criterion of homology-"the correspondency of a part or organ, determined by its relative position and connexions, with a part or organ in a different animal".

Jardine has formulated a logical model for correspondence of parts of things with respect to a set of relations along lines suggested by Woodger³, and from it has derived a general method for finding such correspondences.

A computer programme based on this method for finding biological homologies has been written. The first stage in the computing of biological homologies is the selection of a set of spatial relations and of a set of parts from each organism. The spatial relations holding between each pair of parts in each organism are then tabulated in matrix form, these being the data for the programme. The programme finds the largest correspondence or correspondences between some or all of the parts selected from each organism in which the spatial relations between parts are preserved. Thus if "anterior to" is one of the relations selected and x is anterior to y in one of the organisms, any parts p and q from another organism found to correspond to x and y, respectively, must be such that p is anterior to q. A symbol for "undecidable" in a given relation may be included in the data, an undecidable relation being allowed to map onto the relation or its converse. The programme has an option allowing for two or more parts to be considered together as a single part, and in this case it may find solutions in which a part in one organism corresponds to an aggregation of several parts in another. Another option allows for partial solutions to be fed in with the data in cases where at least some of the homologies of parts are not in dispute; this may save computer time.

The programme has been used in the study of the homologies of the bones of a variety of vertebrate skulls. For a number of simple cases it has been shown to yield satisfactory results. For example, the correct homology between the bones of human, cat, rat and dog skulls was found using the relations "anterior to", "dorsal to" and 'distal to". The success of the method in these simple cases suggests that it may be used in more complex cases



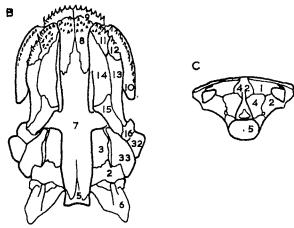


Fig. 1. Diagrams of the skull of *Amia calva*. A, Lateral aspect; B, ventral aspect; C, posterior aspect. (Partly after Allis^{7,8}.)

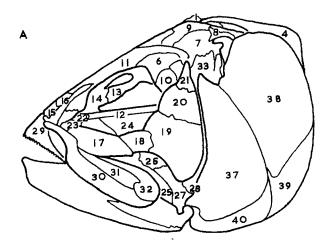
Table 1. HOMOLOGIES COMPUTED BETWEEN THE SKULL BONES OF Amia calva (HOLOSTEI) AND Clupea finta (TELEOSTEI)

Amia Clupea Amia Clupea	
1 — 5 Epiotic 22 — 8 Scalebo	one
2,4 — 2 Opisthotic, (extr	ascapular)
exoccipital 23 — 6 Spheno	tic
3 — 10 Pro-otic 24 — 7 Pteroti	c
5 — 3 Basioccipital (sup	ratemporal)
6 — 4 Post-temporal 31 — 31, 32 Supran	naxilla
7 — 12 Parasphenoid 32 — 28 Symple	ectic
5 — 3 Basioccipital (supple of the first of	ındibular
9 — 29 Premaxilla 34 — 35 Ceratol	hval
10 — 30 Maxilla 35 — 34 Epihya	
11, 12 - 23 Palatine 36 - 36 Basihy	al
13 - 25 Pterygoid 37 - 38 Opercu	
14 — 24 Entopterygoid 38 — 37 Preope	
15 — 26 Metapterygoid 39 — 39 Subope	
16 — 27 Quadrate 40 — 40 Interes	percular
	sphenoid
18 — 16 Nasal 42 — 1 Suprao	ccipital
19 — 11 Frontal (cart	ilaĝe in
20 — 14 Prefrontal-pareth- Amic	a)
moid	•

The bones are numbered as in Figs. 1 and 2. The nomenclature follows that of $Gregory^{11}$.

which involve large numbers of parts where the recognition of homologies by eye is difficult.

The homologies of the skull bones of the holostean Amia calva and of the teleost Clupea finta, shown in Figs. 1 and 2, were investigated in this way. The homologies of the skull bones of *Amia* with those of teleost fishes have been much disputed⁷⁻¹¹. The relations "anterior to", "dorsal to" and "distal to" were used, and the parts selected were the bones numbered in Figs. 1 and 2. No unique largest correspondence was found, but the several equally large correspondences differed only in the ways in which the bones of the suborbital series (25-29 in Amia and 17-21 in Clupea) were paired. This indicates that skull topography alone is insufficient to determine the homologies of these bones which are, at least in Amia, rather variable in number and relative position. correspondence computed for the other bones is shown in Table 1. The homology suggested by Gregory¹¹ is largely supported, although Gregory considers 16 and 32 in Amia to constitute a single bone, the quadrate, in contrast to Allis⁷ and Goodrich¹⁰, who recognize, as we do here, a distinct symplectic bone.



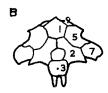


Fig. 2. Diagrams of the skull of Clupea finia. A. Lateral aspect; B, posterior aspect. (Partly after Ridewood¹⁸.)

A further study of a disputed homology, the homology of the dermal skull-roof bones of the crossopterygian fishes Osteolepis and Holoptychius, will be described elsewhere.

We feel that the demonstration that biological homologies can be derived numerically is of importance for tworeasons. First, an empirical concept of homology as correspondence in relative position escapes the circularity implicit in phylogenetic definitions of homology, but is in line with the widely prevalent use of the term in comparative anatomy. We have shown that the method for computing homologies outlined here may be of practical use in studies of comparative anatomy. Instead of defining homology in terms of derivation from common ancestral parts it should be possible, by studying fossil series, to investigate the extent to which homologies, in the sense defined here, may be used to infer the structure of ancestral organisms. Second, it clears up a difficulty in the rationale of numerical taxonomy. One of the criteria for the selection of characters in numerical taxonomy is that the characters should be, in some sense, the same in all the organisms studied (compare Sneath and Sokal¹²). In other words, the characters selected should be such that their states are identical attributes either of whole organisms or of parts homologous in all the organisms studied. For example, the character "petal colour", having as states "petals red", "petals white", etc., should only be used in a numerical taxonomic study when the parts called petals are homologous in all the plants studied. The explication of homology in terms of correspondence in relative position removes the suspicion that phylogenetic assumptions may be involved in the initial selection of characters.

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GENERAL

Character Recognition by Holography

THE application of holography to character recognition has been receiving a great deal of attention. A recent report by Gabor¹ introduced a somewhat different approach to the problem, based on a principle "that if two coherent waves are made to fall simultaneously on a photographic plate, one coming from an object A, the other from an object B, the photograph links these together in such a way that if the hologram is illuminated by A alone, B will appear too, and vice versa". I now present some experimental investigations based on this approach.

This principle may be re-stated in a mathematical form as follows. If A and B are two coherent disturbances

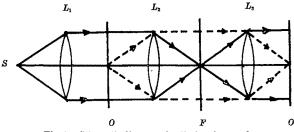


Fig. 1. Schematic diagram of optical system used.

falling on a photographic plate, then the recorded intensity of the interference pattern is

$$I = |A+B|^2 = |A|^2 + |B|^2 + A^*B + AB^*$$

where superfix * denotes a complex conjugate function.

When the photographic plate is developed such that its amplitude transmission is proportional to recorded intensity and then illuminated by disturbance A above, the complex amplitude distribution, S, leaving the plate is given by

$$S = A \cdot |A + B|^2 = A \cdot [|A|^2 + |B|^2] + A \cdot A \cdot B^*, + A \cdot A^* \cdot B$$

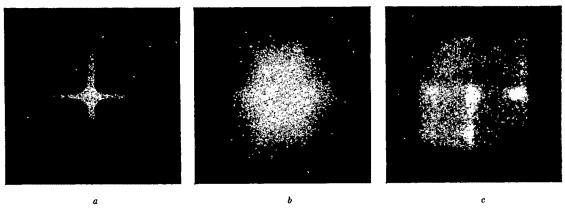


Fig. 2. Auto-correlation functions of: a, a cross +; b, a triangle \triangle ; c, a square \square .

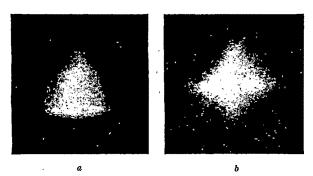


Fig. 3. Reconstruction of (a) the triangle and (b) the cross from the interference pattern of the triangle and the cross.

It may be seen that the last term, in the foregoing equation, will yield B, provided $[A A^*]$ is approximately constant. This is sometimes the case, as will be shown

The experimental work was carried out using Fourier transform holography in order to avoid the more serious optical alignment problems. The optical system is shown in Fig. 1. A transparency containing two objects, in this case two block letters A and B, was placed in the object plane O. The resultant interference pattern between the Fourier transforms of A and B was photographed in the Fourier plane F. The developed plate was then replaced in exactly the same position and the output was observed in the image plane \bar{O}' .

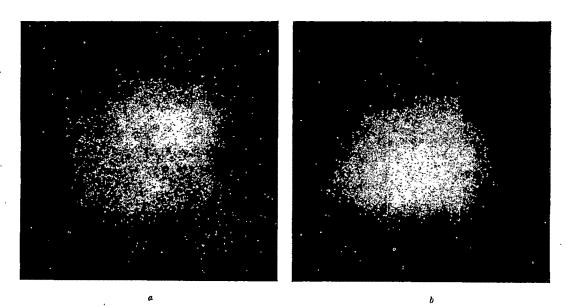


Fig. 4. Recovery of (a) the square and (b) the triangle from an interference pattern between the triangle and the square.

It is not necessary to print a positive from the recorded hologram, for this only reverses the contrast of the interference fringes. This change of contrast amounts to phase shift of π which does not influence the final intensity in the observed image.

Thus the recorded intensity in the plane F is given by

$$I = |ae^{ikr_1w} + be^{ikr_2w}|^2$$

where a and b are Fourier transforms of A and B, respectively, r_1 and r_2 are the distances of the centres of area of patterns A and B, respectively, from the optical axis and w is the co-ordinate in the Fourier plane F. Now, when pattern A is covered in the input plane and only B illuminates the filter at F, the complex amplitude distribution just behind it is given by

$$T_{P} = be^{+ikr_{2}w}|ae^{+ikr_{1}w} + be^{+ikr_{2}w}|^{2} =$$

$$= be^{ikr_{2}w}[|a|^{2} + |b|^{2}] + b \cdot b^{*} ae^{ikr_{1}w} + b \cdot a^{*} \cdot be^{ik(r_{1} + r_{2})w}$$

The lens L_3 takes the Fourier transform of light distribution at F giving the output at O' as

$$O'(z) = [\alpha \beta]_{z = -r_2} + [B^*B^*]_{z = -r_1} \oplus A + [B^*A^* \oplus B]_{z = -(r_1 + r_2)}$$

where α is an approximately constant attenuation factor due to $|a|^2 + |b|^2$ and A_*B^* and $A \oplus B$ denote the cross-correlation and convolution of A and B, respectively. The subscripts z=-r, etc., denote the position of the centres of the patterns.

Thus the first term in the last equation is the geometrical image of B attenuated by α. The second term occurs at the position where the geometrical image of A would have been. This term will also represent the reconstructed image of A, provided that B*B* tends to a Dirac delta function. Thus in order to recover faithfully the missing pattern from the combination of the two, the illuminating pattern must have a narrow autocorrelation function, ideally tending to a Dirac delta function. The third term is a complicated pattern which may be interpreted as cross-correlation between A and B convoluted with B.

A number of patterns were chosen with various autocorrelation functions ranging from very narrow to very broad. These are shown in Fig. 2 along with their autocorrelation functions. Only some combinations of patterns were used.

Fig. 3 shows the reconstruction obtained from the interference pattern of the first pair, that is, a cross and a triangle. The triangle (Fig. 3a) is very well reconstructed when illuminated by the cross, which has a narrow auto-correlation function. On the other hand, the reconstruction of the cross is very poor (Fig. 3b). Fig. 4 shows the same for a triangle and a square. Neither of the two objects is well reconstructed, the reconstruction of the triangle being especially poor because the autocorrelation function of the square is very far from a narrow peaked function.

The experimental evidence presented shows that patterns A and B may be retrieved from the interference of A and B but with an important qualification. Pattern A may only be faithfully recovered if B, illuminating the combination, has a narrow auto-correlation function, and vice versa. Thus the method described is not very suitable for character recognition because many letters do not have a narrow auto-correlation function. The method could be used more efficiently with specially designed patterns.

Van Heerden² in 1963 proposed a somewhat similar approach for information retrieval. He used what he calls an intensity filter in the Fourier plane; it was simply a positive of a photograph of the Fourier spectrum of a pattern present in the input plane O (Fig. 1). When such a filter is illuminated with a fragment of the same pattern

a "ghost" image of the complete pattern may be seen.

Stroke et al.³ have also used a similar filter for what they call "holography with extended sources" and arrived at the same conclusion that the illuminating pattern must have an auto-correlation function tending to Dirac delta function.

I should like to thank Mr D. A. Gregory for the help with experimental work.

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Identity for the Product of Two Positive Definite Quadratic Forms

THE problem of generalizing the following basic identity

$$\sum_{1}^{p} a^{2} \sum_{i>j}^{p} b^{2} = (\sum ab)^{2} + \sum_{i>j} (a_{i}b_{j} - a_{j}b_{i})^{2}$$

given in Hardy, Littlewood and Pólya¹, remains to be considered. We shall show that the generalized Cauchy inequality follows immediately from this generalization. The resulting identity has many fruitful applications in problems of multivariate ratio estimation in sample survey theory.

Let $C = [c_{ij}]$ (i, j = 1, 2, ..., p) be a symmetric matrix possessing a non-singular inverse $C^{-1} = [c^{ij}]$ (i, j = 1, 2, ..., p). Denote the jth column vectors of C and C^{-1} by c_j and c_j , respectively. Let $H = C_1^2$, so that H is a $p \times p$ symmetric positive definite matrix and let $z = (z_1, z_2, \ldots, z_n)$ z_p) and $u = (u_1, u_2, \dots, u_p)$ be any two non-null row vectors of length p. Then we have the following identity

$$(zHz')(uH^{-1}u') = (zu')^2 + \sum_{i>j} \{z(c_iuc_j - c_juc_i)\}^2$$
 (1)

which is the generalization of the basic identity given here, the summation in the last term being over the C_2^p pairs of (i, j).

The proof is quite simple. In matrix form the basic identity can be expressed as

$$aa'.bb' = (ab')^2 + \sum_{i>j} (a_ib_j - a_jb_i)^2$$
 (2)

where a and b are non-null row vectors of length p. Setting a=zC and $b=uC^{-1}$ in the identity (2) and remembering that $H = C^2$ and that the scalars $a_i = zc_i$ and $b_1 = uc^j$, the identity (1) will be obtained.

The generalized Cauchy inequality follows by noting

$$(zHz')(uH^{-1}u') \ge (zu')^2 \tag{3}$$

equality holding if and only if $zc_i = \theta uc^i$ for all i, where $\theta \neq 0$ is a constant, which implies that we must have $zC = \theta uC^{-1}$ or $zH = \theta u$.

Finally, in identity (1), if we set M=I the identity matrix, and remember that $I = I^2$, we get back to the basic identity.

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¹ Hardy, G. H., Littlewood, J. E., and Pólya, G., in *Inequalities* (Cambridge University Press, 1934).

BOOK REVIEWS

BIRTH OF THE CALCULUS

The Mathematical Papers of Isaac Newton Vol. 1, 1664-1666. Edited by D. T. Whiteside. With the Assistance in Publication of M. A. Hoskin. Pp. xlvi+590. (London and New York: Cambridge University Press, 1967.) 210s. net; \$40.

WHEN the Royal Society took the decision of limiting the publication of the Newton papers to the correspondence, it was apologetically pointed out that editing of the manuscripts "would require long labour of many patient and profound experts such as it is hard to find today". It is therefore a matter of great satisfaction that one at least of such patient and profound experts has had the pluck to accomplish single handed one of the most important of the tasks shunned by the Royal Society: the critical examination and publication of Newton's mathematical manuscripts. This took him ten years of labour, if we include in the reckoning the painstaking and profound study he carried out, as a preparation, of the whole background of contemporary mathematical knowledge and thinking, in which Newton found the starting point and the inspiration for his own discoveries. The results of this study are embodied in a thesis (Archive for History of Exact Sciences, 1, No. 3; 1961) which ranks as a major contribution to the history of seventeenth century mathematics.

With the present volume—the first of eight—the undertaking enters its last stage, and there is every reason to congratulate the author and his collaborator as well as the publishers for a superb achievement, the completion of which will now be impatiently awaited. In format and lay-out the book resembles the volumes of the correspondence, and like the latter is a masterpiece of typography; in details the transcription of the documents is even better than that of the letters. The delicate problems raised by the dating and arrangement of the single items have been solved with great competence and ingenuity. In principle, all the extant material is reproduced, with only trivial exceptions. We thus dispose of every scrap of evidence to help us to retrace the steps that led Newton to his conception of the calculus. In this case, we have even more: in the attempt to present a systematic account of his methods, Newton never managed to proceed further than to drafts, and only one or two of these were eventually published; we are now in a position to read and compare these successive drafts, and to judge, much more easily than from the published pieces, how early Newton acquired a full mastery of the new methods.

The first volume covers only three years, but these were just the decisive ones, 1664–1666. From the notes scribbled in the books Newton used as a student, we can for the first time determine with full certainty the sources of his discoveries, the authors that had the greatest influence on him, and the way in which these influences combined to start him very soon on untrodden paths. This analysis has been done by Dr Whiteside, and he gives us his results in abundant footnotes as well as in the introductions to the four parts (counting the appendix as one part) in which he has distributed the material. Of these, the

first two are perhaps the most interesting, although there is much of value also in the last two, devoted to algebra and geometrical optics respectively. From the first part, reproducing Newton's annotations in books, we learn above all of the paramount influence of Descartes' analytical geometry, and especially the commentary added to the Latin edition by F. van Schooten, that able Dutch mathematician who was Huygens' preceptor. It is from the Dutch Cartesian school that Newton got the example of an algorism—a time-saving general prescription—for finding the tangent to a curve from its equation. This rule was limited to the so-called "geometrical" curves, that is, those whose equation could be written in algebraic form.

At the same time, however, Newton learned from the British school, taking its origin in Napier's invention of the logarithms, how to treat non-algebraic functions "mechanically", that is, by series expansions; he had only before him Wallis' crude "interpolation" procedures, and his establishment of the general binomial expansion was a truly creative effort. The crucial point was (to put it in modern terms) to pass from the definite integrals considered by Wallis to the corresponding indefinite integrals: thus was obtained a series in powers of the variable upper limit, with a clear algorism for the computation of the coefficients, instead of Wallis' obscure arithmetical operations. What Newton further learned from Wallis was the method of integration developed by Cavalieri, who, with that other Galilei disciple, Torricelli, had founded a brilliant, though short-lived, school of mathematics in This method of indivisibles could be readily incorporated in the new calculus as soon as Newton realized that integration and derivation are inverse operations.

The fundamental element in Newton's method, however, the concept of fluxion, came last in the picture. It arose from the need to solve the tangent problem in full generality, that is for the non-algebraic curves called by Descurtes "mechanical", because they were mostly defined as loci of the intersection of two straight lines translated in two different directions according to given laws of motion. To construct the tangent in such cases as the diagonal of the velocity parallelogram is not a deep idea: indeed, it is indicated in the pseudo-Aristotelian "Problemata". At any rate, we find a method of construction of tangents on this principle elaborated about 1650 by Torricelli, and further developed, in direct continuation of Torricelli's work, by R. de Sluse and by Roberval. Now, in Newton's notebooks the same method is discussed and applied to various problems, but there is no indication of its source. It is quite possible that he heard about Torricelli's method through Barrow's lectures, though he later declared that he did not remember it. It is also possible that he rediscovered this rather simple approach independently. However this may be, the decisive step he took was to translate the geometro-mechanical construction in analytical terms. How essential this last step was is shown by the case of R. de Sluse who, like Newton, was conversant both with Torricelli's approach and with the algorism for finding the derivative of an algebraic function (which he had found independently): he missed the fluxion idea, however, because it did not occur to him to combine the two methods.

These very fragmentary remarks may perhaps convey some idea of the treasures opened to the historian's view by this monumental publication. Thick as it is, however, this book reflects only a part of the lonely youth's astounding intellectual activity: his studies of Cartesian philosophy and mechanics, his awakening interest in optics, astronomy and chemistry could be documented by volumes of equal importance. Could one hope that Dr Whiteside's brilliant example might stimulate other 'patient and profound experts' to undertake for the other domains in which Newton has left his mark a similar labour of love, a labour bringing with it such rich rewards?

L. Rosenfeld

DE MINIMIS NON CURAT LEX

The Natural History of Viruses

By C. H. Andrewes. (The World Naturalist.) Pp. viii+237+41 plates. (London: Weidenfeld and Nicolson, 1967.) 55s. net.

More than a hundred years has gone by since the first publication of the Origin of Species, a work whose contents have been so frequently quoted as an excuse for man's inhumanity to man that it has become one of the seminal books of world literature. But during that same century it has also been shown many times that even the unremitting pressures of natural selection sometimes help the meek to survive. Nature is not always red in tooth and claw, and parasites, in particular, exemplify the principle. A parasite which is successful from the point of view of the survival and dissemination of its own species can be one that does little harm to its host. The dividing line between successful parasitism and commensalism—the concept of the tolerated guest—is as arbitrary as the iron curtain.

In this book Sir Christopher Andrewes calls on a lifetime of experience of the devious ways of viruses to sustain the contention that even they are subject to the inexorable Darwinian law: that they, just like other organisms, have their natural history; hence the title of this book, written with wit and a clarity of style that should be a model to those that follow him. Although writing primarily for laymen, he never compromises on fact, or takes refuge in obscurity, so that his main thesis is wholly acceptable, even to professional colleagues. A wealth of data is assembled to show that viruses have to struggle for existence like any other organism. They use all sorts of dodges to obtain access to new hosts and to survive hard times. But viruses differ from all other parasites in that they do not replicate by themselves. They are copied by the living cells they inhabit. Moreover, it is a cliché of pathology that virus lesions do not appear until virus replication is already over. So the primary target of natural selection must be the multicellular organism of which the infected cell forms a part. Selection will only affect the virus within it if the host succumbs before sufficient virus can be made and disseminated, and the successful virus will be one that can persuade its host to produce and excrete a vast quantity of virus particles. Whether the host survives or not is irrelevant in this context; if enough rats succeed in leaving the ship, who cares if it sinks or not? Host mortality will only affect the evolutionary fate of a virus if its onslaught on the host species is so fierce that later virus populations die out through lack of new victims to sustain them. It can be argued that a virus which is less totally destructive, but which still kills a large proportion of its hosts, may even have an evolutionary advantage. First, it will avoid a too rapid spread of immunity through the host species; and second, the more virus there is, the greater will be the chance that new mutants may arise, capable perhaps of attacking new species, or of surviving until new susceptible individuals of the original host species become available.

But when I had finished his book I began to wonder whether in fact this was the only way to assess the importance, in nature, of this fascinating collection of infectious agents. Do viruses obey evolutionary laws because they are organisms? Or is it that such laws describe the behaviour of all things that have reached a certain level of complexity? In this sense, evolution is as inevitable a description of the behaviour of organized matter as Newton's laws of motion. The mere presence, within the primeval prebiotic soup, of a minor constituent with a thermodynamic advantage will ensure its eventual dominance. No one would suggest that such compounds are organisms, or indeed living in any sense, and it is perhaps more appropriate to describe their behaviour in physico-chemical terms.

All this would be mere quibbling were it not that one of the dangers of the "organismal" approach to viruses is that it can blind one to their unique character. Their special position was recognized many years ago by S. E. Luria when he referred to the life of viruses as "parasitism at the genetic level", and it is this aspect of their behaviour that has fascinated virologists over the past ten years. Sir Christopher Andrewes, however, rightly reminds us that there is much more to virology than gift-wrapped packages of rogue nucleic acids. The law of evolution may not care for such little things, but we must never abandon his way of looking at them.

F. K. SANDERS

NMR, NQR AND EPR

Magnetic Resonance Spectroscopy

By Harry G. Hecht. (Wiley Series in Pure and Applied Spectroscopy.) Pp. viii + 163. New York and London: John Wiley and Sons, 1967.) 45s.

VIRTUALLY the whole of NMR, NQR and EPR spectroscopy is covered in 160 pages, and thirty of these summarize a considerable amount of quantum mechanics. The book is the latest of a series on pure and applied spectroscopy and deals with a larger area than any of its predecessors in less than half the space of the shortest of them! Here is the book's weakness—too much in too little space. Topics mentioned include solid state NMR, pulsed radiofrequency fields, electron-nucleus double resonance, electron and nuclear relaxation, as well as the commonly used concepts such as chemical shift, spin coupling, g tensor and so on.

The book is aimed at the undergraduate-graduate overlap region as a "survey of the field of magnetic resonance spectroscopy as a whole that is geared to the interests of a chemist..." and to such students who "find it necessary to rely on magnetic resonance data...." There is no doubt that a general discussion of the relevance to chemistry of this form of spectroscopy should be part of a chemist's education, but the considerable amount of mathematical derivation limits its value here. Only forty references (mainly to books) are given, but these simply follow chapters without specific connexion to the text, so the reader will find original papers only with difficulty.

The author has briefly discussed most of the areas of the subject chemists are likely to meet, so the book will serve quickly to widen the perspective of mathematically inclined students with a limited acquaintance of magnetic resonance.

R. Bramley

STUDENT'S INTERFEROMETRY

Interferometry

By W. H. Steel. (Cambridge Monographs on Physics.) Pp. ix+271. (London: Cambridge University Press, 1967.) 60s. net; \$11.50.

This well written book attempts to cover quite a formidable subject in eleven chapters and succeeds reasonably well in doing so. The whole approach differs somewhat from that of older long accepted texts of interferometry. It is certainly well up to date. The second, third and fourth chapters are mathematical, dealing essentially with Fourier transforms (so popular today), diffraction and coherence. This then is an introductory theoretical basis of wave theory leading on to what is more usually meant by interferometry. This kind of approach is a growing practice now in optics. Whether it helps to clarify the situation for a student is another matter. I am not sure that it does. This introductory theoretical section is well written and compact but will certainly not be easy for the beginner.

Fringe visibility in two-beam systems occupies the fifth chapter. In the following chapter Michelson's interferometer is treated comprehensively, and here I welcome the introduction of this interference system as used for microwaves as well as for light waves. The seventh chapter, which deals with multiple-beam interference systems, is right up to date with regard to refinements of theory, new instrumentation and bibliography. Yet, curiously enough, the author, like so many others expert in interferometry, fails to refer to Boulouch, the real inventor of the multiple-beam fringe sharpening principle, indeed creating in effect what we now call both Fabry-Perot and Lummer plate interferometers. This historical detail deserves rectification if a second edition be called for.

As might of course be expected from a writer who works at a National Standards Laboratory, the section on interference comparators, the measurement of length and the interferometric assessment of refractive indices is very good. Here, too, I welcome brief, clear descriptions of microwave analogues to optical interferometers. ninth chapter covers a great deal of ground, including the use of multiple-beam interference methods for the study of surface microtopography, the testing of optical surfaces, shear interferometers and also the rapidly growing new field of interference microscopes. This latter section might with advantage have been expanded somewhat, now that transmission interference microscopes of a variety of types are coming on to the market for use by biologists, who more and more are beginning to appreciate what interferometry can offer them.

Although the tenth chapter, which deals with interference spectroscopy, is certainly up to date and includes many recent refinements in instrumentation, it is rather lame with respect to the actual optical requirements involved in practice when combining an interferometer with a spectrograph. This involves numerous pitfalls, and the student hoping to become a high resolution spectroscopist is not going to find any real "know-how" here to help him. No tricks for adjusting interferometers, no warnings about possible varieties of ghost or reflected images, no hint at all about how to make the essential high reflecting films and so on. For this the student would have to look elsewhere. Indeed, because of the gloss over such details, interference spectroscopy is made to sound a good deal easier than in practice it is. Nevertheless there is sufficient of very real value in this book for every student of optics and spectroscopy, and the final chapter, entitled "Interference Imagery", contains a valuable and clear discussion of stellar interferometers, both of the optical and radio types.

Altogether this is a most readable text and, although the introductory part makes it heavy weather for the undergraduate, it is to be well recommended to any student who wishes to specialize in optics, metrology or spectroscopy. The book is particularly well illustrated with line diagrams, but regretfully has no plates at all—a pity, because interferograms are often photogenic. The comprehensive fourteen-page bibliography with well over five hundred references will be of real value to the student who wishes to go further.

I welcome this new text.

S. Tolansky

DIFFERENTIAL EQUATIONS

Introduction to Ordinary Differential Equations By Albert L. Rabenstein. (Academic Press Textbooks in Mathematics.) Pp. xii+431. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 80s.

This book represents a well organized introduction to what used, in textbooks, to be a somewhat disorganized subject. In the main, apart from the basic existence

theorem for solutions of ordinary differential equations. all the results are proved.

There is a discussion of the various cases of Frobenius's method of solution in series and a considerable treatment of results concerning sets of orthogonal polynomials, both general sets and particular sets. Sturm-Liouville theory is discussed in one chapter with a brief mention of some singular cases, but it is odd to note that the two originators of the theory are not mentioned at all.

Other topics included are systems of differential equations, Laplace transforms, separation of variables, and autonomous systems, and there is a short excursus into the theory of partial differential equations.

There are sufficient exercises together with answers to about half of them to provide an eminently readable textbook for a second or third year undergraduate mathematics or engineering student.

R. L. Perry

MORE DIFFERENTIAL EQUATIONS

Elementary Partial Differential Equations

By Paul W. Berg and James L. McGregor. (Holden-Day Series in Mathematics.) Pp. xv+421. (San Francisco, London and Amsterdam: Holden-Day, Inc., 1966.) \$13

This book is formed by a revised collection of notes of lectures given at Stanford University, and is suitable for mathematics students or mathematically inclined physicists and engineers.

It is based on the concept of separation of variables which is adapted to cater for various boundary conditions and various initial conditions. There are several introductory chapters containing concepts and theory needed later, covering among other topics Fourier series and some ordinary differential equation theory including Sturm-Liouville theory. The theory is mostly in respect of the heat, wave and Laplace equations in two or three dimensions and seems somewhat repetitive, but the methods and theorems can apply to similar equations. Extensions to semi-infinite and infinite intervals are also included. Each chapter contains exercises and some but not all answers are supplied.

The authors have produced a book which covers those aspects of partial differential equation theory which are perhaps those of most interest to the physicist and engineer.

R. L. Perry

CENTRES OF IMMUNITY

Germinal Centres in Immune Responses

Edited by H. Cottier, N. Odartchenko, R. Schindler and C. C. Congdon. (Proceedings of a Symposium held at the University of Berne, Switzerland, June 22–24, 1966.) Pp. xvi+499. (Berlin and New York: Springer-Verlag, 1967.) 78 D.M.; \$19.50.

This book contains fifty-seven contributions on various aspects of the role of germinal centres in immunity. In addition, a considerable amount of space has been devoted to the discussions which followed each session, and a valuable concluding summary of the whole proceedings is also provided. The name "germinal centre" was coined more than 80 years ago, to describe the prominent clusters of pale staining cells found in lymphoid tissues. These centres show intense proliferative activity, and so they have been thought to be sites of small lymphocyte production. Although this assumption is no longer wholly tenable, there is a growing body of evidence that they play an important part in certain immune responses.

One of the aims of the book, as stated in the preface, is to combine this evidence "in a single package". It succeeds in being comprehensive, but this is at the expense of repeating data which have been published in the proceedings of other recent conferences. The need to repeat

information in this way is open to question.

In reviewing a volume of this length it is impossible to appraise separately each individual contribution, but the overall impression is that the book provides pointers to possible lines for future research rather than a body of firm facts. In an opening session the attractive concept of separate thymus and bursa dependent central immune systems is reviewed and it is concluded that the development of germinal centres is most closely related to the bursal system (or its mammalian equivalent). In this context, the centres are probably more concerned with antibody responses (mainly bursa dependent) than delayed hypersensitivity reactions (probably thymus dependent). Unequivocal evidence is presented for the localization of antigens and immunoglobulins within germinal centres, but it is not certain whether the antibody is actually produced within these sites.

Clearly, much hinges on the elucidation of the origins, functional properties and ultimate fate of lymphoid cells within the centres. It is unfortunate that in the session dealing with ultrastructural studies, some contributors attempt to draw up schemes of cellular transformations by means of the static appearances seen in tissue sections. Appropriate labelling techniques (radioisotope or chromosome marker) are always needed to determine cellular transformations with certainty. Further knowledge of the kinetics of the cells of the centres must await the

successful application of these methods.

In conclusion, this book contains a valuable account of current progress in research on germinal centres. Ample evidence is provided for the importance of these centres in immune responses and in the actiology of a number of disease processes. The centres probably result from a proliferative response to prolonged stimulation by antigen. The precise relationship of this response to antibody formation and other immunological phenomena remain problems for future study.

J. J. T. OWEN

TOXICOLOGY AND TOXINOLOGY

Animal Toxins

Edited by Findlay E. Russell. (A Collection of Papers presented at the First International Symposium on Animal Toxins, Atlantic City, N.J., USA, April 9-11, 1966.) Pp. xiii+428. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 120s.

The publication in book form of the proceedings of symposia was deplored some years ago in the pages of Nature (193, 724; 1962). This volume is described as "a collection of papers" presented at a symposium. This may mean that some contributors did not submit suitable manuscripts; it certainly means that the reader is spared the trivia and undocumented pronouncements commonly found in recorded discussions. The papers certainly have the accepted format for those appearing in the publishers' scientific journals. About 2,000 pages of their Biochemical Pharmacology costs a library £35 and a private subscriber £5. The 400 pages of this bound volume cost £6—a marginal bargain for the library, but not for the individual!

There are forty-four papers dealing with the poisons produced by arthropods (eight), marine organisms (thirteen), snakes and amphibia (twenty-two), together with a final paper on the action of any of these toxins on the nerve, the muscle and the neuromuscular junction of the guineapig. The papers deal chiefly with pharmacology and toxicology (twenty), chemistry (nine), immunology (four), and biochemistry (three) of the toxins as well as the anatomy (four) of the poison glands, and zoology (three) of the poisonous animals.

It would be mere effrontery for a toxicologist to undertake a detailed critical review of this book, which embraces the subject of toxinology. In toxicology, the essential

chemistry of the poison, be it synthetic or of plant origin, is usually not a problem. The fact that much less is known about the chemistry of animal poisons is not solely the result of a preference of chemists for ragwort rather than rattlesnakes as a source of their starting material but also of the innate complexity of many animal secretions. The precise modes of action of animal toxins, however, remain neither more nor less of a mystery than those of most other natural or synthetic poisons. Here toxinology and toxicology are on common ground. Meanwhile bacterial toxins will presumably remain outside the fields of both toxicology and toxinology and continue to be studied by microbiologists.

It is difficult to know what place a book such as this has in overcrowded libraries. To the general reader interested in learning something of a subject new to him or her there is too much detail in the papers. The specialist will probably have seen the detailed work reported elsewhere. As was said in 1962, there is probably a place for the recording of critical reviews and survey papers presented at symposia, and only one of this type is found in this volume.

J. M. Barnes

FAILURE STORY

Investigations into Generation 1651-1828
By Elizabeth B. Gasking. (The History of Scientific Ideas.) Pp. 192. (London: Hutchinson and Co. (Publishers), Ltd., 1967.) 30s. net.

From the time of the Greeks until the nineteenth century the word "generation" was used for what we would now call reproduction. It included a penumbra of other subjects: genetics, embryology and the anatomy of the reproductive organs; but the central issues concerned the contribution of the sexes and the mystery of embryological development.

A number of accounts of this complex historical topic have been written, the most renowned of which is Professor Cole's Early Theories of Sexual Generation (1930). In her presentation of this subject Mrs Gasking has been concerned to avoid the pitfalls of the dramatic approach—accounts painted in the lurid colours of the clash of rival theories; and of the discovery approach—the documentation of the first discovery and first adumbration of facts and ideas which still find a place in modern biology; for she is interested in theories of generation in the seventeenth and eighteenth centuries for their own sake. She was puzzled by the naivety of supposedly eminent scientists who accepted so implausible a theory as the preformation of the germ (egg or sperm). This puzzlement led her to carry out the research which is described in this book.

What has her presentation to offer? First, it contains much of the work already gone over by Cole. Because Cole's book is out of print and because Gasking comes to different conclusions than he on a number of points, this part of the new book is very welcome. Her book also contains an account of the more recent work carried out by Professor Glass on Réaumur, Maupertuis and Adanson. She has also described her detailed work on Harvey and Prévost and Dumas.

Mrs Gasking holds that Harvey must not be considered as an orthodox representative of the views of the ancients on generation, but as one who broke away from their authority as a result of his observational and experimental work. Coming now to preformation, Mrs Gasking advances three reasons for the acceptance of this implausible theory. (1) That there was no plausible alternative explanation at the time. (2) That the doctrine of preformation was in harmony with the organ-ridden physiology of the day. (3) That empirical discoveries, particularly in the eighteenth century, tended to support the theory.

While accepting that these three points were important, I wonder whether the religious foundations of the doctrine of preformation were not much more important, and were more central to the problem of the naivety of eminent biologists.

Like the other titles in this series on "The History of Scientific Ideas", this book is very well produced. those who enjoy reading about the interplay of facts and theories, about the plausibility of a theory in terms of the knowledge and world view of the day, and who have the patience to work through poorly organized but scholarly material, Gasking has provided a detailed and thorough case history. The reader will learn, as the author did, "more about what happens when scientists become interested in aspects of a problem which they lack the means to solve". ROBERT OLBY

READINGS IN ANTHROPOLOGY

Human Evolution

Readings in Physical Anthropology. Edited by Noel Korn and Fred W. Thompson. Second edition. Pp. xiii + 466. (New York and London: Holt, Rinehart and Winston, Inc., 1967.) \$4.95 paperbound.

IT has long been customary in university teaching to supplement textbooks and organized lecture series by readings in the literature of the given subject. This may be done in science by reference to the original technical literature, by reprints of semi-technical papers or by monographs on specialized sub-topics. In the United States, particularly, supplementary materials are increasingly provided by books which are selections of previously published papers and essays. Anthropology has been well served in this way by a variety of yearbooks, for example, one published by the Wenner-Gren Foundation, and by more occasional books of readings. for example one edited by William Howells (1962) or another by J. D. Jennings and E. A. Hoebel (1955 and 1966). The present collection by Korn and Thompson is of the latter sort. Although titled as a second edition, it is new for the most part, about half of the selections being from publications originally issued in 1963 or later.

Review of a book like this should be directed rather to editorial features and to adequacy for an intended purpose than to the contents of the individual chapters, all of which have already been published elsewhere. In the first place, the sub-title of this volume is inaccurate, for one of the seven parts ("Evolution, Genetics, and Natural Science", three essays) is not on any aspect of anthropology as such and another whole part ("Studies of Primates", confined to primate behaviour, four essays) and several others of the twenty-six separate essays do not meet any

accepted definition of physical anthropology.

Selections are for the most part by competent experts and adequately represent the current status of the subject. There are unfortunate exceptions; for example, the essay on the extremely touchy subject of skin colour is not by an anthropologist and does not represent the most recent and soundest views of anthropologists. Many of the essays are abridged, and the extent of curtailment is not adequately indicated by a symbol (...) for omissions. For example, less than a third of Goodall's remarkable essay on chimpanzee behaviour is reprinted. (It is interesting that the deletions include the section on sexual behaviour.) Most of the illustrations have been redrawn ("adapted"), not always skilfully, and figures have even been interpolated without notice; for example, the two very poor figures (17-2 and 17-3) of fossil hominids apparently attributed to J. T. Robinson are not based on anything in his original publication. Citations given by the original authors have all been deleted, sometimes with mystifying results; for example, two figures (23-3 and 23-4) attributed in the text to myself are completely strange to me.

The noted shortcomings are surely no more than venial, and the selected essays are well worth reading. It is to be hoped, however, that both teachers and students can do better. G. G. SIMPSON

ADVANCED LOOK AT EVOLUTION

Process and Pattern in Evolution

By Terrell H. Hamilton. (Current Concepts in Biology.) Pp. x+118. (New York: The Macmillan Company; London: Collier-Macmillan, Ltd., 1967.) 15s.

Thus is a useful little book in the "Current Concepts in Biology" series, apparently aimed at first-year under-graduates and higher. The author says "There can be no doubt that the first-year undergraduate now has a better secondary education than we previously assumed, and that he is capable of more independent work than we usually demand". One can hope that this is so, but I think many will find the book too stiff. It deals, after a brief chapter on Darwin and Wallace, with the elements of population genetics and population ecology, types of natural selection, speciation and adaptive radiation as the consequence of selection and species specialization. Only geographical speciation is treated, and no plants and usually only the higher animals are mentioned. The discussion of highly controversial points is often oversimplified and too exclusively theoretical. The title is much too wide, but the book does form a coherent whole. English workers will in general be familiar with the line of approach, although some will welcome the discussion of population ecology and the attempt to bring it into relation with selection and population genetics. series is almost wholly American and, because Ernst Mayr describes the approach as novel, it is presumably expected to be thought rather advanced in viewpoint in the United States, as indeed is likely—it is rare to find an American author so free from the prevailing dogma of the importance of genetic drift in evolution. The author has some of the usual weaknesses of American terminology; adaptive value (fitness for a particular purpose) is equated with selection coefficient (relative representation in the next generation); but only once is something that is necessary. desirable, or useful described as mandatory, and the book is very readable and well laid out. A. J. CAIN

EVOLUTION OF PINE TREES

The Genus Pinus

By N. T. Mirov. Pp. viii + 602. (New York: The Ronald Press Company, 1967.) \$15.

This is a fascinating book to anyone working with pine trees. It sets out not to give full detailed data on individual species, although many details are given as examples. but to trace the genus from its origins in time and place and thereby explain the current distribution and potentialities of the pines. Silvicultural and wood quality studies are omitted.

After a chapter on the historical references to pine in literature from the days of Theophrastus to the end of the nineteenth century, the palaeobotany and geography of the genus are traced by means of fossil records and pollen grain studies. This reveals the possible migration of pines during 200,000,000 years from a northern hemisphere centre around the Bering Sea through North America to Nicaragua, through China and south-east Asia to Sumatra and through the Himalayas and Turkey to Europe and the Canary Islands. In Cretaceous times (100,000,000 years ago) pines had already become differentiated into two groups, haploxylon and diploxylon, but had not travelled further south than 32° N. in Texas and 35° N. in Asia. No fossil pine remains have been found in the tropics or south of the equator. From Cretaceous times Mexico has become a secondary centre of proliferation of diploxylon pine species, owing to its wide range of habitats within short distances.

Many small scale outline maps and excellent photographs cover the distribution and appearance of 105 species, the areas where hybridization between species has occurred and the world pine regions. Some species have greatly expanded their distribution since the ice ages, for example Scots pine; others are senile and isolated in parts of California and elsewhere; some can be crossed easily with related members of the genus although widely separated for many million years; others are difficult to hybridize.

There are chapters on genetic aspects, on morphology and reproduction, and on physiology and ecology. Much is still unknown and, interested in tropical pines, I found no explanation of the grass stage of seedlings common to several species or apical dominance so characteristic of *Pinus caribaea* and some others when grown in favourable conditions.

The relatively new study of the chemical aspects of pine cell contents has been a special interest of Mirov's and receives full treatment in the seventh and eighth chapters. He points out that chemical evolution may not have run parallel to morphological evolution but that it can provide valuable evidence to supplement morphological data in attempts to explain the relationships between species and provenances of species.

All these threads of enquiry are drawn together in a chapter on taxonomy where the older system based on type specimens is contrasted with the multiple aspect approach favoured by Mirov and the radically different system of Gaussen which is criticized.

From their fossil history pines are shown to be adapted to seasonal climates with severe dry periods, but unsuited to equatorial zones of high humidity.

The book is well produced. An extensive list of references is given at the end of each chapter and there is an index.

A. F. A. LAMB

HERTFORDSHIRE FLOWERS

Flora of Hertfordshire

The Wild Plants of the County of Hertford and the Adjoining Areas included in Watsonian Vice-County 20. By John G. Dony, with a foreword by Edward Salisbury. Pp. 112+56 maps. (Hitchin: Hitchin Urban District Council, 1967.) 42s. net.

The last Flora of Hertfordshire, that of A. R. Pryor, was published in 1887. It is now amply superseded by Dr Dony's work, very different in style and reflecting the recent trend towards mathematical impartiality seen in other regional floristic studies. We cannot fault the precision with which the records have been assembled, but we may be less confident as to the purpose lying behind such a publication.

The 656 distribution maps, using a 2 km grid square as unit, are a novel feature of this work, and they invite direct comparison with other mapping methods, such as those used by Good in his studies on the Dorset flora. Whilst documentation is easier with a regular grid, some distribution patterns (for example riparian species) are rather obscured. But, whichever method is used, why map a species distribution at all? Surely the scientific justification is to display a pattern which can be related to possible causative agents, historical or ecological? I feel, reluctantly, that Dony has missed an opportunity here in not attempting to relate distributions to any such controlling factors. As an example, he has not included any geological map. I expect superficial geology to be at least as important as precipitation in a small lowland

county, and this is a loss compared with Pryor's work of 80 years ago. Six regions are delimited, precisely mapped and given pseudo-geological names, but they do not coincide with geological outcrops. Furthermore, these regions are not employed in the body of the Flora.

In his previous Flora of Bedfordshire, Dony used "habitat studies", detailed lists of species in well localized These attempt a compromise between the traditional British vagueness in floristic description, and the much greater precision in those emanating from the continent of Europe. They are intended to help in assessing the scale of future vegetational changes. Are they really detailed enough for this? Virtually no data are provided about soils, and the use of "frequent", "occasional" and so on is still too vague. Linking these lists is an account of the main features of plant distribution in Hertfordshire, but the actual factors responsible for this are covered in little more than thirty lines on page 24. Dony, an acknowledged expert on adventive species, could well have devoted more space to the effects of man in an intensively farmed county, and less to an account of the history of botanical exploration.

Apart from such important and more fundamental issues, I have a few small criticisms to make. There are no definitions of "colonist", "denizen" and so on, it is ambiguous to say that *Lathyrus nissolia* has "no distribution pattern", *Berberis vulgaris* does not "cause" wheat rust, and why not omit the grid reference to habitat study 51, instead of including a deliberately falsified version?

As a detailed and reliable factual record of plant distribution, Dony's Flora of Hertfordshire is admirable. But I fear it is only a skeleton. Is there not a danger of overlooking the wood for the trees ... and an account of plant distribution for a card index of impeccably documented dots?

D. H. Dalby

EVERYMAN'S GUIDE TO CRIME

Crime and the Social Structure

By John Barron Mays. New and revised edition. (Society Today and Tomorrow.) Pp. 256. (London: Faber and Faber, Ltd., 1967.) 35s. net.

UNLIKE other books in this series, which report original researches, this volume is a summary review of research into crime, presented within the author's overview of the problem. Its main appeal therefore should be to the socially aware layman, the student and those working in disciplines other than, but related to, sociology. For all, and the latter in particular, it should be salutary. It is well written and very readable.

The analytical framework is simple but persuasive, and would be supported by most sociologists. Our society is crimogenic, in that our social structure produces most crime, which must therefore be regarded as one "normal" response to social stimuli. For an adequate understanding of its causation, and of appropriate remedial action, we must therefore rely primarily on sociology, with the assistance of psychology. But the latter has substantial relevance only to the minority problem of the hardened criminal and the psychopath. The author makes it clear that most crime is confined to the younger age groups, is predominantly against property and not too serious, and is amenable to containment and reduction by effective community action. His prescription mainly emphasizes the desirability of a restructuring and fuller integration of the educational service in the widest sense, embracing the schools, youth service and the corrective institutions for the young. This is admirable as far as it goes, but many sociologists would look for a more extensive approach in view of the author's correct emphasis on social structural analysis in the fullest sense.

. 1.

There are a number of minor blemishes, which a more meticulous revision (for this is a revision of the original 1963 edition) would have avoided. On page 30, for example, we are told, "... in 1938 those found guilty per thousand of the population numbered 78.5 . . . ", whereas a later Table (page 36) shows this to be both a typographical and factual error. In a number of places sources are not given (a glaring example on page 212: "Clearly the existing educational and recreational services available for 70 per cent of the young people . . . are inadequate"), and a number of footnotes are incomplete. These are unfortunate, but the book remains a most readable introduction to the subject. W. H. SCOTT

OBITUARIES

Professor B. G. Peters

BERNARD GEORGE PETERS, professor of parasitology at Imperial College since 1955, died at his home in Sunninghill on September 9 after a few months' illness. He was born at Isleworth in 1903 and was educated at Bristol Grammar School and the University of Bristol. After graduating, he worked at the London School of Hygiene and Tropical Medicine and at the Institute of Agricultural Helminthology, St Albans, first on potato-root eelworm, then on the vinegar worm, obtaining his doctorate. He continued work at the institute (which had become the Imperial Bureau) on the helminth parasites of various birds and mammals, in particular on the liver fluke of sheep and cattle. At the same time he was lecturer in helminthology at the London School.

Dealing with taxonomically difficult material the relations of which with the environment were often obscure, he found it necessary to acquire a greater knowledge of statistical methods than was common at that time. These qualifications led to his spending more than two years during the war conducting operational research at the headquarters of RAF Bomber Command. After the war he was attached to the Imperial Bureau and also to Rothamsted Experimental Station first as principal scientific officer and, from 1952, as head of the department of nematology. From then onwards his research was concerned entirely with eelworms, animals which are responsible for about half of the annual losses from British crops. Peters, however, had never lost his interest in parasites of all kinds, and when a chair in parasitology was established at the Imperial College in 1955 it was to him that the College naturally turned as its first incumbent. At that time no detailed course dealing with eelworms was available anywhere in the British Commonwealth, so that besides organizing a sub-department of parasitology which was not tied exclusively to medical or veterinary problems, he started a course in nematology leading to a diploma (now M.Sc.). The course was taken by staff seconded from the Ministry of Agriculture as well as by students from all over the world. With the aid of funds from Shell Research, Ltd., and from the University of London a new laboratory was established at the college field station, where the course, as well as much parasitological research, was carried out.

An increasing interest in parasitology in British universities led to the establishment of the British Society for Parasitology, of which Peters was an active founding member. He was a most successful teacher who soon acquired the devotion of his students. As an expert in the field of plant nematology he is irreplaceable and it will not be easy to find another parasitologist with such broad interests and so well able to inspire students. He will be greatly missed by his friends and colleagues and by his old students now scattered all over the world.

O. W. RICHARDS

A. E. Ingham

A. E. Ingham died suddenly on September 6, at the age of 67, while on holiday in Switzerland. He was reader in mathematical analysis in the University of Cambridge and a fellow of King's College.

Albert Edward Ingham was educated at Stafford Grammar School and Trinity College, Cambridge, which he entered as a Scholar in 1919. He was elected to a prize fellowship in 1922 at his first attempt. From 1926 to 1930 he was reader at the University of Leeds; he then returned to Cambridge as a university lecturer and a teaching fellow of King's College. He was made reader in 1953.

Ingham was a world authority on the theory of the distribution of the primes, and his Cambridge tract of 1932 (reprinted 1964) is still the standard account of the subject. To this difficult theory he made several notable contributions. I select for mention here the two which are probably best known to mathematicians generally.

(1) If p_n denotes the *n*th prime (so that $p_1=2$, $p_2=3$, and so on), one may ask how large the "gap" $p_{n+1}-p_n$ can be, in relation to p_n , when n is arbitrarily large. Hoheisel proved in 1930 that there is some exponent $\alpha < 1$ such that

$$p_{n+1} - p_n < p_n^a$$

 $p_{n+1} - p_n < p_n^a$ for all sufficiently large n. Ingham made a considerable advance in 1937 when he proved that the result holds with $\alpha = \S$, or indeed a little less. His method has still not been improved on.

(2) In 1919 Pólya put forward the conjecture that of the numbers $1, 2, \ldots, x$ there are always more with an odd total number of prime factors than with an even number, or at least as many. This has been verified up to 600,000. The conjecture would imply the truth of the Riemann hypothesis. In 1942 Ingham showed² that it would imply much more, namely, that the ordinates of the zeros of the ζ-function would be connected by infinitely many linear relations with integer coefficients. He proved a more specific result, involving only a finite number of the zeros, and so opened up the possibility of disproving the conjecture by computation. This was done in 1958 by the late C. B. Haselgrove (formerly a research student of Ingham), who showed further3 that the conjecture fails for some x less than e^{832} . Ingham's method is not limited to the investigation of Pólya's conjecture and is one of great generality.

Ingham made many other advances towards the solution of difficult problems in the analytic theory of numbers, but his work was not confined to this field; in fact, about half of his papers are on questions of analysis which have no direct relevance to the theory of numbers. He had a complete mastery of many difficult and delicate aspects of analysis, and in particular he added greatly to our understanding of Tauberian theorems4.

For a man of his standing, Ingham published relatively little: some thirty papers in addition to the tract. One has the impression from his papers that before publishing he had to satisfy himself that everything that could be relevant to his topic had been fully taken into account. When he did publish, every word and symbol was the subject of serious consideration, and the malpractices of editors and printers in changing what he had written were a source of much annoyance to him.

Ingham's lectures were always a pleasure to listen to, as many generations of students can testify. thought must have been devoted to their substance and to their presentation. Examining was another duty which he took very seriously; he retained throughout his life an interest in all branches of mathematics, and was often able to point out errors and obscurities in question s on subjects remote from his special field.

H. DAVENPORT

¹ Ingham, A. E., Quart. J. of Math., 8, 255 (1937).

Ingham, A. E., Amer. J. of Math., 64, 313 (1942).
 Haselgrove, C.B., Mathematika, 5, 141 (1958).
 See, in particular, Ingham, A. E., Proc. London Math. Noc. (3), 14 A, 157 (1965).

University News:

Birmingham

DR S. H. HOLLINGDALE, at present head of the Department of Mathematics in the Royal Aircraft Establishment, has been appointed director of computer services.

Manchester

DR F. H. SUMNER, at present senior lecturer in computer science, has been appointed professor of computing science in succession to Professor D. B. G. Edwards, who has been appointed to the ICT chair of computer engineering.

Massachusetts Institute of Technology

MR RICHARD G. MILLS has been appointed to the new position of director of information processing services, a post which is to provide co-ordination between all computer facilities in the institute.

Miami

DR R. G. BADER, head of the Division of Oceanography at the Hawaii Institute of Geophysics, University of Hawaii, has been appointed associate director of the University of Miami Institute of Marine Sciences.

Ulster

DR M. F. GRUNDON, at present reader in organic chemistry at the Queen's University of Belfast, has been appointed professor of chemistry.

University College of Townsville, Queensland

DR C. BURDON-JONES, senior lecturer in marine biology in the University College of North Wales and deputy director (marine biology) of the Marine Science Laboratories, Menai Bridge, Anglesey, has been appointed to the chair of marine biology.

Appointments

DR. JOHN M. HILL has been appointed chairman of the United Kingdom Atomic Energy Authority. Dr. Hill is at present member for production of the authority, with direct responsibility for the authority's Production Group. He succeeds Lord Penney, who is retiring in order to take up the rectorship of Imperial College.

MR M. V. TRACEY, at present leader of the CSIRO Wheat Research Unit, has been appointed chief of the CSIRO Division of Food Preservation, in succession to Dr J. R. Vickery.

DR RONALD S. PAUL has been appointed deputy director of Battelle-Northwest.

MR T. A. MANGELSDORF, a retired oil company executive from New Kent, Virginia, Dr C. G. Hurst, jun., a speech and hearing expert from Washington, DC, and Dr J. E. Harris, an ophthalmologist and university professor from Minneapolis, have been appointed to serve four-year terms on the US National Advisory Neurological Diseases and Blindness Council.

Meetings

SIXTH International Congress of Allergology, November 5-11, Montreal (Dr Samuel O. Freedman, Chairman, Organizing Committee, VI International Congress of Allergology, 1390 Sherbrooke Street West, Montreal 25).

GLOBAL Impacts of Applied Microbiology, November 6-11, Addis Ababa (Dr Aklilu Lemma, Dean, Faculty of Science, Haile Selassie I University, P.O. Box 1176, Addis Ababa, Ethiopia).

Engineer in Management, November 10-12, Scarborough (Mr B. Taylor, c/o Greenwood and Batley, Ltd., Armley Road, Leeds 12).

ERRATUM. Throughout the communication "In vitro Increase in Virus Infectivity", by C. E. Yarwood (page 269 of this issue), potassium sulphate should read potassium sulphite.

ERRATUM. In the communication "Late Pliocene-Pleistocene Stratigraphy in Deep Sea Cores from the South-central North Atlantic", by W. A. Berggren, J. D. Phillips, A. Bertels and D. Wall (page 253 of this issue), reference 14 should be inserted at the end of the third paragraph.

CORRIGENDUM. In the communication "Possible Instability in the Self-closure Phenomenon in Gravitational Collapse" by W. Israel (Nature, 216, 148; 1967), equation (3) should read

$$\lambda = -\frac{1}{2} \int_{-m}^{+m} (R^2 - 2 Rx \cos \theta + x^2)^{-\frac{1}{2}} dx \qquad (3)$$

CORRESPONDENCE

Pink Spots Galore

SIR,—I am replying to a recent comment entitled "Pink Spots Galore", which is a discussion of studies initiated in my laboratory. As a result of those studies, we reported, in 1962, that 3,4-dimethoxyphenethylamine (DMPEA) is a constituent of urine from schizophrenic patients^{2,3}. The writer states that "hats were thrown in the air" when these findings were reported, but that they have now been contradicted by the study of Boulton et al.4. In point of fact, I believe that reference to our publications will demonstrate that we have made a deliberate, and rather successful, attempt to prevent undue optimism or elation in regard to our work. It has been our attitude from the outset that these findings, while of considerable interest, may or may not be relevant to the aetiology and pathogenesis of schizophrenia. I do not feel that any additional findings made thus far, either positive or negative, have been sufficient to justify an alteration in this point of view.

The title of the editorial suggests that there are many compounds producing "pink spots". This is, of course, true and was pointed out by us in our first publication about this so-called "pink spot" test⁵. This test was developed by us to detect certain phenethylamines and developed by the course of the spot of the course of the spot of the course of the spot of the course of for nothing more. We went to great effort to identify the compound peculiar to schizophrenics (DMPEA) in order to avoid misunderstandings among various workers and to prevent ambiguous references to chromatographic "spots". We would therefore recommend strongly that the term "pink spot", which has come into use despite our efforts, be dropped and that each investigator should properly characterize and identify any compounds with which he is concerned.

It is my opinion that Boulton's conclusion that DMPEA is not present in urine of schizophrenic patients is incorrect, and that his inability to identify this material results from deficiencies in the methods that he used. However, not all divergent scientific findings can be reconciled at every point in time. It is my hope that each of the investigators involved in these studies will continue along his own lines, even though all of the results may not be consonant at the present time.

Sincerely.

ARNOLD J. FRIEDHOFF

New York University Medical Center, 550 First Avenue, New York, NY 10016.

¹ Nature, 215, 115 (1967).

² Friedhoff, A. J., and Van Winkle, Elnora, Nature, 194, 867 (1962).

³ Friedhoff, A. J., and Van Winkle, Elnora, J. Nervous Mental Dis., 135, 550 (1962).

Boulton, Alan A., Pollitt, R. J., and Majer, J. R., Nature, 215, 132 (1967). ⁵ Friedhoff, A. J., and Van Winkle, Elnora, *J. Chromatog.*, 11, 272 (1963). ⁶ Friedhoff, A. J., *Lancet*, ii, 1188 (1966).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 23

INSTITUTION OF INDUSTRIAL SAFETY OFFICERS (in the Lecture Theatre' enstitution of Civil Engineers, Great George Street, London, SW1), at 2 p.m. -Mr. W. A. Wood: "The Application to Industry of Recent Advances in accident Prevention in the Mining Industry" (Alexander Redgrave Memorial

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, SW7), at 5.30 p.m.—Mr. D. North: "Eclipses and Eclipse Computers in the Ptolemaic Tradition".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, Londou, WC2), t 5.30 p.m.—Mr C. M. Van der Burgt: "Materials for Ultrasonic Delay Ines".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), t 5.30 p.m.—Professor A. Stratton: "Inertial Navigation".

UNIVERSITY COLLEGE LONDON (in the Botany Theatre, Gower Street, andon, WC1), at 5.30 p.m.—Dr R. J. Smeed: "Traffic Studies and Urban Congestion".*

University of London Institute of Education (in the Great Hall, King's College, Strand, London, WC2), at 5.30 p.m.—Professor G. H. Mantock: "The Idea of a Liberal Education".*

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, WC2), at 6.30 p.m.—Dr A. H. Cookson: Compressed Gas as High Voltage Insulation" (Chairman's Address).

Tuesday, October 24

UNIVERSITY COLLEGE LONDON (in the Anatomy Theatre, Gower Street, London, WC1), at 1.20 p.m.—Dr D. R. Harris: "Corn, Cucurbits and lultures in the New World—the Origins of American Agriculture".*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), it 5.30 p.m.—Dr J. M. Linke, Mr I. F. MacDiarmid, Mr J. A. Taylor and dr D. A. Coles: "Correction of Linear Distortion in Wide Band Links for full-Channel Telephony and Waveform Transmission".

Institution of the Rubber Industry, London Section (at the Eccleston Totel, Victoria, London, SW1), at 5.30 p.m.—Mr R. C. Haines: "Golf-Balls—Old and New"; 7 p.m.—Mr A. Dibbo: "Role of Sulphur Donors in Compounds for High Temperatures".

RESEARCH DEFENCE SOCIETY (in the Physiology Lecture Theatre, University College London, Gower Street, London, WCI), at 5.30 p.m.—Proessor A. S. Parkes, CBE, FRS: "Animals in Captivity" (Thirty-sixth itephen Paget Memorial Lecture).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5.30 p.m.—Professor R. L. Wain, FRS: "The Chemical Control of Plant Growth". (Lecture for Sixth Form Boys and Girls from Schools in London and the Home Countles. To be repeated on October 25, 31 and November 1.)

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr J. M. Barnes: "Toxic Substances and the Nervous System". (Third of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Cars for Cities".

Wednesday, October 25

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (at Exhibition Road, London, SW7), from 2.30 p.m. to 7.30 p.m.—The work and new buildings of the City and Guilds College will be on view: Aeronautics, Chemical Engineering and Chemical Technology, Civil Engineering, Electrical Engineering, Mechanical Engineering, Centre for Computing and Automation.

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, SW7), at 5 p.m.—Annual General Meeting, followed by 'Rear Admiral Sir Edmund Irving, KBE, CB: "The Work of the Institute" (Presidential Address).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr E. C. Rippon: "Power Plant for the 1970's" (Chairman's Inaugural Address).

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT IERE/IEE COMPUTER GROUPS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 6 p.m.—Dr G. Wooldridge and Mr T. J. Stakemire: "A Survey of Digital Data Display Systems".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Mr H. G. Conway, CBE: "Engineering on the Air".

PLASTICS INSTITUTE, BUILDING SUBCOMMITTER (at the Building Centre, 26 Store Street, London, WC1), at 6 p.m.—Meeting on "Plastics in Internal Lighting".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (joint meeting with the Royal Society of Health—Food and Nutrition Section, at 14 Belgrave Square, London, SWI), at 6.15 p.m.—Dr A. G. Kitchell and Dr A. R. Hobbs: "Psychrophilic Bacteria in Foods".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, EC4), at 6.30 p.m.—Discussion Meeting on "The Quantitative Analysis of mgm-Samples of Metals and Alloys".

Thursday, October 26

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m.—Professor Hermann Bondi, FRS: "Relativity and Cosmology" (Civil Service

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W1), at 5 p.m.—Professor A. L. Takhtajan (USSR): "Classification and Phylogeny, with special reference to the Flowering Plants".

University of London (at the Institute of Child Health, Guilford Street. London, WCI), at 5.30 p.m.—Dr D. B. Hope: "Storage of Polypeptide Hormones". (Fourth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5.30 p.m. - Dr S. Keiser-Nielson: "Forensic Dental Work: Cases and Comments".*

Friday, October 27

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Dr. E. Reiser: "Photolysis of Aromatic Azides".

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London. SW1), at 6 p.m.—Discussion Meeting on "How Can Mechanization Reduce the Costs of Distributing Documents in Office Blocks?"

ROYAL INSTITUTION (at 21 Albemarie Street, London, W1), at 9 p.m.—Sir Paul Chambers, KBE: "Controlling an International Chemical Group".

Saturday, October 28

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at the National Hospital, Queen Square, London, WC1), at 10 a.m.—Symposium on "Chemical Pathology in Relation to Neuropathology".

Monday, October 30

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr D. L. Thomas and Mr E. P. G. Wright: "The Impact of the C.C.I.T.T. No. 6 Signalling System on Telecommunications".

ROYAL INSTITUTION (at 21 Albemarle Street, London, Wi), at 5,30 p.n..-Mr Arthur T. Gill: "Faraday and Photography".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5.30 p.m.—Dr N. A. Mitchison: "Control of the Immune Response".*

University of London Institute of Education (in the Great Hall. King's College, Strand, London, WC2), at 5.30 p.m.—Professor A. V. Judges: "The Idea of Equality in Education".*

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "The Implications of Going Metric".

Monday, October 30-Wednesday, November I

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2) — Conference on "Metering and Apparatus for Modern Electricity Supply Tariffs".

Monday, October 33-Thursday, November 2

INSTITUTE OF WELDING (at 54 Princes Gate, Exhibition Road, London, SW7)—Autumn Meeting on "Welding in Non-Ferritic Materials".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN THE BIOBNGINEERING UNIT IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C1 (October 28).

TENFORARY LECTURER/SENIOR LECTURER (graduate with a good honours degree or Dip. Tech. or equivalent in mathematics) in Mathematics at Britannia Boyal Naval College, Dartmouth, Devonshire—Ministry of Defence, Navy Department CEI(N) 368, Old War Office Building, London. SW1 (October 30).

LECTURER OF SENIOR LECTURER IN PATHOLOGY—The Registrar and Secretary, The University, Leeds, 2 (October 31).

RESEARCH ASSISTANT (with a degree, preferably in mathematics and preferably some knowledge of computer programming) in the School of Mathematics, to collaborate with Professor P. H. Roberts in research in theoretical hydrodynamics—The Registrar, The University, Newcastle upon Tyne, 2 (October 31).

SENIOR LECTURER IN PHARMAGEUTICALS; a SENIOR LECTURER IN PHARMACOLOGY—The Clerk to the Council, The School of Pharmacy (University of London), 29:38 Brunswick Square, London, WCI (October 31).

TECHNICIAN IN THE LOW-SPEED WIND TUNNEL LABORATORY, to be responsible for the maintenance of the wind tunnel and maintenance and construction of electronic and other measuring equipment—The Secretary. University of Edinburgh, Old College, South Bridge, Edinburgh, quoting Ref. No. NP[7]10/67 (October 31).

LECTURER (preferably with a Ph.D. degree, but those with a good first degree and relevant industrial experience will be considered) in the Micro-Biology Section of the Department of Applied Min and Robinstown, South Africa—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (South Africa—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (South Africa—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (South Africa—The Association

character) in the Basser Computing Department, School of Physics, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, November 20).

Chair of Prisical Chemistry—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, 8 (November 30).

Chair of Prisical Chemistry—The Registrar, The University, Newcastle upon Tyne, 1 (November 30).

Fellow and a Sanior Fellow in Social Anthropology and Sociology at the Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, November 30).

Chairs (2) of Mathematics at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, December 1).

Assistant Experimental Officer (preferably with a degree or equivalent qualifications) to work on embryonic growth and differentiation in cell and organ culture—The Secretary, Poultry Research Centre, West Mains Road, Edinburgh, 9.

Grade B Assistant Lecturer in Biological Sciences—The Principal, Medway and Maidstone College of Technology, Horsted, Maidstone Road, Chatham, Kent.

Physioist (with a good honours degree in physics) to carry out radiation

GRADE IS ASSISTANT LECULULAR IN DIGINAL STATES AND MEDIAN AND MEDI

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Edinburgh School of Agriculture—The Edinburgh and East of Scotland College of Agriculture. Calendar for 1967–1968. Pp. 68. (Edinburgh: The Edinburgh School of Agriculture, 1967.)

Commonwealth Collections of Micro-Organisms. Directory of Collections and List of Species Maintained in Canada 1967. Pp. 70. (London: H.M. Stationery Office, 1967.) 9c. net. [178 Ministry of Power. Safety in Mines Research 1966—Forty-fifth Annual Report of the Safety in Mines Research 1966—Forty-fifth Annual Report of the Safety in Mines Research 1966. For 1967. 78. 6d. net. [218 How do I det my GCE or Degree at Home? Pp. 43. (Cambridge: National Extension College, 1967.) [218 Topology. By Brian Griffiths. (A reprint of articles from Mathematics Teaching, Nos. 27, 28 and 29.) Pp. 44. (Nelson: The Association of Teschers of Mathematics, 1967.) 5z. National Parks Commission. The Coasts of Hampshire and the Isle of Wight: Report of the Regional Coastal Conference held in Southampton on June 30th, 1966. (Coastal Preservation and Development: a Study of the Coastline of England and Wales.) Pp. v+82+14 plates. (London: H.M. Stationery Office, 1967.) 22s. 6d. [218]

Other Countries

Other Countries

Bulletin of the National Museum, Singapore. No. 33, Part 1: A New Form of Saqitta bedoti Beraneck Found in the Littoral Waters Near Penang. By T. Tokloka and D. Pathansali. Pp. 1-6. M. 31. No. 33, Part 2: A Review of the Brackish Water Prawns of Malaya. By D. S. Johnson. Pp. 7-12. M. 31. No. 33, Part 3: Some Fresh-Water Oligochaeta of Singapore. By K. V. Naidu. Pp. 13-22. M. 31. No. 33, Part 4: Notes on the Biology of the Anchovy, Stolephorus pseudoheterolobus Hardenberg. By Tham Ah Kow. Pp. 23-26. M. 30.50. No. 33, Part 5: A Collection of Land Mollusca from Limestone in Ulu Kelantan. By A. J. Berry. Pp. 27-30. M. 30.50. No. 33, Part 6: Deboutevillea marina n. gen., n.sp. (Collembola, Sminthuridae) from the Inter-Tidal Zone of Singapore. By D. H. Murphy. Pp. 31-34. M. 30.50. No. 33, Part 7: The Anatomy of Calamaria multipunctua (Boie). By R. A. M. Bergman. Pp. 35-58. M. 31. No. 33, Part 8: Acetes (Sergestidae) from the Malay Peninsula. By D. Pathansali. Pp. 50-64. M. 31. No. 33, Part 9: A New Species of Balanus (Crustacea: Cirripedia) from Singapore. By Artffin Suhalmi. Pp. 56-68. M. 80.50. No. 33, Part 10: Nesting Beach Preferences of Malayan Turtles. By J. R. Hendrickson and E. Balasingam. Pp. 69-76. M. 31. No. 33, Part 11: Some Helminths from Malayan Wild Birds with Descriptions of Two New Species. By O. P. Lee, Pp. 77-82. M. 31. No. 33, Part 12: A Taxonomic Study of the Malayan Corixidae (Hemiptera-Heteroptera) with the Description of Micronecta malayana sp. nov. By C. Y. Leong. Pp. 83-90. M. 31. No. 33, Part 13: On Acanthocephalus bujonis (Shitjely), a Common Parasite of Malayan Amphiblans. By P. H. Yuen and C. H. Fernando. Pp. 91-94. M. 80.50. No. 33, Part 15: Berndtia nodosa sp. nov. (Cirripedia, Acrothoracica), a New Burrowing Barnacle from Singapore. By J. Tomilinson. Pp. 101-106. M. \$1. No. 33, Part 16: A New Species of Sesarma from Singapore By E. Serene and C. L. Soh. Pp. 107-110. M. \$0.50. (Singapore: National Museum, 1965, 1966 and 1967.)

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NATURE Volume 216 OCTOBER 28, 1967

All Planners Now

THE second report of the Council for Scientific Policy (see page 322) is more cautious than the first. The passage of time seems to have worn down a good many potentially rough edges. When the council was first appointed the best part of three years ago, it seemed adventurous and open-minded, ready to put the world, or at least a part of it, to rights. The first report seventeen months ago bristled here and there with signs of willingness to rush to the defence of the scientific community against all kinds of threats and impediments, from parsimony at the Treasury to indifference among the administrators. Now, however, the council seems more firmly under the influence of conventional doctrines. It seems a little over-anxious not to rock the boat—not even the other fellow's. On the face of things, at least, it seems to be suffering from an excess of statesmanship of the kind which has afflicted the Science Research Council in the recent past (see Nature, 215, 1215; 1967). And the council is still asking what its function should be. This may be a bad sign, but it is a good question.

It would be wrong not to give the council credit for several important innovations. Soon after taking office, the council embarked on a number of special studies of matters affecting the relationship between science and the community, and many of these have now brought valuable benefits. The Dainton Committee seems, for example, to have done valuable work, and its final report should have an important influence in the potentially fruitful negotiations on which the schools and the universities are soon to be engaged if only the Department of Education and Science can publish it in time. The interim report of the Swann Committee was a healthy shot across the bows even if it has sometimes been quoted in evidence by those who wish mistakenly to prove that technology is both distinct from and in some sense morally superior to science. But in the long run the influence of the Jones Committee on the brain drain could be more lasting and more profound, for that document has provided a splendid analysis of the causes not merely of emigration but of the discontents of an industrial society as well. For these good works, and for others yet to come, the council deserves full credit. It has done this part of its job more thoroughly than it has ever been done before.

The council can also claim credit for the eloquent justification in its most recent report of scientific research in an industrial community. It is too easy to overlook the ways in which seemingly academic work can often, but unpredictably, bring great benefits of all kinds. It is true, of course, that some societies are able to sustain quite rapid economic growth without

spending large sums of money on scientific research, but this is probably a transient phenomenon. For countries like Britain, to turn back could easily bring disaster of some kind, cultural if not economic. It is particularly welcome that the council insists on the difficulty of deciding in advance which kinds of academic research are likely to be more profitable than others. There may be some who ask why the council did not add that research is in any case a necessary part of teaching, so that some of what is at present spent in the universities should be counted as education money; the council could certainly have done more to construct objective yardsticks with which to determine the proper scale of various kinds of expenditure. Should there not, for example, be some kind of link between the postgraduate population and the cost of university research? The working party under Sir Harrie Massey which is now studying the support of scientific research in the universities may yet have useful suggestions to make on matters like these. With luck, the council's exhortation on the value of research should help to bridge the gap.

The council is also to be commended for its attachment to planning, even if interpretations of what this means are sometimes unimaginative. The argument is that budgets which are determined from year to year are bound to mean that valuable resources are wasted, and that big projects always seem impossibly expensive. No doubt the nasty summer which the National Science Foundation has spent before the congressional committees in Washington is another reason why the council and its dependent research councils welcome the promises which seem to have been won about the scale on which scientific research will be supported in the three years ahead. Better a bird in the bush than two on the wing is what they are no doubt saying to themselves. At the same time there is probably a good case for thinking that academic research should not come to too much harm if the money spent on it increases (in real terms) by 9 per cent a year for the next decade or so. This would imply that the annual expenditure of the research councils would increase from £80 million now to just over £200 million at the end of the seventies, by which time it will be a great surprise or a great scandal if the cost of higher education as a whole does not exceed £1,000 million every year. Obviously the council's wishes are not outrageously ambitious. For the time being, it has probably struck as good a bargain as any to be had.

So why should sensible men complain? Everything hangs on the question of what the Council for Scientific Policy is meant to do. There is an impassioned

sentence in the new report which says that "if we could summarize the purpose for our existence in a single sentence, it would be to understand the scientific environment, to advise on its preservation in current difficult conditions, and to convince both scientific and lay opinion that the nation wills its proper support". This implies more than mere budget-making. Taken at its own valuation, the council should be a public influence, as much concerned to see that reasons for its recommendations are widely understood as to ensure that its recommendations are sensible. This, no doubt, is what Mr Patrick Gordon Walker, the Secretary of State for Education and Science, had in mind when he spoke on Tuesday of the way in which the council should engage in a continuing dialogue with the scientific community. Yet there seems a serious danger of the council lapsing into the secretive and passive ways of those who equate public discussion with unwelcome criticism.

The council's justification of the growth rates agreed for the years immediately ahead is one example of this unhealthy tendency. Is the 10 per cent increase for the year ahead intended as a recommendation or is it merely the best that the council was able to negotiate with the Government? And how much was the council influenced by its espousal of the fashionable but potentially misleading view that industry will benefit if life is made less comfortable for those who work in universities? The council's latest report would have been more valuable if it had been more explicit on these points. It would then, for example, have been easier to understand why the Science Research Council was the other day lamenting the way in which desirable projects have had to be postponed for lack of money. It would then also have been easier for the scientific community in Britain to have participated in the kind of dialogue for which Mr Gordon Walker is hoping.

Reticence about the exact status of the forward estimates of growth is nothing like as damaging as the decision not for the time being to publish the full report of the committee on the 300 GeV accelerator. By keeping it secret, the council and the Government between them will only create the impression that there is something to be hidden. No doubt the objective is somehow to save the Government from embarrassment if it should eventually decide not to accept every detail of the council's advice on the 300 GeV machine. But would this really matter? Governments are always repudiating or ignoring their advisers, sometimes with good reason. The council's advice on the 300 GeV machine might well be overridden by wider financial or political considerations, for example. Its function as a sounding board for public discussion of the principles on which science policy should be determined would be enormously strengthened if on this, as on other issues, it had been able to make a greater show of openness and independence.

The habit of public reticence about matters of public importance is not, of course, a fault possessed only by the Council for Scientific Policy. It runs through

the whole of public life in Britain, no doubt because of the way in which it is assumed that the Civil Service is answerable not to the public but to the ministers in the Government. The results, nevertheless, are frequently absurd. The existence of the committee which has been examining the 300 GeV project was something of a secret until the publication of the second report earlier this week. In other fields, reticence is not merely an impediment to public understanding but positively harmful as well. Why, for example, should those who are in charge of the sensible scheme for sharing expensive apparatus among colleges of the University of London (see page 320) be unwilling to explain how the system operates? Why, for that matter, should Dr Husband be able to explain (see page 319) to the conference of radio-telescope designers in Massachusetts last week a good deal about the design conception of the radio-telescope he may yet be building for the Science Research Council when the council itself says that the design must be confidential for the time being? Reticence on simple matters like these can serve no useful purpose and yet is not merely an irritation but a sign of how far there is to go before the relationship between the bodies which administer science in Britain and the scientists for whom they do so is anything like as open as it should be.

The advice which the council has actually given on the 300 GeV machine also leaves a great deal to be desired. It is fair, of course, to make reservations about the damage that would be done to the pattern of expenditure in Britain if the costs of the 300 GeV machine should increase dramatically, and the council is also on sure ground in saying that a decision to contribute to the 300 GeV machine sets a lower limit to the pace at which the budget for civil science as a whole must continue to increase. On the assumption that the conditions will be met, however, the council could have given a more straightforward statement of whether it thinks the 300 GeV machine is desirable or not. Does the council, for example, consider that a nuclear physics budget increasing at 7 per cent a year is reasonable? And if it does, should not the council also have given the project to build the machine more enthusiastic backing?

The most serious cause of disappointment in the council's new report is, however, the way in which it seems not to have tackled a great many important What, for example, is happening to the National Library for Science and Invention? Is it really safe to think that the scale on which computers are being employed in universities is sufficient to meet the real needs? Although the report of the Flowers Committee on university computers has had a beneficial influence, complaints abound of shortage of facilities and there is in any case a great deal to be done before undergraduates at universities have access to up to date machines. The relationship between Government establishments and universities is also urgently in need of close attention—the council's first attack on this important problem turned out to be an exceedingly damp squib. And then, of course, there is the

whole question of the development of higher education in the years ahead. Will the new polytechnics carry out research on a substantial scale, and, if so, who will pay for it? What about the need for more post-graduate education? It is a long time now since the Robbins Commission raised this issue, yet very little has been done. In these and many other ways, the

council could become much more vigorous and constructive. It could also in the process help not merely to awaken the interest of the scientific community in its work but also to extend its influence on the development of scientific research in Britain. That, so far, has been a good influence. The need now is that there should be more of it.

Assessing the AGR

IT has never been easy to feel optimistic about the export prospects of the British nuclear power consortia, committed as they are to the advanced gas cooled reactor. It is clear for a start that the AGR is an expensive piece of equipment compared with an American boiling water reactor. But in the past it has been possible to hope that the greater capital cost of the AGR could be outweighed by its high thermal efficiency and fuel economy. This notion has come under increasing attack since 1965, when an AGR won the contract for Dungeness B power station against competition from a boiling water reactor. clear from the latest annual report of the Kjeller Laboratory of the Norwegian Institutt for Atomenergi that Norwegian companies, at least, are unlikely to be convinced by it.

Since the beginning of 1966, the institute has collaborated with the Norwegian firm Norsk Hydro on a study of different reactor systems for a 500 MW nuclear power station. A preliminary costing study shows that the AGR is the most expensive type of all. For an AGR of 600 MW(e) the total plant cost would be £43.5 million, excluding initial fuel charges. (In Britain a similar station would cost more, the report says, because of "the very special requirements of the Central Electricity Generating Board".) Generating costs would be 0.41 pence/KWh. For a boiling water reactor the same size, initial costs would be £34.6 million, and generating costs would be 0.393 pence/ The other types studied, which included the pressurized water reactor and the heavy water pressure tube reactor, fell in between these two extremes. The fast reactor, the report suggests, would be the cheapest of all, supplying electricity at a cost of 0.264 pence/ KWh, but the figures are admittedly speculative.

In the larger sizes, the gap between the AGR and the BWR narrows slightly; for a 1,000 MW station, generating costs for the AGR would be 0.38 pence/KWh and for the BWR 0.367 pence/KWh. As a result of this study, the institute has decided to carry out a more extensive study to compare the light water boiling reactor with the heavy water boiling reactor.

The UKAEA is inclined to doubt the validity of the assessments. The figures for the AGR, which the AEA itself supplied to the institute, are accurate, but the BWR costs were taken from a catalogue issued in 1964 by the General Electric Company (US). Since then, the AEA says, GE costs are up by 20–30 per cent; an adjustment of this order would bring the figures into line with the Dungeness B assessment. In any case, although calculations of this sort can give a general feeling for the costs, they are not accurate enough for final decisions to be taken, and the decision to go ahead with the BWR and the heavy water boiling reactor is probably "an unreasonable deduction from the strength of the exercise". Only the discipline of tendering can

really establish costs, the AEA believes; a conclusion shared by the delegates at a recent IAEA symposium in London.

Rescuing the Dragon

DURING 1967, the Dragon reactor at Winfrith Heath became the first system to demonstrate the feasibility of high temperature reactors. In the process, it also became the first system in Britain to demonstrate the cliff-hanging financial arrangements of Euratom. The Dragon is a collaborative reactor supported by the countries of OECD; most of the cost falls on the United Kingdom and the Euratom countries. During 1967 Euratom was unable to agree to an extension of the Dragon programme into 1970, and as a result the UK declared itself willing to bail out Dragon for the whole of 1968 if necessary, to give Euratom more time. Euratom now has two decisions to make; the immediate one is to decide whether to accept the British offer. If Euratom refuses, the UK (together with the other OECD countries not in Euratom) will go ahead for at least one year, and take over the whole project. If Euratom accepts the interim British offer, it will then have to decide, some time next autumn, whether to go on into 1969. If the answer then is yes, Euratom will repay to the UK the share of the 1968 costs: if it is no, then the UK will take full charge of the project. The UK will either get its money back or be left with a project which is almost completely paid for, and is now producing useful information. The UKAEA believes it has a very good deal.

The reactor has operated well, despite a leak which developed in the heat exchangers, allowing helium to leak from the primary to the secondary circuit. The fuel charge developed for the reactor, consisting of particles of thorium and enriched uranium carbides coated with layers of silicon carbide and pyrolytic carbon and contained inside graphite tubes, has worked perfectly, the report says. The fuel reached a temperature of 1,250° C and a burn-up of 25 per cent of the uranium-235. A second fuel charge has been developed, with improved particle coatings and graphite, and it has been possible to do without the arrangements for purging the fission products which were incorporated in the first fuel charge. During the year the work of assessment on a full scale power station using the Dragon concept has been continued. One design, called the feed and breed concept, calls for a mixture The breed fuel contains fissile uranium-235 and fertile thorium-232 in such proportions that the ²³³U which is bred replaces the original ²³⁵U at the same rate as it is burned. To maintain the reaction, since breed fuel cannot be made critical, part of the core contains highly enriched uranium, the feed

Hard Words about Pollution

DR E. F. SCHUMACHER, economic adviser to the Coal Board, was brave enough last week to attack the safety standards in nuclear power stations. In a speech to the conference of the National Society for Clean Air (Nature, 216, 219; 1967) Dr Schumacher claimed that nuclear power stations would gradually contaminate the environment, by "silently leaking radioactivity into the ground". The increasing use of nuclear fuel would add to the dangers of accidents in transport, and what would be done with nuclear power stations when they came to the end of their active lives? Dr Schumacher pictured them standing idle all over the country as mute witnesses to the folly of allowing economic criteria to dictate policy. No insurance company, he added, would take on third party risks for nuclear power stations.

The spokesmen for the Ministry of Power and the Electricity Board responded with the alacrity born of long experience of the Coal Board. Mr Peter Williamson, for the CEGB, managed to get his reply in first, by responding to Dr Schumacher's speech at the conference. Reactors once out of use would be sealed up and covered up with a mound of earth, he said, and would be no danger. Accident-proof containers have been designed for the transport of dangerous materials, and insurance companies did accept the third party risks for nuclear stations. The Ministry of Power was slower to react, but no less decisive. Dr Schumacher's statements, the ministry said, "are so inaccurate that they cannot be regarded as a serious contribution to any discussion of the subject". External radiation from power stations, it added, is virtually non-existent.

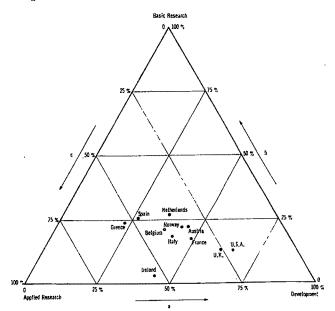
Later, Dr Schumacher claimed that his remarks had been misinterpreted. He has no criticism of the way in which dangerous materials are handled at the moment, and regrets that certain points used to illustrate the theme of his address should have been taken as an attack on standards of public safety. The caution Dr Schumacher made was against too much emphasis on economics. Too often the cheapest production methods for commodities are used without enough consideration of the human factors involved. A massive increase in nuclear power installations might not be matched by a greater knowledge of how to protect man from dangerous radiation.

Know your loneses

THE amount a country spends on research and development is probably the most confusing index of economic performance ever devised. Even so, British ministers will doubtless feel encouraged when they read a recent report prepared by the Organisation for Economic Co-operation and Development (The Overall Level and Structures of R and D Efforts in OECD Member Countries, OECD, 9s.). The fact that Britain spends far more than other European countries on research is likely to be regarded by British supporters as a further reason why Britain should be admitted to the Common Market. But it would be equally easy to say that a large research budget, combined with a stagnant economy, merely emphasizes how loth Britain is to

The United States, of course, leads the field by a very substantial margin. It spends more both in real

terms-more than \$21,000 million-and as a proportion of gross national product—3.4 per cent. Great Britain spends \$2,160 million, 2.3 per cent of the GNP, while France and Germany manage \$1,299 million (1.6 per cent) and \$1,436 million (1.4 per cent) respectively. Other countries of the EEC trail well down the field; Italy spends only 0.6 per cent of the GNP on research and development. The Netherlands, on the other hand, spends 1.9 per cent and Sweden 1.4 per cent.



This diagram, after the style familiar to metallurgists and physical chemists, shows the amounts spent by countries on basic research, applied research and development.

The report does nothing to encourage illusions of the value of these figures. "International comparisons of GNP percentages are not good yardsticks for science planning. Such an evaluation can be made only in the light of the research and development aims a country sets itself, some of which are more costly to realize than others." For this reason the report makes a useful analysis of the proportions spent on different areas of research. About two-thirds of the expenditure in the United States, for instance, is devoted to nuclear, space and defence research and development. France spends 45 per cent of her total in these three fields, and Britain just under 40 per cent. According to the figures, Japan spends nothing at all on these activities.

The United States employs as many qualified scientists, engineers and technicians as the rest of the OECD countries put together. A full-time equivalent of 700,000 qualified people is estimated for the United

RELATIVE EXPENDITURES ON RESEARCH AND DEVELOPMENT IN SELECTED INDUSTRIES

	(US equ	als 1,000)		
	Air- craft	Elec- trical	Chemical	Manu- facturing industry
United States	1,000	1,000	1,000	1,000
United Kingdom	76	103	112	96
Germany		94	175	62
France	28	63	80	44
Japan		57	93	41
Italy		6	29	11

States, while Japan comes second, with 187,000 qualified people. The formula used assesses people in terms of full-time equivalent, to take account of those who spend only a proportion of their time on research. The United Kingdom has 160,000, West Germany 105,000 and France 85,000.

The report also compares the expenditures in various industries with that in the United States (see Table), and this comparison is even more striking; if the whole of Europe were united overnight, the research and development expenditures in the aircraft, electrical and chemical industries would amount to less than half the totals in the United States.

American Technology

How should applied research be supported? The National Academy of Sciences in the United States, having dealt with basic research in a report called Basic Research and National Goals, has now turned its attention to applied science. The result, Applied Science and Technological Progress, is a free-ranging collection of essays tied—sometimes rather tenuously—to the basic theme. The report, prepared for the Committee on Science and Astronautics of the House of Representatives, also includes an introduction which bravely attempts to find common themes in the essays and to draw conclusions.

The mission-oriented laboratory, most members of the panel agree, is a happy invention for applied science. One of the recurrent themes is the difficulty of maintaining the vitality and spirit of these laboratories; Dr Alvin Weinberg, director of Oak Ridge, is daring enough to suggest that they should be turned over to the solution of social problems—pollution, race and crime. What, he asks, is to become of the fine laboratories of NASA after the Apollo mission has been completed? Agency laboratories have a way of becoming obsolete, especially when the agency is organized around a technology. This is recognized in the introduction to the report, as is the difficulty of changing the direction of a laboratory—this preoccupation echoes the problems of the United Kingdom Atomic Energy Authority.

The university, on the other hand, is not a good place for applied research, although the panel sees some hope that the advent of the computer will allow university workers to engage in the problems of real life, at least by proxy. Close association between universities and applied research laboratories is important, and there is at least an undercurrent of fear that the increasing influence of university attitudes in American life is causing people to regard applied science as a second-class activity—and this in a country where, the introduction rightly points out, industrial research has been a magnificent triumph.

At least two of the essays give fascinating blow-byblow accounts of these triumphs. Donald Frey and Jacob Goldman from the Ford Motor Company discuss innovations ranging from high pressure metal forming to micro-electronics, and C. Guy Suits and Arthur Bueche from General Electric do the same for their own company. The case studies show that there are few common factors in the process of innovation except determination; some successes are the result of deliberate investigation but others, equally important, are a mixture of luck and good observation. This has been said often enough, but rarely demonstrated as clearly as Suits and Bueche manage to do. discovery that fission tracks in mica can be etched out to form tiny holes, for example, was originally intended as a filter for blood, but has been applied to a great variety of scientific and commercial uses, from nuclear physics to the age determination of tektites. Faced with this diversity, it is hardly surprising that the panel was unable to establish hard and fast rules for applied science, although some general principles did emerge. Secrecy in applied science is a bad thing, and therefore the patents system must be preserved, for without it greater secrecy would be inevitable. Personal mobility is also important, and may, the panel considers, be where the United States differs from less successful countries. The technical entrepreneur, the man whose enthusiasm for a project may often exceed his technical understanding, is frequently a vital element in successful innovation. Finally, the panel has a good word to say for technological forecasting, but adds a caution-forecasting can do more harm than good if its results are treated as more than rough first approximations.

Who Builds What?

THE construction of a 400 foot radio-telescope somewhere in Britain seems to have been carried one step forward in the past few days with the decision of the Science Research Council to negotiate a contract for design and construction with the consulting engineering firm Husband and Company. The principal of the firm, Dr E. C. Husband, has been deeply engaged in the design of radio-telescopes since his involvement with the 250 foot instrument which has been operating at Jodrell Bank since 1957. The decision to negotiate with Husband and Co. rather than with the other firms which have submitted proposals has been taken the recommendation of the Atomic Energy Authority, which has been acting as an agent for the Science Research Council during the feasibility study now completed. Some confusion seems to have been caused by the way in which Dr Husband let it be known, at a conference on the design of radio-telescopes at the Massachusetts Institute of Technology last week. At that stage it seems that the decision had not been communicated to the unsuccessful firms.

The council's immediate commitment is to the detailed design of the telescope, on which work will now begin. In the first half of 1968, it should be possible for the council to make a final decision to go ahead with construction of the telescope. By then it should also be known just what the instrument will cost. With luck, the council hopes to build the new instrument for a good deal less than £5 million. The intention is that the dish should be constructed accurately enough for the whole of its area to be usable at the wavelength of the 21 cm hydrogen transition, and at the slightly shorter wavelengths now being used increasingly by radio-astronomers. The choice of Dr Husband's company implies that the new dish will be supported on an open steel beam itself held above the ground by vertical legs. Husband says that this type of design is preferable to that in which the dish is supported at the centre by a single column because of the more sensitive control of position which is then possible.

Research Service

THE University of London Intercollegiate Research Service was first discussed about ten years ago. After nearly three years of hard work by a committee under Professor W. Klyne, the final scheme has been announced, with details of the allocation of funds. Altogether the Science Research Council is providing £170,000 to set in motion the service "for chemistry and related sciences".

Sophisticated scientific instruments are now so expensive that it would be impracticable to install and maintain all types of instrument at every college. The intercollegiate research service has been designed so that research workers from colleges inside and outside the University of London can have access to all Seven colleges are housing and the instruments. running the machines, making about 50 per cent of the operating time available to outside departments. The general principle is that samples are submitted, probably by post, to the department in question. technician working with the machine makes the readings and the results are returned. Detailed arrangements may vary to make the best use of each type of machine.

The SRC grants have been awarded as follows: £14,560 for X-ray crystallography and £25,248 for laser Raman spectroscopy at Imperial College; £19,974 for Raman and long wavelength infrared spectroscopy at King's College; £26,925 for nuclear magnetic resonance spectrometry at Queen Elizabeth College: £23,519 for electron spin resonance spectrometry at Queen Mary College; £36,425 for mass spectrometry at the School of Pharmacy; £14,025 for mass spectrometry at University College; and £9,100 for optical rotatory dispersion and circular dichroism at Westfield College. Two-thirds of the machines are already installed.

A report was made to Dr G. Johns of the SRC in September on the machines in use, including some which have been operating for nearly a year. There seems to be no particular reason why an official announcement of the service should have been made at this stage in the proceedings, and there seems to be an ambivalent attitude to publicity amongst those involved with organization. Although parts of the service have been running smoothly since January, Professor Klyne is reluctant to describe the facilities in detail, possibly for fear of an influx of samples to be tested. grapevine has been working in the chemistry departments of the University of London, but physicists and biochemists who might be keen to use the new instruments have had no official information. individual colleges are anxious to help where they can, and, when time allowed, have run spectra for various industries. Professor B. C. L. Weedon, head of the University Board of Studies in Chemistry, thinks the service deserves publicity, especially in other universities which could well benefit from co-operative organizations of this kind. Professor Klyne's committee is hoping to produce a booklet in the near future, which will give instructions on how to send in samples, and the various facilities available.

New Role for BISRA

DOUBTS about the future of the British Iron and Steel Research Association (Nature, 214, 867; 1967) have

now been resolved. The Council of BISRA have accepted the offer by the British Steel Corporation to take over responsibility for finance and operation of BISRA from October 31, 1967, when the dissolution of the British Iron and Steel Federation takes place. BISRA will then become the central laboratory of the BSC, and will concern itself with problems common to more than one of the four groups of the corporation. At the same time, the research done by BISRA within an agreed scope will be made available to the private steel companies, now represented by the British Independent Steel Producers Association.

The future work of BISRA will probably break down into three broad categories. As well as the co-operative research programme which has to be agreed by a technical collaboration committee jointly appointed by the BSC and the private companies, there will be sponsored work-which will doubtless include work for the corporation and for the private companies—and there will be work which falls outside the co-operative programme and is therefore available only to the corporation. The association is out to disprove the notion that collaborative work is impossible if one group dominates the others in size—and, in this case, in nature. In effect, BISRA had little choice but to accept the corporation's offer, since the fourteen nationalized companies supply as much as 80 per cent of the association's income.

BISRA sees its work as the central laboratory of the corporation as being slightly different in emphasis from that carried out by the individual groups of the corporation. BISRA will concern itself with problems common to several groups, and to the general improvement of processes, plants and products. The group research departments are likely to be concerned with more immediate problems in the day to day running of the plant, and problems specific to the product manufactured by the group they represent.

Mental Health Research

PEOPLE with cheque books will be interested to learn that the Institute for Research into Mental Retardation is now registered as a charity. The institute, whose chairman is Lord Francis-Williams, was founded in April 1966 by the National Society for Mentally Handicapped Children which donated the first grant of £5,000. Its function is to co-ordinate research, to provide a centre of information and to promote collaboration between workers of various discplines. Eventually it hopes to attract support from all kinds of charities.

Although throughout the world a mentally retarded child is born every twenty seconds, the governors of the new institute are enthusiastic and optimistic. Dr D. Morris, vice-chairman of the institute and a practising paediatrician, suggests that prevention is possible in about half of the cases whose deficiency originates during the perinatal period. Five projects in primary prevention are already being sponsored by the institute, aided by a grant from the Wates Foundation. example, research workers are anxious to know what causes the well known extra chromosome (chromosome 21) in the cells of mongols and how this shows itself in the clinical symptoms. The incidence of mongolism increases with age of the mother and this could possibly be attributable to the ageing of ova. Another promising

line of work involves mosaics: some mongols have an extra chromosome in all of their cells, others in only a few. Although it is not feasible at present, if some mongols could be made into mosaics, their appearance and behaviour would resemble more closely those of a normal person. Other puzzling questions include the number and nature of genes carried by the extra chromosome.

Prevention apart, the institute aims to make life a lot better for the mentally retarded. Already it has been effectively demonstrated that after appropriate training mentally retarded individuals can be economically productive, not only in sheltered workshops but also in open employment. It is, however, both unfortunate and surprising that the information service provided by the institute is well financed, while the actual research work is dependent on charities and private donations.

More about Pollution

Most records of air pollution are from towns and industrial areas and there is much less information on background levels of pollution in agricultural districts. To remedy this state of affairs, the Agricultural Research Council has now compiled a comprehensive review of the literature on the effects of air pollution

on plants and soil.

The report describes a number of toxic elements and pollutants; how they originate and how they should be treated. The total emission of smoke in Britain, for example, has been declining for some years but reaches a higher concentration in winter. Deposited matter, including soot, tar, dust, grit and ash, is mainly derived from the combustion of solid fuels. So, too, is sulphur dioxide; in 1963, 68 per cent of sulphur dioxide came from burning coal, 7 per cent from coke and 25 per cent from oil. At Battersea and Bankside power stations in London, flue gases are washed with water from the Thames to which chalk has been added: this process removes 90 to 98 per cent of gases but it is costly, and more attention has been paid to dry processes in recent years. Fluorides are emitted by some brickworks and possibly by some potteries and cement kilns, but fluoride pollution is rarely reported in Britain now. It remains to be seen whether photochemical or oxidant smog, often referred to as Los Angeles smog, will be troublesome in Britain in future.

According to the review, the toxic effects of many pollutants vary with species and variety, with environmental conditions, with nutritional levels and with the stage of growth of the plant. Smoke and deposited matter have an adverse effect on photosynthesis by reducing the light intensity, blackening leaves and blocking stomata. Conifers are particularly sensitive to sulphur dioxide, which causes acute damage to leaves. In soils which have suffered heavy pollution for prolonged periods, oxidation and solution of the gas in rain water result in increased acidity and sulphate content of soil, with a corresponding reduction in calcium and other gases and reduced activity of micro-organisms. Fluorides, on the other hand, act as cumulative plant poisons and the consumption of such vegetation can cause fluorosis in cattle and sheep. Oxidant smog produces silvering and bronzing of the lower leaf surface, although it can cause physiological

disturbances and reduced growth in some species without producing any visible leaf symptoms.

Landing on Venus

from a Correspondent

THE drama at Jodrell Bank last week when the Soviet Union Venus 4 spacecraft reached the planet recalled the days of the early sputniks ten years ago.

Signals from Venus 4 were picked up at 0417 BST, some 40 minutes after Venus rose above the horizon. They were described as consistent with the signal strength recorded in July, with 2-tone telemetry similar to the Luniks' transmissions but speeded up four times. Besides recording, Jodrell was also measuring Doppler shift. At 0440 the distance of Venus 4 was estimated at 30,000 km and speed change at 30 cm per second per second. The received signal strength was 10^{-17} watt, assumed to be 1 watt transmitter power boosted to 200 watts effective power by the 2-metre directional antenna on the spacecraft.

It was estimated by the Jodrell team that if a Venus orbit or a main spacecraft soft landing was aimed for, the signal shift would slow between 0550 and 0530, the expected time of arrival at the surface. No such signal modifications occurred while the spacecraft continued to transmit and show enhanced frequency shift. At 0515 it was estimated to be 10,000 km from the surface. At 0538 05, the signals ceased, compatible with the probe ceasing to transmit at about 100 km from the surface.

As is now known, this was in fact the beginning of the main experiment, but further signals were not expected, and though the equipment continued to observe no recordings were made of the early part of the new transmissions. These began 15 seconds after the end of those from the main spacecraft. There was a hundred-fold drop in signal strength, compatible with the loss of the 2-metre high gain antenna, and the same telemetry as before was apparent but with many fewer data, as would be expected from the power reduction. The apparent stillness of the new transmissions led the Jodrell team to assume that the signals were coming from an ejected capsule actually resting on the planetary surface.

Some time later, it occurred to the Jodrell team as well as to some other commentators that the data had been transmitted in real time as the ejected capsule descended from 25 km to the surface of Venus in 90 minutes. Soviet sources gave 90 minutes as the period taken to descend and this exactly corresponds with the transmission registered at Jodrell Bank. The capsule transmission cut out sharply "as if on command" at 0714 BST. There has as yet been no conclusive evidence that the capsule did make a soft landing on the surface, or that its transmissions continued after touchdown, or that it directly measured surface conditions. It was certainly designed to do all these things-it had the same basic configuration as last year's first successful Soviet lunar soft landing by Lunik 9, with a weighted base to ensure it settled right side up. Indications are that this was not the main object of the experiment but a possible bonus. A sounding of entire Venus atmosphere from top to bottom which must have included very near-surface measurements makes a significant addition to planetary knowledge.

Planning Ahead

The second report of the Council for Scientific Policy raises questions of the problem of research in Britain.

THE principal centres of interest in the report of the Council for Scientific Policy, published on October 24 (Cmnd 3420, HMSO, 4s. 3d.), are the estimates of what will be spent by the four research councils between now and the financial year 1969-70 and the council's recommendations to the British Government about British participation in the building of the 300 GeV accelerator within CERN. On the first score, the council has agreed with the Government provisional figures for the two financial years ahead which represent annual increments of 10 and 9 per cent respectively. On the accelerator, the council has urged that the British Government should participate in paying for it on the assumption that the British contribution towards CERN is in future proportionately no greater than it has been in the past and provided that "assurances can be obtained that resources will be made available for science on a scale sufficient for both the 300 GeV machine as part of the nuclear physics programme and the proper development of other fields of science". The council comes near to specifying what it would consider a sufficient assurance with its estimate that the cost of other kinds of science than nuclear physics will grow at about 9 per cent per annum in real terms over the decade ahead.

Planning and the need for it is indeed the central theme of the council's report. As an earnest of its intentions, the council is already discussing with the research councils plans for expenditure in the financial years 1970-71 and 1971-72. Several benefits are expected to accrue from developments like these. For one thing, the council expects that the ability to make firm commitments will enable the research councils to make more efficient use of available re-The council's deliberations on the 300 GeV machine have plainly served as a telling object lesson of how the long time-scale on which large capital projects must be planned implies that other kinds of expenditure should also be determined in advance for a comparable interval of time. But there is more to be said than this. In the peroration to its report, the council emphasizes that long-term planning is the only way of creating confidence in the continuing development of science. "We believe that much of the problem of emigration of scientists can be dealt with by these means.

The council also acknowledges that the rapid growth of the budget for civil science in Britain since the war—from £6.6 million in 1945 to £295 million in 1967—implies that the council has the responsibility of justifying continuing growth. For one thing, the council points out that if science budgets are measured by the proportion of a nation's GNP devoted to them, the British performance may have been deteriorating by comparison with the United States in the past five years or so. British expenditure on research and development since 1961 has been static at 2.7 per cent of the GNP, but research and development in the United States has risen from 3.0 per cent of the GNP in 1961 to 3.2 per cent in 1964.

The council's chief justification of expenditure on scientific research is more philosophical than this, however. It argues against too rigorous an attempt to assess the potential value of particular programmes in basic research by estimating what measurable economic or humanitarian benefits might accrue. It points to the way in which work in electron physics has led to practical applications in electronics, and to the potential benefits which may come from recent developments in molecular biology. There is a passage in the report about the way in which medical research has brought "an immeasurable gain to humanity in terms of happiness and health", and numerical calculations of what this means in terms of more people at work and fewer people in hospital. In agricultural research, the council mentions the increase of agricultural output from British farms of 44 per cent in the decade from 1954 in terms which attribute some of this improvement to agricultural science, and the council also says, in a reference to the work of the Natural Environment Research Council, that "in no field more than the natural environment is the potential gain from applied research more spectacular or more neglected".

But this is only a part of the story. Basic research is the only assurance of a continuing flow of original discoveries "from which all other progress flows". The council considers that the way in which countries such as Japan have been able to make rapid progress in technology without spending as great a proportion of the GNP on research and development is proof of the need in Britain for closer relationships between industry and research. "We have seen no evidence that we must write off our long-term insurance for the future in order the better to exploit the already existing results of science".

As it happens, the council does not quote in this most recent report the argument which appeared a year ago that the pace of growth in scientific research would somehow have to be matched with the pace of growth in other sectors of the economy. also some evidence that the council has moderated its demands on the public purse by its recognition that there is a need to bring about a "reorientation of objectives towards the application of science for some of the ablest young men emerging from the universities". The estimates of what will be spent by the agencies under the control of the Council for Scientific Policy in the years ahead are shown in Table 1. Fluctuations from one year to another in the spending of individual research councils are accounted for by the concentration of certain items of capital expenditure in particular years—for example, the comparatively high rate of growth for the Agricultural Research Council in the current year is explained by the construction of two laboratories for food and meat research.

One of the most striking uncertainties in this analysis is that there is at present no way of knowing just what will be the contribution of the University Grants Committee to research expenditure in the universities. In 1966–67 this source contributed close on £50 million towards the cost of research, compared with the

Table 1. THE GROWTH AND DISTRIBUTION OF SCIENCE VOTES EXPENDITURE

		196667	,		1967–68			1968–69 Provision	n1	1	1969–70 Provisions	. 1
	£m Esti- mate	Per cent of total	Per- centage growth	£m Esti- mate	Per cent of total	Per- centage growth	£m Pro- posed	Per cent of total	Per- centage growth	£m Pro- posed	Per cent of total	Per- centage growth
Agricultural Re- search Council	10.312	16.1	7.3	11.974	16.5	13.3	13-1	16-4	9.8	13.8	15.8	5.0
Medical Research Council	11.885	18.5	11.6	14.232	19-6	15.1	15.3	19-3	7-6	16.8	19-4	9.5
Natural Environ- ment Research Council	C·186	9-6	17.8	7.657	10.5	20-1	8.8	10.9	14-8	10-1	11.5	14-6
Science Research Council	33.919	53 ·0	13.2	36.584	50.4	7.0	40.0	50.0	9-4	43.0	49.3	7.6
British Museum (Natural History)	C-931	1.5	1.3	0.957	1.3	_	1.0	1.3	6.6	1.5	1.7	42.7
Science: Grants and Services	0.878	1.4	41.2	1-203	1.7	31.2	1.6	2.0	36.1	1.7	1.9	2.6
Total	64-111	100.0	12.2	72.607	100.0	11-0	79-8	100-0	10.0	87.0	100-0	9.0

£23 million or so which the research councils spent in or around the universities in the same year. It follows that much will depend on the settlement soon to be reached for the financing of the universities in the five years from September 1968. There is also some doubt about the way in which the sums available will be affected by the inevitable increase of the cost of carrying out scientific research in the years ahead. The report says that the council's working party on what is called "sophistication" should be ready soon, but there seems no prospect of being able to obtain a simple rule of thumb which can be applied across the board to anticipate the increasing cost of research.

The council takes a cautious line on space research. Its particular concern is to ensure that the relationship between British universities and ESRO should yield as much good science as possible, and the council gives a warning that it will be alarmed if resources provided for scientific research in space should be diverted to technological development. It does not deny the potential value of space applications but says that these must be considered and financed separately from the present budget. The council also resists the suggestion that space research should be transferred from the responsibility of the research councils and the UGC—a comment on the recent report of the House of Commons Select Committee on the Estimates, which recommended that the Ministry of Technology should assume direct responsibility for all forms of space research and development. Not merely, says the council, would such a step expose funds provided for science for use on a very large scale for nonscientific purposes, but it would violate the principle that the research councils should decide scientific priorities on the recommendations of scientists.

The council's argument about the proposal to build a 300 GeV proton accelerator within CERN is cautious. It acknowledges the success of CERN, at present based on the 28 GeV machine at Geneva and the accompanying storage rings. But participation in the construction of the 300 GeV machine would entail that the British investment in nuclear physics, in Britain and abroad, would work out at £34 million a year in 1977, which implies an annual increase of 7 per cent. The council has based its decision on the deliberations of a working

group under Professor Michael Swann with Dr J. B. Adams, Dr F. S. Dainton, Professor B. H. Flowers, Dr J. C. Kendrew, Sir Harry Melville and Professor A. B. Pippard as members. The report of the working group is not to be published, for the time being at least, but its argument is that the justification of the project rests entirely on its intrinsic scientific interest. One surprising aspect of the argument is that the council has been convinced that there would be no economic benefit in having the proton accelerator built in Britain, although of course there would be significant scientific benefits. The council's feeling is that the building of the new machine should not interfere with plans for development in medical, agricultural and environmental research. It adds its caveat about the need to avoid escalation before suggesting that the Government should participate.

Among the other activities of the council, the report singles out the work of the computer board, saying that a continuous review of the employment of computers in universities is being undertaken. There is to be a review of biological research in British universities based on a survey carried out in February this year and this, it is hoped, will reveal the present balance of activity in different fields of biology. The council is pleased with the way in which European science fellowships are being developed in collaboration with the Royal Society. A total of £50,000 has already been allocated to the Royal Society for planning a programme of scientific exchanges at post-doctoral level and the council says that £200,000 has provisionally been reserved for each of the two succeeding years. The council is also carrying out a study of the value of the British Antarctic Survey, although it is not clear from its report whether it has it in mind that the scale of the survey's operation should "continue at its present level, be run down or be increased". On the Naples Zoological Station (see Nature, 213, 221; 1967), the council has recommended that the British Government should contribute £28,570 a year so as to secure a seat on the council. On EMBO, the council is in favour of supporting the current programme of exchanges when the grant from the Volkswagen Foundation comes to an end in 1968, but it considers that the case for a European laboratory is not yet proved.

NEWS AND VIEWS

Nobel Prizes for Vision

THE award of the Nobel Prize for Medicine to Professors George Wald of Harvard, H. K. Hartline of the Rockefeller University and R. Granit of Stockholm is a welcome and imaginative step. The three people concerned are distinguished for their separate contributions to three separate parts of contemporary understanding of the processes of vision. One way and another, all of them have done a great deal to stimulate work in other laboratories. The result is that the physiology of vision has been transformed, in a comparatively short time, from what seemed to be a narrow and introspective specialism into a rapidly growing field of interest. And the objective, of course, is not merely an understanding of the mechanism of vision but a deeper insight into the nature of sensory processes of all kinds.

Professor Wald is a chemist by origin, and his contribution has been the identification and the study of the visual pigments and their chemical precursors. He, for example, was for a long time the driving force in the solution of the problem of rhodopsin. It is interesting and important that much of his influence on the development of this subject stems from his flair for expressing the results of his research with enormous clarity, and from his eagerness to spend time on doing this. Whatever the means, however, the result has been a dramatic advance in understanding in molecular terms of how light is absorbed by the visual receptors. One important by-product has been a deeper insight into the processes of colour vision. But the study of the visual receptors also promises to link up in important ways with work elsewhere in molecular biology on enzyme systems which involve the conduction of electrons along sheets of molecules the enzyme systems in mitochondria, for example. Nobody should be surprised that Professor Wald's work has posed as many questions as it has answeredthey are all of them important questions.

Professor Hartline works in a different line. He has made a lifelong study of the visual receptors in the

Behavioural Mutants

from our Cell Biology Correspondent

When it seemed likely that studies of the genetic basis of behaviour and memory were likely to yield some quick results, many molecular biologists toyed with the idea of changing to the new field, but as the complexity of the problems emerged all but a few lost their enthusiasm. Benzer, famous for his work on T phage, was one of those who determined to make the change. He chose to work with Drosophila; a shrewd choice for anyone trying to define the structures and events which underlie behaviour, for Drosophila, as well as

eye of the horseshoe crab Limulus, chosen for what seems to be the simplicity of its eye. He was, for example, the first to demonstrate that it is possible to make electrical recordings from single nerve cells. The study of Limulus eyes has provided all kinds of object lessons for the understanding of more complicated systems. Hartline and his collaborators have been concerned above all to use knowledge gleaned about the eye of Limulus as a means of constructing a mathematical representation for the functioning of an intact retina. Evidently this has important links with the way in which physiologists of all kinds are now preoccupied with understanding the ways in which living systems process information.

Professor Granit was awarded the prize in recognition of his work on the vertebrate eye, in the course of which he demonstrated inhibition in the retina, and revealed the principles of colour vision. Born in Finland, he graduated from the Swedish Normalyceum in Helsinki. After holding a fellowship of the University of Pennsylvania and a chair at the University of Helsinki, he became professor in the Caroline Institute in Stockholm in 1940. He was appointed to his present position of director of the Nobel Institute for Neurophysiology in 1945. He was president of the Royal Swedish Academy of Science from 1963–5, and is a foreign member of both the Royal Society of London and the American Philosophical Society.

In 1954, seven years after the publication of his first major work, Sensory Mechanisms of the Retina, Professor Granit was invited, on the fiftieth anniversary of Sherrington's own lectures in the same series, to deliver the Silliman Lectures at Yale. These were published as Receptors and Sensory Perception. In more recent years, Professor Granit turned his attention to motor physiology; an account of some of his work in this field, which brought him the Jahre and St Vincent Prizes, can be had from his contribution to the report of the first Nobel Symposium on Muscle Afferents and Motor Control, which he also edited.

offering the advantages recognized by the classical geneticists, has a well mapped genome and well documented behavioural patterns which potentially can be analysed with mutants. Starting with an inbred strain, it should be possible to study the direct relationships between individual genes and the nervous system if mutants with single step changes in a behavioural pattern can be isolated. The difficulty, of course, is devising a selection procedure which isolates such mutants in a single generation rather than progress-

sively over several generations. Progressive selection yields recombinant strains with multiple gene changes so it is virtually impossible to determine the effect of any one particular mutation on the nervous system.

In Proc. US Nat. Acad. Sci. (58, 1112; 1967), Benzer now reports the isolation in one generation of two mutants with changed phototactic behaviour. He devised a most ingenious selection technique, analogous to the counter current procedure used to separate mixtures of molecules, to isolate the two mutants. In essence, this involves putting Drosophila at the bottom of a tube over which is inverted a second tube. After arousing the flies a light is shone at either end of the joined tubes and the flies move in response to it. After 1 min the tubes are separated and new tubes attached to each and the process repeated for fifteen cycles. In model experiments, mixtures of wild type and wing deficient mutant flies, which also have poor phototactic response, were separated in this way.

Benzer then mutagenized with ethyl methane sulphonate (EMS) a population of male flies-strain Canton, Ohio-Standard—which he found forms a homogeneous population with strong phototactic response and then mated them with virgin X chromosome attached females. He used this unusual female strain as a genetic trick to ensure that all the male progeny received their X chromosome from the mutagenized male parent, not, as is usual, from the female. The twenty-six male progeny of this mating which lacked phototactic response were isolated by the counter current procedure. After a second mating of these males individually with virgin X attached females to distinguish between true behavioural mutants and flies which, although phenotypically non-phototactic, are genetically normal, he was left with two sex-linked mutants which had lost phototactic behaviour in one generation. Furthermore, the site of these two mutations must differ, for one mutant has abnormal wing veins whereas the other appears normal.

Phototaxis is obviously a complex behavioural pattern involving reception of stimuli, transmission and integration of nervous signals and the generation of a motor signal telling the fly to move towards light. A mutation altering any of these elements would alter phototaxis, but by isolating many different mutants it should be possible to analyse the wiring diagram of the nerve circuits and the biochemistry of the impulse transmissions involved in phototaxis. Moreover, the counter current selection method can obviously be adapted for such stimuli as gravity, odour and sound and, by using a two stage procedure, any two factors can be selected for independently. Although this remarkable work is only a beginning, it provides a method for the isolation of many behavioural mutants and ultimately the genetic analysis of behaviour.

Family of Proteins

from our Molecular Biology Correspondent

CERTAIN families of proteins—particularly the haemoglobins, the cytochrome cs and the lysozymes—have been the target of biochemical attacks in depth on broad fronts. Large numbers of these proteins have been sequenced, and it has been possible to draw broad conclusions about the number of positions in the chain which must remain invariant and the nature of the substitutions permissible elsewhere, and here and there some information has accrued on the effects of particular substitutions on aspects of the activity and structure

Sequence data on a considerable number of lysozymes are available, thanks mainly to Jollès and his collaborators, and his latest review (Bull. Soc. Chim. Biol., 49, 1001; 1967) draws together the large number of facts which have been uncovered. The vertebrate lysozymes are basic proteins, which are substantially similar in amino-acid composition, but show considerable differences in their sequences. It is possible to make some assertions at least about residues not specifically involved in the activity or in maintaining the conformation. Thus, one lysozyme (duck egg) has been described which is devoid of histidine. This appears to put paid to schemes involving histidine in the enzymic mechanism. The residues near the N-terminal end of the chain are also not involved, since two or three of these can be removed with an aminopeptidase without detriment to the function. The X-ray structure of hen's egg lysozyme indicates that three of the six tryptophan residues are clustered around the active centre; Jollès reports that two tryptophans, including one of the above three, try-108, can be chemically destroyed without observable diminution of activity. Even the number of disulphide bonds is variable. In hen's egg lysozyme, which by virtue of the X-ray structure is implicitly taken as the norm, there are four, in human lysozymes (milk and tears) three and in goose egg lysozyme two. This is also the order of thermal stability of the enzymes, the last, which also has a low tryptophan content, being very unstable. The structural lability is correlated with high enzymic activity, which one would like to believe reflects a readily responsive conformation (in terms, for example, of the induced-fit concept). A bacteriophage lysozyme, sequenced last year by Inouye and Tsugita, has the same substrate specificity as the mammalian lysozymes. but has very different sequence and composition, and may well be unrelated.

The most unexpected observation comes now from Brew, Vanaman and Hill (J. Biol. Chem., 242, 3747; 1967), who have determined the sequence of the milk protein α-lactalbumin. This is a component of the lactose synthetase system, and catalyses the formation of the same glucopyranosyl linkage which is broken by the lysozymes. It is astonishing to find an extensive and unmistakable sequence homology with hen's egg lysozyme. Forty of the 123 residues of α-lactalbumin are identical with those in the corresponding positions in lysozyme. A further 27 residues are of similar type ("conservative" substitutions). In both proteins there are four disulphide links; two of these only have so far been determined in lactalbumin, and these are identical to their counterparts in lysozyme. To achieve the best homology, two double-residue and four single-residue gaps must be allowed in the lactalbumin sequence, and two double-residue gaps in lysozyme. A remarkable point of difference is that, despite the homologies, α-lactalbumin is an acidic protein, whereas lysozyme is basic (the isoelectric points are pH 5 and 10.5). It seems then that the two proteins derive from a common precursor, and that a duplication of genes occurred at some stage, which was followed by the separate evolution of α-lactalbumin in the mammals.

Hybrid Cells and Gene Location

from a Correspondent

When animal cells from two different species are mixed and grown in culture, a few hybrid cells are formed. By using parent cells with appropriate genetic markers, the hybrids can be selected for further study. These newly formed hybrids have a chromosome complement derived from both parents. The hybrids are not just bizarre novelties—one of their uses, as Weiss and Green show (*Proc. US Nat. Acad. Sci.*, **58**, 1104; 1967), can be to locate genes to particular chromosomes. These authors used as parents cells of mice deficient in thymidine kinase and resistant to 5-bromodeoxyuridine (BUdR) and human cells self-sufficient for the kinase. The hybrids were selected and they too are now self-sufficient for thymidine kinase because of the presence of human kinase genes. After about 20 generations, the hybrids still have all their mouse chromosomes, but have only 2-15 of the original 48 human chromosomes. If gene markers are also lost, can these be assigned to the lost chromosomes? Addition of BUdR kills these hybrid cells which are self-sufficient for thymidine kinase, while cells lacking the kinase survive. Chromosomal analysis of the hybrids has shown that human chromosomes of the group 6-12-X were rare in the BUdR-selected hybrids, but more frequent in cells susceptible to BUdR. Gene mutation at the kinase locus was ruled out and so the human gene(s) controlling thymidine kinase was concluded to lie on a 6-12-X group chromosome.

Analysis of cell surface antigens, cell morphology and chromosome complement of the hybrids has indicated that genes for surface antigens are widely distributed throughout the human genome, but genes for cell morphology are less widely so. Scaletta et al. (Genetics, 57, 107; 1967), using hamster-mouse hybrids, show that a similar approach to gene mapping is feasible in other species. But in this hybrid the chromosomal changes, especially in the mouse genome, make it difficult to locate genes to chromosomes. This hybrid contains an enzyme (glucuronidase) of hybrid structure, so enzyme sub-units can be used as another genetic marker. Other hybrid enzymes have been found by Weiss and Ephrussi in rat × mouse hybrid cells.

An important feature of the mouse-human hybrid is the preferential loss of human chromosomes. The human cells were of a cell strain where cell death occurs naturally after about 50 generations, while the mouse cells were of an established cell line and are potentially capable of surviving independently. Although the hybrid seems to have cell line properties, the human complement has not had immortality conferred on it by its association with the mouse environment.

To extend work on mammalian genetics, many more marker genes are needed; also mutant cells must then be confidently isolated. Puck and Kao (Proc. US Nat. Acad. Sci., 58, 1227; 1967) show how the latter requirement can be very simply achieved with nutritional mutants. They use a technique by which the wild type cells are selectively killed off, leaving the mutants. But chromosomes must also be more specifically identified than is possible at present, especially in man and mouse. Moreover, if gene location is to be possible it will be necessary to recognize structural genes from their operators and regulators, and indeed to show that these latter exist in mammalian cells.

Viral Nomenclature

The latest edition of *Progress in Medical Virology* (9, 476) describes how the International Committee on the Nomenclature of Viruses was set up. Until recently the nomenclature of viruses has been governed by a subcommittee of the International Committee on Bacteriological Nomenclature. This unsatisfactory state of affairs was brought to an end by the voluntary dissolution of the subcommittee and its replacement by a Provisional Committee on Nomenclature of Viruses which has now become the permanent ICNV responsible to the International Association of Microbiological Societies.

The committee was established by inviting each national microbiological society to nominate up to five delegates, who met for the first time in Moscow in July 1966. Under its president, Professor P. Wildy, the ICNV agreed to examine all true viruses, to define evident groups at the generic level and to suggest names for them. Four working subcommittees were established to deal with viruses of vertebrates, invertebrates, bacteria and plants; a fifth is concerned with the general utility and practicability of cryptograms in virology.

There is considerable disagreement within the ICNV; some favour an attempt at phytogenetic classification—despite the inevitable mistakes—and a binomial nomenclature, while others believe that the extent of knowledge about viruses is too small for this to do anything more than fossilize the mistakes. The subcommittees are also beset with difficulties of language—English must be used because Anglo-Saxons and Americans cannot express themselves in anything else, but this poses problems for abbreviation of names and their transliteration into Cyrillic and other scripts.

Despite difficulties and disagreements the subcommittees are working towards a tentative binomial nomenclature. They are studying criteria and suggesting definitions for species genera and subgenera. They are to select a type species for each genus and subgenus and to consider generic names. The cryptogram is also likely to be tried experimentally and the members of the subcommittee considering this are representatives of different branches of virology.

The ICNV has produced twelve rules so far. The code of bacterial nomenclature shall not be applied to viruses and the new code shall be international and universally applied to all viruses. An effort will be made to produce a latinized nomenclature, and where possible existing latinized names shall be retained. There will be no law of priority, no sigla shall be introduced, nor shall person's names or nonsense names. For pragmatic purposes the species is considered to be collections of viruses with like characters, and the genus is a group of species sharing certain common characters. Some orthographic rules have been approved. The ICNV is continuing its work towards a viral nomenclature.

Satellite Geodesy in Europe

from a Correspondent

For more than a year, laboratories in fourteen countries in western Europe have been collaborating in a programme of simultaneous observation of the Echo 1

and 2 satellites. About twenty observing stations scattered throughout the member countries are regularly making photographic observations of the two satellites so that knowledge of the size and shape of Europe may be improved. The camera stations photograph one of the satellites against the star background and, when this is achieved simultaneously at two or more stations, the rays to the satellite from the cameras form the sides of a triangle or a polyhedron from which it is possible to deduce something about the relative positions of the stations. For example, at about 22.25 U.T. on April 16 this year, the cameras situated at Graz in Austria, Meudon and Strasbourg in France, Frankfurt and Munich in western Germany, San Fernando in Spain and Malvern in England all photographed the Echo 1 satellite. Observations such as this establish strong geodetic ties between the respective camera stations. The observations need to be extremely accurate, about a millisecond in time and a few seconds of arc in position, if any improvement is to be made on the existing knowledge of the shape of Europe.

Photographic observations of satellites can only provide an angular measurement, and, in order to determine the distances between the stations, a scale must be introduced into the system. This can be done by assuming that the distance between two stations of the network is known or by measuring the distance of the satellite directly. One method of ranging a satellite is by the use of a pulsed laser and work in this field is being actively pursued in the United States and France. At a conference on Satellite Geodesy in Paris in May and at the recent COSPAR meeting in London, American and French workers estimated that accuracies of a few metres in the measurement of range are now possible by the use of lasers, although this is, of course, restricted to satellites which carry specially constructed corner reflectors. Eventually, an accuracy of about 10 metres will be possible for the position of each of the camera sites. Because each site can be carefully placed within a national geodetic datum, the relative positions of any two places in Europe should be determined with comparable precision.

This programme of observations of the Echo satellites, organized by the Western European Sub-Commission of the International Commission for Artificial Satellites of the International Association of Geodesy, is not the only one which has been carried out recently in Europe. The United States Coast and Geodetic Survey has used portable BC4 cameras to establish sites at several points in Europe, including Edinburgh in Scotland and Munich in western Germany. These sites are occupied for a few weeks while observations are being made and the cameras then move to another This European programme is part of a location. larger world wide triangulation programme of the United States Coast and Geodetic Survey. In addition, the Smithsonian Astrophysical Observatory has camera stations at San Fernando and Athens which are actively participating in a geodetic programme. The National Aeronautics and Space Administration is also undertaking geodetic studies which include observations from Europe. The Geos A flashing light satellite has also been observed on several occasions from European camera stations, particularly Zimmerwald in Switzerland and Malvern in England. In general, satellite geodesy in Europe seems to be growing well on the observational side; the great need now is for work on the analysis of observations so as to make more widely available the results of what has been done.

Meteor Showers Ahead

from a Correspondent

THE two important meteor showers of November and December—the Leonids and the Geminids—are markedly dissimilar in character. The Geminid shower provides one of the most reliable meteor displays, with only statistical variations in the rate from year to year, but the Leonids are typical of another type of stream in which remarkable showers occur periodically, indicating an intense localized swarm of particles in the orbit.

Some Geminid meteors can usually be seen between December 7 and 15, but the maximum occurs on December 13 or 14, when an observer may see as many as fifty meteors an hour with the naked eye if the sky conditions are good. Thus the Geminids are one of the richest showers of the year. Oddly enough, however, there are no records of a shower on these dates in antiquity, and the shower does not seem to have appeared with sufficient strength to be recognized until 1862.

Its origin is an enigma. The formation of meteor streams is now generally supposed to be a result of the disintegration of comets as they swing around the Sun. In favour of this view, several meteor streams are known to move in the same orbits as certain comets —in fact, one of the first cometary associations to be demonstrated was that of the Leonids and Tempel's comet. The Geminids have never been satisfactorily accounted for by this hypothesis. Their orbit is peculiarly small, smaller than that of any known meteor stream or comet and of most asteroids. Geminid meteors are also unique in having densities two to three times greater than the average density of meteoroids. The explanation of these abnormalities by an old parent comet in which the meteoric dust is likely to be more compact, coupled with perturbations of the orbit by close approaches to the planets, cannot be ruled out, but the exact mechanism remains a mystery.

Interest in the Leonids, which flagged somewhat as a result of the disappointing maxima of 1899 and 1932, has revived after the historic display over North America in 1966. Last year the Soviet astronomer I. S. Astapovich predicted that the maximum of the 1966 Leonid shower would occur on November 17 at 10.00 U.T.—a forecast which was very nearly correct. Observing stations in northern Siberia, the only region of the Soviet Union where the radiant was above the horizon during darkness, recorded maximum activity between 11.50 and 12.30 U.T. on November 17, and a zenithal hourly rate at peak activity of 130,000, in close agreement with observations in the United States. This year, Russian astronomers are expecting the maximum to occur on November 17 at 17.00 U.T. No predictions are being made of the strength of the shower, and perhaps astronomers are remembering that the failure of the shower to return as predicted in 1899 was, in the words of the American meteor physicist C. P. Olivier, "the worst blow ever suffered by astronomy in the eyes of the public".

Bacterial Inhibitors in Milk and other Biological Fluids

by B. REITER J. D. ORAM

In common with other body fluids, milk contains several bactericidal and bacteriostatic factors.

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THE bactericidal and bacteriostatic properties of milk were first described at about the same time as those of blood, but naturally have not attracted the same attention. Most of the relevant literature consists of observations that various pathogenic and saprophytic bacteria are killed or their growth temporarily inhibited by cow's milk¹-8. It has become increasingly evident that milk contains several inhibitors ("lactenins") which differ in their antibacterial specificities and, moreover, are not confined to milk.

In this article some of the inhibitory systems in milk are considered in relation to those present in other biological fluids.

Lactoperoxidase/Thiocyanate/Peroxide

In 1924 Hanssen¹ suggested that the bactericidal properties of milk against Salmonella typhosa and S. paratyphosa are the result of its peroxidative activity. A close correlation between the inhibition of group N streptococci and the progressive heat inactivation of lactoperoxidase (LP) (ref. 5) indicated that the lactenins active against various streptococci (group A, F, G, H, K, L and N) are associated with lactoperoxidase 6-9. It is now well established that streptococci and other bacterial species are inhibited only in the presence of thiocyanate, indican or iodine 9-14, which are found in milk and other biological fluids. The concentration of NaSCN required for inhibition is only 0.2 μg/ml. in the presence of lactoperoxidase, but about 100-200 μg/ml. in its absence 9,10. Lactoperoxidase in the presence of SCN- and H₂O₂ completely inhibits hexokinase and partially inhibits other glycolytic enzymes of resting streptococci. Moreover, strains of streptococci which resist this inhibition possess an enzyme which catalyses the reduction of the intermediate oxidation products of SCN- by NADH, or NADPH. The inhibitor in milk is very unstable and has not been isolated, but it resembles sulphur dicyanide, a minor product in the non-enzymatic oxidation of SCN-by H₂O₂ (ref. 15); both inhibit hexokinase but not phosphohexokinase and both are reduced in the presence of NADH₂ or NADPH₂, by extracts of LP-resistant but not LP-sensitive strains. These results led us to suggest that the inhibitory product formed in milk during the oxidation of thiocyanate is either S(CN)2 or a substance similar to it11.

Xanthine Oxidase

Green and Pauli¹⁶ demonstrated that a variety of pathogenic Gram-positive and Gram-negative bacteria were inhibited by $\mathrm{H}_2\mathrm{O}_2$ produced by xanthine oxidase in media containing xanthine. But because milk contains only low concentrations of the substrates for xanthine oxidase it is unlikely that bactericidal concentrations of $\mathrm{H}_2\mathrm{O}_2$ are formed. The small amounts of peroxide produced either by xanthine oxidase or by the non-enzymatic oxidation of the ascorbic acid in milk, however, could cause the inhibition of organisms which do not normally produce $\mathrm{H}_2\mathrm{O}_2$ or are not susceptible to inhibition by peroxide itself if the peroxide is utilized in the lactoperoxidase-catalysed oxidation of thiocyanate.

Lactotransferrin

Milk contains an inhibitor against B. subtilis and B. stearothermophilus which is very heat stable (90° C for

60 min) but is inactivated by trypsin, FeSO₄ and less effectively by MgSO₄ (refs. 17 and 18). This inhibitor has been identified as the red iron-binding protein, lactotransferrin¹⁹. During the main period of lactation cow's milk contains only low concentrations of lactotransferrin, but the concentration gradually increases during the drying-off period (Table 1). An inhibitor fraction purified from the dry period secretion by filtration through 'Sephadex G-200', followed by zone electrophoresis on a block of 'Pevikon G870', was found to have two components in the ultracentrifuge with S_{20} values of about 12Sand 20S. Immunoelectrophoresis revealed that these fractions contained lactotransferrin and casein. Purified lactotransferrin inhibits the growth of B. stearothermophilus and B. subtilis and its inhibitory activity is suppressed by ferrous ions. When not fully saturated with iron, lactotransferrin (but not serum transferrin) has no effect on the activation of spores of B. stearothermophilus but inhibits their germination.

Table 1. INHIBITION OF Bacillus stearothermophilus by the secretion of NON-LACTATING UDDERS

	Number of cows	Titre*
Milk before drying off	2	1:20
	2	1:80
	3	1:160
Secretion 14 days after drying off	1	1:80
• • •	3	1:160
,	3	1:320

*Inhibition expressed in units/the reciprocal of the highest dilution of 0.25 ml. of material which gives a visible zone of inhibition when placed in a well in agar containing a standard inoculum of heat shocked spores of Bacillus stearothermophilus.

Complements and Antibodies

Because sheep erythrocytes are not haemolysed by raw milk it is generally assumed that bovine milk does not contain complement. As early as 1907, however, it was demonstrated 20 that while sheep erythrocytes are not suitable for the detection of bovine complements guineapig erythrocytes are readily haemolysed by raw milk. Using a conglutinin test (suggested by Dr P. J. Lachmann, Department of Pathology, University of Cambridge) we frequently detected complement in bulk milk, but not in all samples of milk from individual cows. It was regularly present towards the end of the lactation period, in the secretion of the non-lactating udder, in the colostrum and in the milk for some weeks afterwards (Table 2).

Milk does not normally contain high titres of antibodies, but it often contains low titres of agglutinins-natural antibodies—against lactic acid bacteria^{5,8,21}. Boyden²² suggested that natural antibodies need not necessarily arise in response to a specific infection but may be induced by contact with "antigenic determinant groups, particularly those occurring on polysaccharides shared among many organisms as well as animal and plant tissues" In ruminants, where non-specific antigens could be derived from plant tissue and rumen micro-organisms, conditions may be particularly favourable for the formation of such natural antibodies. It is now evident that in many instances the bactericidal activity of raw milk against Gram-negative organisms3 may be attributed to the killing effect of complement and antibodies. Raw milk also reduces the count of Gram-positive organisms through agglutination or increase of the length of the streptococcal chains by an end-to-end agglutination3,5,23

Table 2. COMPLEMENT TITRES IN BLOOD SERUM, MILK AND SECRETION OF NON-LAGFATING QUARTERS

	Number of cows	Titres
Blood serum	6 6	$1:16 \\ 1:32$
	Number of quarters	Titres
Milk before drying off	16 6 2	<1:2 1:2 1:4
	Number of quarters	Titres
Secretion after drying off (14 days	2 9	<1:2 1:2 1:4
	10	1:8

Although the occurrence of immune globulins in colostrum and in the secretion of the non-lactating udder is well authenticated, little attention has been paid to their specificity against udder pathogens. We examined the levels of agglutinins and complements in blood sera, milk and dry udder secretion of fifty cows over a period of 2 yr. Although Staphylococcus aureus and Streptococcus uberis were the only endemic organisms in this herd, agglutinins were also detected against Streptococcus pyogenes, Streptococcus dysgalactiae, Streptococcus agalactiae, Escherichia coli and Corynebacterium ulcerans at titres up to 1:640 in the blood, milk and secretion of different animals.

Leucocytes and Mastitis

The number of leucocytes in milk is important in the defence against mastitis pathogens, but the experimental evidence on the relative importance of cellular and humoral factors in the prevention of udder infections is conflicting. We have challenged individual udder quarters containing various levels of leucocytes, agglutinins and complement with S. aureus. When ten colony forming staphylococci (S. aureus, strain m, phage type 6/7/54/75/77/52B) were placed beyond the teat canal into the teat cistern of normal udder quarters containing 104-105 leucocytes/ml. (a few per cent at the most being polymorphonuclear leucocytes (PMN)), the udder invariably became infected. When, however, leucocytosis was induced by infusing 50 ml. of 0.14 molar NaCl into each quarter before the introduction of the staphylococci the greatly increased number of leucocytes, particularly PMN, prevented infection (Table 3). Table 3 is representative of experiments performed with ten cows.

Dry period quarters containing 10⁵–10⁶ leucocytes/ml. (up to 25 per cent PMN) seemed to be more resistant to infection than lactating quarters. When six dry quarters were each infused with twenty staphylococci, only one quarter became infected; infusion with two thousand staphylococci resulted in the infection of thirteen out of eighteen dry quarters. Prior infusion of eleven quarters with saline increased the number of leucocytes to a maximum of 10⁸/ml. (up to 80 per cent PMN) and on subsequent inoculation with staphylococci only one quarter became infected. In two quarters which became infected after saline infusion the number of PMN failed to increase above 10⁶/ml.

For 2 years we attempted to correlate the presence of complements and/or antibodies with the resistance of the udder against infection with staphylococci or streptococci and came to the conclusion that high numbers of PMN prevented infection irrespective of the presence of complements or antibodies.

Comparison of Bacterial Inhibitors in Milk with those in some other Biological Fluids

There is now increasing evidence that two of the inhibitory systems in milk are also minor defence mechanisms in animals and man.

Thioeyanate is widely distributed in body fluids and, because the mammary gland, like the salivary gland, is of ectodermal origin, we suggested^{7,8,11} that the inhibitory system found in milk also operates in saliva; this has recently been confirmed^{12,13}. Besides streptococci and lactobacilli, *E. coli* and *S. aureus* are also inhibited in vitro by milk¹³ if an exogenous, non-microbial H₂O₂-generating system such as xanthine oxidase and xanthine is available. Because peroxidase and lysozyme are both present in the mammary, salivary, Harderian and lacrimal glands—all of ectodermal origin—they may, under aerobic conditions, control the bacterial floras of the nasal, oral and ocular areas²⁴.

Thiocyanate is also oxidized by myeloperoxidase²³ and because the rate of H₂O₂ formation by PMN is greatly enhanced during phagocytosis we suggested that this could lead to the formation of antibacterial substances¹¹; this seems to have been recently confirmed²³, although the authors interpret their results differently. Thus the LP/SCN-/H₂O₂ inhibitory system may act not only in milk and saliva but also in phagocytes, and may explain why complements and agglutinins have not been demonstrated to effect the elimination of staphylococci by PMN in the udder.

In addition to its antibacterial activity, the LP/SCN-/ H_2O_2 system also affects bovine spermatozoa. Unheated milk was once used as a diluent for bull semen and its pronounced spermicidal effect has been ascribed to "lactenin". Because the spermicidal and bactericidal properties of milk are similarly affected by heat and reducing agents, we examined the effects of LP and SCN-on bovine spermatozoa and found that their penetration into cervical mucus was impaired by the addition of peroxidase and thiocyanate²⁸. These observations may help to explain the widespread but unconfirmed belief that feeding cattle with kale (an excellent source of glucobrassicin, a precursor of thiocyanate²⁹) affects the conception rate in cows³⁰.

An antibacterial action of lactotransferrin similar to that in milk has also been demonstrated in saliva, bronchial and nasal secretions, tears, hepatic bile, pancreatic cyst fluid, seminal fluid, cervical mucus and urine in man. With fluorescent-labelled antibodies, lactotransferrin was detected in the glandular acini but not in the epithelial lining of the bronchial wall and submaxillary glands; it has also been demonstrated in the lumen of the galactophorus ducts of a human non-lactating mammary gland^{31,32} and may protect the mucosa against bacterial invasion.

Since the original report³³ that the iron-binding protein of human blood sera inhibits Salmonella dysenteriae the bacteriostatic activity of transferrin has been repeatedly confirmed. The lack of activity of bovine serum transferrin against B. stearothermophilus is surprising and as yet unexplained. More work is needed to determine the reason for the differences between the effects of transferrin and lactotransferrin on bacteria. It is of considerable interest, however, that Rogers³⁴ recently found that

Table 3. Effect of induced leucocytosis in the infection of single lactating quarters with low numbers of Staphylococci

Cow 297	Leuco- cytes/ml.	RF* % PMN	Staph./ml.	Leuco- cytes/ml.	RH % PMN	Staph./ml.	Leuco- cytes/ml.	LF* % PMN	Staph./ml.	Leuco- cytes/ml.	LH % PMN	Staph./ml.
Before saline infusion After saline infusion Days after infection:		0 53	_	2×10^{5} $2 \cdot 6 \times 10^{5}$	4 0	*******	2.5×10^{5} 8×10^{6}	6 48	_	7 × 10 ⁴ 6 × 10 ⁴	0	_
1 2 5	3×10^{5} 4×10^{5} 2×10^{6}	24 10 0	0 0 0	1.5×10^{5} 4×10^{6} 4×10^{6}	0 62 63	1×10^{2} 6×10^{4} 7×10^{2}	3×10^{6} 4×10^{6} 1×10^{6}	47 26 44	0 0 0	1 × 10 ⁴ 4 × 10 ⁷ 4 × 10 ⁶	0 61 57	2×10^{1} 1×10^{6} 1×10^{4}

^{*}Infused with 50 ml, of physiological saline.

Inoculum Staphylococcus aureus m phage type 6/7/54/75/77/52B, 10 colony forming units/quarter.

RF, right fore; RH, right hind; LF, left fore; LH, left hind.

Clostridium welchii type A is inhibited by unsaturated serum transferrin in the presence of $\beta 2$ or $\gamma\text{-globulin}$ or a mixture of both; these findings should help to clarify the nature of the various blood sera inhibitors.

We thank Dr G. C. Cheeseman for the determination of the sedimentation constant of the lactotransferrin.

Note added in proof. It has been found that the infusion of saline into udders produces leucocytosis because of the presence of pyrogens. Infusion of pyrogen-free water fails to induce leucocytosis and less than 1 µg of purified lipopolysaccharide (E. coli 055: B5, Difco) fails to produce leucocytosis in 4-5 h.

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Linked Groups of Residues in Immunoglobulin K Chains

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Crossing over theories have been advanced to explain antibody variability, and the simplest involves recombination between the elements of an "antibody gene pair" containing two versions of the variable section of the molecule. Data now seem to require at least three versions of the same section.

It is now generally accepted that the remarkable degree of variation in the primary sequence of the immunoglobulin chains is related to combining specificity, especially because the successful refolding of antibody molecules in the absence of antigen has provided strong evidence that such specificity is dependent on covalent structure. The present understanding of the heterogeneity of the peptide chains has been derived mainly from studies of the monoclonal immunoglobulins produced in association with myelomatosis of men and mice. Thus it seems that immunoglobulin light chains are made up of a C-terminal section, with a sequence that is characteristic of the chain type and which is common to many different clones, and an N-terminal section specific to the clone of origin. Specific antibody activity is seldom associated with specific chain types, and so antibody specificity should derive primarily from the alternative amino-acid sequences of the N-terminal sections. The variability of the Nterminal half of light chains seems thus to be of fundamental importance to the understanding of the mechanism of antibody specificity.

Of the 107 residues (reviewed in ref. 1) of the N-terminal section of human x chains, forty-two have been found to be identical in the individual proteins studied. Other residues, however, change only in isolated cases, such as residue No. 2 which was substituted in one out of seventeen proteins studied. In these cases the substitutions involve homologous residues (Ser/Thr; Val/Ile, and so on). Thus about half of the residues in the N-terminal section vary only in isolated cases or not at all.

Of the others there are many positions which contain one or two alternative residues. Occasionally a third residue is also present in these positions. In several instances these two alternatives involve homologous residues (Asp/Glu; Val/Leu; Thr/Ser), but in others these are certainly non-homologous (that is, Arg/Pro at position 18; Gln/Arg at position 24).

There are, on the other hand, positions that are extremely variable, and the best examples are found in two clusters. The first occurs around residue 30/32 where several extra residues have been observed in some proteins^{2,3} and the second is in residues 92, 93, 94 and 96 (ref. 4), where both homologous and non-homologous variants occur. Other clusters may still appear in sections of the molecule less extensively investigated.

The presence of positions that contain chiefly two alternatives is particularly interesting. Two alternatives are commonly found in certain positions of many proteins

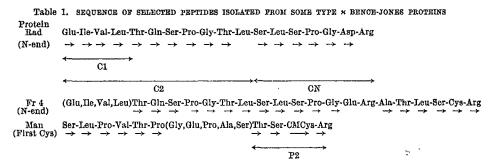


Table 2. RESIDUES 1-24 IN SEVERAL × CHAINS

Residue No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Ref.
Basic sequence I		Asp-	Ile-	Gln-	-Met-	Thr-	Gln	-Ser	-Pr	o-Sei	- Ser	-Leu	-Ser-	Ala-	Ser-	Val-	Gly-	Asp-	Arg-	Val-	Thr	-Ile-	Thr-	Jys-	Gln	
Basic sequence II		Asp	Ile-	Val-	Leu-	Thr-	Gln-	Ser	Pro	o-Leu	-Ser-	Leu-	Pro-	Val-	Thr-	Pro	Gly	Glu-	Pro-	Ala-	Ser-	Ile-	Ser-C)ys	Arg	
Basic sequence III		Glu-	Ile-	Val-	Leu-	Thr-	Gln	Ser	Pro	o-Gly	-Thr	-Leu	-Ser-	Leu	Ser-	Pro	Gly-	Glu-	Arg-	Ala-	Thr-	-Leu	-Ser-	Cys-	Arg	1
Protein									I	Basic	seque	nce	I													0
Roy Ag Ker BJ			Vəl																	Ile						5 4 4
BJ10 BJ6 BJ1 BJ4		_		Leu						- Thr																3 3 3
Day									Ι	Basic	sedne 	nce :	II						-							
BJ3 Cu Man	Glu	_			-I			hr-																		-3 2
Rad									Ва	sic s	equen	ce II	Ι					r –						-		4
BJ5 HS4		_								Asx																3

Residues identical in all proteins have been omitted. Variants of basic sequence are given as breaks of solid line; Roman numbers indicate that the variant is the same residue of the corresponding basic sequence. All variants can be one step mutations from basic sequence.

resulting from allelic genes, and the first question to answer is if these two alternatives are allelic forms of the same structural gene. A typical case is the N-terminal residue. Both Asp and Glu are found in that position, Asp being present in about 60–64 per cent of the cases. Because λ chains with N-terminal Asp or Glu have not been reported, the N-termini of normal light chains should represent the N-termini of their \varkappa chains. A search of light chains of twelve normal individuals showed that all of them contained both Asp and Glu as N-termini, suggesting that these do not behave like allelic markers (Feinstein and Milstein, unpublished).

The second question to ask is whether there is linkage between the individual residues in the positions along the chain which have a choice of two (or mainly two) variants. I have selected stretches at both ends of the N-terminal half of x chains to do further studies of the nature of the variations. New peptides, related to residues 1-24, are shown in Table 1. When these results are added to other known sequences (Table 2) of the N-terminal quarter of the x chains variable stretch, strong linkage seems to be quite apparent, especially if one assumes three linkage groups. The first twenty-four residues in all the known proteins fall into one of the three basic sequences, with the exceptions shown in Table 2. Smithies has recently proposed that only two linkage groups appear to be involved, and so I would like to discuss the reasons for proposing at least three linkage groups. The combination Glu...Thr...Leu is present in the proteins that are

assumed to belong to the third linkage group, and none of these residues occurs in proteins defined as of groups I or II. Furthermore, the Leu of Rad in position 21 is also found in protein Fr 4 but in none of the proteins listed as from the basic sequence I or II. For one group of proteins to originate by crossing over of the other two,

several crossings over in identical places plus identical point mutations should have occurred, while the general patterns of the proteins of a group show very isolated examples of crossing over. If three basic sequences are assumed only two residues (No. 4 of protein Cu and 17 of Rad) could originate from crossing over (Table 2), while the other six variants appear as isolated point mutants. Another peptide, related to the stretch of residues 62–77, does also fit in the three basic sequences pattern (Table 3).

I have isolated in several proteins a peptide sometimes referred to as "switch peptide", which appears to belong to residues 104 to 107. This is of interest to provide information of the variability at the other end of the N-terminal half. Here also either of two residues occurs in positions 104 and 105, but in this part of the molecule no linkage is disclosed between these two (Table 4). It is as if, unlike the N-terminal section, frequent crossing over gave rise to these random combinations.

The occurrence of residues that do not fit in a crossing over pattern between two genes is too frequent to be overlooked. Such residues are often non-homologous and are scattered along the chain, although some are clustered after residue 90 (Table 4). The occurrence of some of the variants between residues 91 and 96 (Table 4) is difficult to interpret, unless one assumes that more than three sequences cross over or that they arose by some mutational event either in the germ line or as a result of mistakes during transcription or translation. It is interesting nevertheless that, for example, all four proteins listed as of sequence I contain Leu in position 94, unlike the examples of sequences II and III. I am not considering here the large differences after position 30 because the occurrence of a deletion (or addition) makes that part of the sequence more difficult to interpret with the very scanty amount of data known.

Table 3. SEQU	ENCE OF A PEPTIDE (RESIDUES 62-77 OF PROTEINS ROY AND AG
Basic sequence I	T Phe-Thr-T 7 ?
Basic sequence II	Phe-Ser-Gly-Ser-Gly-Ser-Gly-Thr-Asp-Phe-Thr Leu-Thr Leu-Thr Leu-Thr Leu-Thr
Basic sequence III	L J _{-Leu-Thr-L} J _{-Arg}
	Basic sequence I
Roy	——Thr —————Ser
Ag	
	Basic sequence II
BJ3	
Cu	A CONTRACTOR OF THE PROPERTY O
	Basic sequence III
Rad	Glu-
Fr 4	- Address - Addr

Only residues that differ are displayed. The results of Fr 4 are based on partial sequence of the peptide.

Table 4. RESIDUES 91-96 AND "SWITCH PEPTIDE" (104-107) IN SEVERAL

Protein	Inv	Basic sequence (Table 2)	Residues 91–96	"Switch peptide" (104-107)
		I I I II III III as Table 2. f basic seq		Val-Asp-Phe-Lys Leu-Glu-Ile-Lys Val-Asp-Leu-Lys Leu-Glu-Ile-Lys Leu-Asp-Ile-Lys Val-Glu-Ile-Lys Leu-Glu-Ile-Arg Val-Glu-Ile-Lys Leu-Asp-Ile-Lys Leu-Glu-Ile-Lys Leu-Glu-Ile-Lys
	5 0		MVMVV 48	

In λ chains there are not many well defined positions offering a choice between mainly two residues (reviewed in refs. 1 and 6); the best one is at position 10. Unlike the comparable stretch of x chains, no significant linkage group can be proposed. The four known Ala at position 2 present with Ser at position 13 are so far the strongest example of linkage. In other words, the situation here resembles the randomness described in the switch peptide of x chains (Table 4). Finally, I would like to emphasize that, if one assumes theories involving crossing over between two strands, the number of variants that remain unexplained in both types of chain is in fact larger than the number explained.

For reasons put forward previously1,3, it is unlikely that the C-terminal half of the x chain is coded for by more than a single gene. Three or more structural genes seem to code for the N-terminal half, and so it seems necessary to assume some mechanism of chromosomal rearrangement or insertion involving "half genes". Unless, however, a very large number of genes is proposed in the germ line3, an added mechanism of somatic hypermutation should operate on the variable section of the gene.

In this article I have used data obtained from manuscripts in press, for which I thank Dr Hilschmann and Dr Hood. I also thank my colleagues for helpful discussions, especially Drs Crick and Brenner.

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Structure at the Hinge Region in Rabbit Immunoglobulin-G

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The hinge peptide of rabbit IgG has an unusual amino-acid sequence. In containing three prolines in a row it is unique among known protein structures.

THE H chain of immunoglobulin-G (IgG) consists of two segments, the Fd and Fc fragments, coupled through a hinge region which is singularly exposed to enzyme attack. The Fd section has a variable amino-acid sequence and participates in antigen binding whereas the Fc section has a constant sequence and does not bind antigen.

Our experiments establish that there is a highly reactive disulphide bridge in the centre of the hinge region which seems to span the two H chains of the four chain IgG

molecule. We have identified the position of the reactive cystine and the amino-acid sequence of the surrounding area (Fig. 1). Galactosamine, a carbohydrate not previously reported in IgG, is present on 35 per cent of the H chains; it is located on a threonine residue immediately preceding the reactive disulphide bridge. The sites in IgG which are sensitive to attack by papain, and by pepsin, have been identified by examining the peptides liberated from the hinge region by the action of these

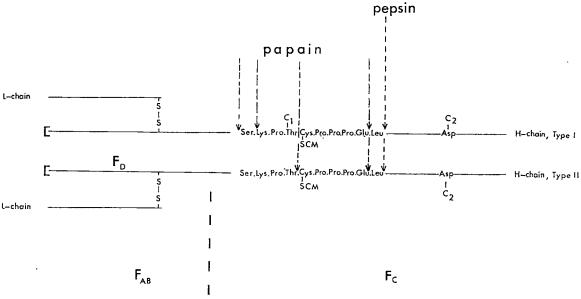


Fig. 1. Chain structure of rabbit IgG, showing detailed structure at the hinge region. The arrows indicate alternative sites of papain cleavage.

Molecules lacking the carbohydrate C₁ undergo additional cleavage at the NH₂ group of the adjacent cystine residue.

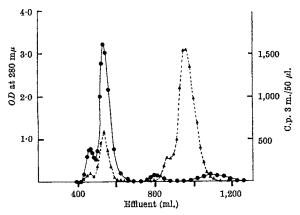


Fig. 2. Gel filtration of a papain digest of reduced carboxymethylated P(ab'), fragment of IgG. F(ab'), (250 mg) was reduced at pH 8·0, 30° C, for 6 h in 0·0014 molar mercaptoethanol, and then was incubated with 0·0022 molar ¹⁴C iodoacetic acid (0·7 $\mu c./\mu$ mole) at pH 8 for 1 h. After removal of the reagents by dialysis, digestion was carried out with crystalline papain at a ratio of enzyme: substrate of 1:100 in the presence of 0·0014 molar mercaptoethanol at pH 7·0, 3° C, for 24 h. The digest was applied to a column (170 × 3 cm) of 'Sephadex G-75' and eluted with 0·2 molar ammonium bicarbonate—ammonium acetate pH 8·7.

enzymes; the presence of the carbohydrate C₁ exerts a controlling effect on the sites of papain cleavage. The hinge peptide containing the reactive disulphide, Cys-Pro-Pro-Pro-Glu-Leu was located at the COOH terminus of the H chain in F(ab')₂. This peptide was extremely resistant to enzyme attack. The region flanking it, on the other hand, was cleaved by papain in a variable manner, perhaps reflecting heterogeneity of structure elsewhere in the molecule.

An immunoglobulin molecule radioactively labelled at the hinge region was obtained by reducing pooled rabbit IgG in mild conditions (0.0028 molar mercaptoethanol for 1 h at $p{\rm H}$ 8) and then blocking the released sulphydryl groups with $^{14}{\rm C}\text{-iodoacetate}$. These conditions were

designed to affect a single disulphide bridge1: approximately 80 per cent of the reduced alkylated IgG, dissociated into half molecules at pH 2.4 (refs. 2 and 3). expected cleavage of the labelled molecule by papain to leave the radioactivity either in the Fab fragment or in the Fc fragment, according to which side of the hinge region the enzyme attacked. When the molecule was digested with the papain, however, neither fragment was substantially labelled; most radioactivity appeared in a peptide fraction. Fab and Fc were recovered in the normal way, and so it was clear that one or more peptides containing labelled cysteine had been split out from the hinge region of IgG, following the action of papain. The peptide fraction, which contained 80 per cent of the initial protein bound radioactivity, was resolved by gel filtration on 'Sephadex G-25' into two principal components: fraction A with 16 per cent and fraction B with 60 per cent of the total radioactivity. Both fractions contained a mixture of peptides. When their structure had been elucidated, a composite sequence could be proposed for the hinge region.

From fraction B, ion exchange chromatography on 'Dowex 50-X2' provided one radioactive peptide and a variety of unlabelled peptides. The radioactive peptide was obtained in a pure state by further chromatography on 'Dowex 1-X2' and was isolated in two forms, one of which had a blocked NH₂-terminal residue:

The blocked end group was identified as a derivative of thiazane carboxylic acid, formed from the carboxymethylcysteine residue (CM-Cys) of peptide I during the isolation procedure. This terminal residue has not previously

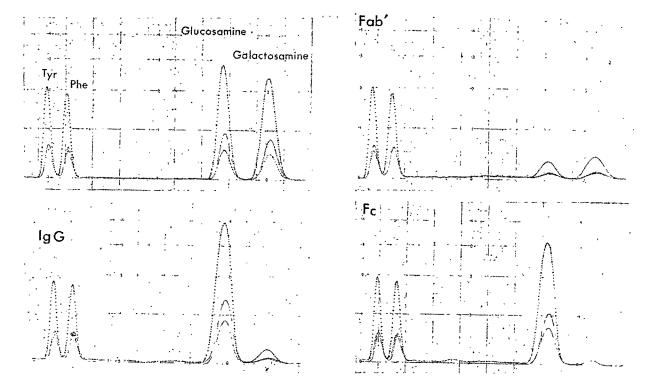


Fig. 3. Localization of glucosamine and galactosamine in rabbit IgG. Rabbit IgG, F(ab'), fragment, or Fc fragment was hydrolysed in 3 normal hydrochloric acid at 110° C for 12 h. After the hydrolysis, residual peptides and amino-acids were removed on a column (10×0.9 cm) of 'Dowex 2-X8' operated with 1 per cent N-ethylmorpholine acetate pH > 0 as eluent. (Details of the method will be published elsewhere.) Tyrosine and bhenylalanine (0.2 μ mole) were added as markers to the peptide-free hydrolysates. The hydrolysates were analysed at 50° C on a column ($bb \times 0.9$ cm) of IR-120 with 0.4 molar sodium citrate pH 5.28 as eluent. For comparison, the first chart shows an analysis of a synthetic mixture containing glucosamine and galactosamine (0.6 μ mole) together with tyrosine and phenylalanine (0.2 μ mole).

been encountered in analytical studies of proteins and peptides. In these experiments, ring closure of terminal CM-cysteine was found to take place in mild conditions, occurring even during chromatography at pH 3·2.

The cyclization reaction could be used to advantage in the purification of peptides that have CM-cysteine at the NH_2 -terminus, and it may account for the frequent difficulties in the analysis of CM-cysteine.

The hinge region represents the portion of the H chain that is present in the peptic fragment, $F(ab')_2$, but absent from the papain fragment Fab; it appears to be the only site of inter-H chain disulphide bridges. An attempt was made, therefore, to isolate the hinge peptide containing the labile disulphide from the 5S divalent fragment, $F(ab')_2$, and accordingly a similar procedure of mild reduction (0·0014 molar mercaptoethanol), alkylation, and digestion with papain was applied to the peptic fragment in place of whole IgG. With 1·4 groups of carboxymethylcysteine in 1 mole of $F(ab')_2$, 95 per cent of the reduced alkylated product was converted to 3S univalent components. After digestion with papain, gel filtration on 'Sephadex G-25' again revealed the two peptide fractions corresponding to those obtained before: fraction A with 35 per cent and fraction B with 50 per cent of the total protein bound radioactivity (Fig. 2).

The principal radioactive peptide in fraction B, however, was not the expected pentapeptide but an overlapping hexapeptide, Cys-Pro-Pro-Pro-Glu-Leu. Furthermore, carboxypeptidase A released leucine and no other amino-acid from the COOH-terminus not only of this peptide but also from $F(ab')_2$ itself. These results suggest some conclusions about the sites in whole IgG attacked by papain and by pepsin. One site of pepsin cleavage is clearly the carboxyl group of the leucine situated five residues from the reactive disulphide bridge: this leucine must form the COOH-terminus of the H chain in $F(ab')_2$. The sites of papain cleavage, on the other hand, can include the NH_2 group of the reactive cystine and the COOH group of glutamic acid situated four residues away (Fig. 1).

Fraction A, liberated by papain from reduced, carboxymethylated Fab', was found to contain a mixture of overlapping peptides:

in this fraction, and by the quantitative and concomitant disappearance of both threonine and galactosamine on mild alkaline hydrolysis^a.

The peptides of fraction A showed no further cleavage by the action of trypsin, papain, pepsin, pronase or subtilisin. In particular, extensive treatment of fraction A with papain failed to release the labelled peptide of fraction B. On the other hand, no amino-sugar was present in the unlabelled peptides isolated from fraction B, which were derived from the region on the NH2 side of Cys-Pro-Pro-Pro-Glu. The resistance of fraction A peptides to enzyme degradation was therefore attributed to the presence of the carbohydrate C₁ situated at a site that otherwise would be susceptible. Furthermore, quantitative analysis of IgG and F(ab')₂ showed that only 35 per cent of the H chains carry a galactosamine residue. Thus two classes of H chain are recognized: one with galactosamine next to the reactive disulphide bridge, the other devoid of galactosamine. These results provide a molecular basis for a recent observation that IgG appears to contain two species which differ in their sensitivity to papain digestion. The suggestion that carbohydrate might influence the pattern of enzyme attack has previously been used to account for a restricted cleavage of IgG, which occurs during limited digestion by papain^{8,8}.

Analyses of hexosamines in whole IgG, F(ab')2, and Fc isolated after a long period of digestion4 reveal at least two carbohydrate moieties (Fig. 3): galactosamine principally on one side of a disulphide bridge in the hinge region and glucosamine on the other, consistent with a previous view of two separate carbohydrate groups, C1 and C2, along the H chain 10,11. The mode of attachment of the galactosamine, coupled glycosidically to the OH group of threonine, contrasts with that of the glucosamine in Fc, which is linked through the β-COOH group of aspartic acid residue¹² (position 150 in ref. 13). The observation that differing sites and linkages are involved in the attachment of two carbohydrates to a single polypeptide chain is relevant to the problems of attachment of the carbohydrate during biosynthesis. That galactosamine is present on only 35 per cent of the H chains raises the interesting possibility that its attachment might be asymmetric in 70 per cent of the four-chain IgG molecules, the remaining 30 per cent being devoid of this carbohydrate side chain.

The unusual composition of the hinge peptide Cys-Pro-Pro-Pro-Glu allows this peptide to be assigned to the NH₂ terminus of the Fe fragment, according to a provisional sequence proposed by Hill et al.¹³. Indeed, the Fe fragment obtained by brief digestion with papain retains the cysteine from a reactive disulphide bridge¹⁴ and we have observed that further digestion of this frag.

The carbohydrate group, C_1 , which contained a single residue of galactosamine, was attached glycosidically to the OH group of a threonine residue preceding the labelled cysteine. All fraction A peptides possess this carbohydrate group; this was shown by the presence of one galactosamine residue per mole of glutamic acid

ment liberates the hinge peptides. These peptides must, therefore, form the link between the Fd and Fc sections of the H chain.

The occurrence of three prolines in a row is unique among proteins of known structure. In view of the striking physical properties of synthetic polyprolyl peptides16 and of the role of proline in forming "corners" in the steric structure of a crystalline protein16, the hinge peptide linking the Fd section to the Fc section of the H chain may well form an essential element in the structure of immunoglobulin. Whether the reactive half cystine residue in the hinge region is coupled to the corresponding half cystine on the complementary H chain or whether this disulphide bridge connects two regions along the same H chain is still undecided. The ease of reduction of this disulphide would generally be taken as evidence in favour of its assignment as an interchain bridge. The isolation of hinge peptides containing the disulphide bridges intact, which will allow their unequivocal assignment, is in progress.

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5-Hydroxytryptamine in the Circulation of the Dog

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Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England Estimation of the relative importance of platelets, lungs, liver and peripheral vascular beds in removing this amine from the circulation of the dog shows that the lungs are most active in this respect, and can remove more than 90 per cent in one circulation. This removal does not depend on monoamineoxidase activity.

5-HYDROXYTRYPTAMINE can be removed from the blood in several ways. Platelets1 and erythrocytes2 take it up by a mechanism which, for platelets, has been extensively studied in vitro3,4. Although it has sometimes been assumed that, in vivo, the rapid removal of 5-hydroxytryptamine from the blood is through platelet uptake⁵, there is little evidence for this. Another mechanism by which this amine can be removed was first observed by Starling and Verney⁶. They noted that the vasoconstrictor activity of shed blood, later shown to be caused by 5-hydroxytryptamine, could be removed by perfusing blood through the lungs of a dog. More recent works, has confirmed this observation. Another organ which removes 5-hydroxytryptamine is the liver and it has been shown that 30-80 per cent of 5-hydroxytryptamine injected into the portal vein disappears in its passage through the liver 10.

We have estimated the relative importance of the platelets, the lungs, the liver and the peripheral vascular beds in the removal of 5-hydroxytryptamine from the circulating plasma of dogs. 5-Hydroxytryptamine was assayed continuously by the blood bathed organ technique11. The assay organs were rat stomach strips¹², which contracted to 5-hydroxytryptamine, and a rat colon¹³, which did not, but instead responded to angiotensin or prostaglandins. While the dog was being prepared the assay organs were suspended in polypropylene chambers and superfused14 in series with Krebs solution. The movements of the organs were recorded on a Beckman Offner 8 channel dynograph with 'Ether' strain gauges attached to auxotonic 15 levers; the initial load was 1-3 g. In some experiments two banks of assay organs were prepared so that the concentrations of 5-hydroxytryptamine in venous and arterial blood could be assayed simultaneously.

Dogs of either sex weighing between 6 and 27 kg were anaesthetized with halothane delivered from a Goldman vaporizer; anaesthesia was then maintained chloralose (100 mg/kg given intravenously) and supple-

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mented when necessary with pentobarbitone (5-10 mg/kg, given either intramuscularly or intravenously). trachea was cannulated and the lungs were ventilated mechanically. Polyethylene cannulae were tied into a femoral or carotid artery and a femoral or jugular vein for the continuous removal and replacement of blood. Mean arterial blood pressure was recorded on the dynograph from a Statham pressure transducer $(P \ 23Db)$ attached to a side arm of the arterial cannula. Heparin (1,000 IU/kg) was injected intravenously and the assay organs were then superfused at a rate of 10 ml./min with arterial or venous blood delivered by a roller pump. The blood was then collected in a reservoir and returned to the dog either by gravity or by a second channel in the roller Catheters were introduced, as described later, either for the continuous infusion of substances or for the removal of blood for assay. In any one experiment only one carotid artery was occluded and, if two arterial catheters were needed, the other was placed in a femoral The positions of all catheters were confirmed post mortem. Platelet counts were determined by the method of Brecher and Cronkite¹⁶ using phase contrast microscopy.

Inactivation of 5-Hydroxytryptamine in Circulating Blood

The inactivation of 5-hydroxytryptamine by blood was measured by passing the blood through a length of silicone tubing of 3 mm internal bore and 45 ml. capacity immersed in a water bath maintained at 37° C. The blood first circulated through this incubating circuit and then superfused the assay organs. By infusing 5-hydroxytryptamine at different points in the circuit, the drug was in the blood for known periods of time before reaching the assay tissues. For example, the contractions of the rat stomach strip to the same rate of infusion of 5-hydroxytryptamine incubated in the blood for 15, 30, 60, 120 and 240 sec could be compared with, and bracketed between, the contractions induced by infusions of 5-hydroxytryptamine made close to the tissues. From this the dis-

Table 1. Estimation of the half life $(t_{1/6})$ of 5-hydroxytryptamine (5-ht) in blood

Experiment No.	Initial concentration of 5-HT in blood (ng/ml.)	<i>t</i> _{1/2} (sec)	Platelet count/mm³
1	40	60	153,000
2	10 20 40	60 60 94	112,000
3* * Venous blood	20 40 80 d.	60 100 120	166,000

appearance and the half life could be calculated. A diagram of the apparatus has already been published¹⁷.

The half life of 5-hydroxytryptamine in circulating blood was estimated seven times in three experiments (Table 1). The concentrations at which the estimates were made depended on the sensitivity of the assay organs. In most experiments, the rat stomach strips detected 2-5 ng/ml. and allowed assay of up to 50 ng/ml. The half life of 5-hydroxytryptamine in either venous or arterial blood was 60 sec at the lower concentrations. In two of the experiments, when the concentration of 5-hydroxytryptamine was raised, the half life also increased to 94 sec in one and to 100 sec in the other. Thus, it seems possible to saturate whatever mechanism it is that removes 5-hydroxytryptamine from blood.

In the present experiments, the count of circulating platelets was only about half the normal, mainly through platelet clumps forming in the return reservoir of the extracorporeal circulation. Despite this relatively low platelet concentration it can be concluded that the disappearance of 5-hydroxytryptamine from the blood, whether it is caused by breakdown or uptake, is too slow a process to account for the very rapid removal of 5-hydroxytryptamine from the circulation.

Disappearance of 5-Hydroxytryptamine in the Portal Circulation

The abdomen was opened in the mid-line and a fine polyethylene catheter was inserted into a tributary of the splenic vein and pushed down until its tip lay in the main portal vein, close to the liver. Infusions were made through this catheter into the portal vein. The amount of 5-hydroxytryptamine which survived passage through the liver was estimated by bathing the assay organs in mixed venous blood (obtained from a catheter advanced down the jugular vein into the right ventricle). contractions of the rat stomach strips which were produced by the intraportal infusions were bracketed between contractions induced by intravenous infusions. In seven experiments (Table 2) more than 70 per cent of the infusions of 5-hydroxytryptamine into the portal circulation disappeared before reaching the heart (mean 75.0 per cent, standard error ± 2.97). A record from one of these experiments is shown in Fig. 1, which shows part of a tracing from an experiment in which one rat stomach

Table 2. Estimation of removal of 5-hydroxytryptamine in one circulation through the liver or the lungs

Percentage removal by the lungs	Percentage removal by the liver
93	
>98	-
{ 97∙5 90*	{ 78 > 75*
{ 88 >80*	$\left\{\begin{array}{c} 75 \\ - \end{array}\right.$
\$ > 98 \$ > 94*	$\left\{\begin{array}{c} 75 \\ - \end{array}\right.$
> 90†	
92.5†	62†
> 90 †	83 †
*****	87†
92.0 ± 1.59	75·0 ± 2•97
	by the lungs 93 > 98 97.5 90* 88 > 80* > 94* > 90†

* Determination made after 200 mg/kg nialamide was given intravenously. † Determination made after 7 mg/kg mebanazine was given subcutaneously on each of the 2 days preceding the experiment.

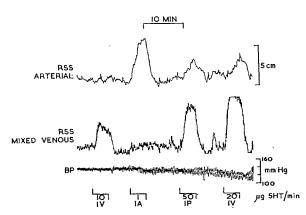


Fig. 1. One rat stomach strip (RSS) was superfused with arterial blood (top record) and another with mixed venous blood (middle record) taken from a 10 kg female dog. Blood pressure is shown on the bottom record. The first response shows the sensitivity of the RSS in venous blood to an infusion of 5-hydroxytryptamine (5-HT, 10 μg/min for 4 min). None of this infusion could be detected in the arterial circulation, despite the high sensitivity of the second RSS which responded to 1 μg/min intra-arterially. An infusion of 5-HT (50 μg/min for 4 min) was then given into the portal vein and, of this, 10-20 μg/min ms detected in the mixed venous blood, and less than 1 μg/min in the arterial blood. Similarly, from the intravenous infusion of 20 μg/min, less than 1 μg/min survived passage through the pulmonary circulation. Time, 10 min. Vertical scales: 5 cm and mm of mercury.

strip was bathed in arterial blood and another in mixed venous blood. An intraportal infusion of 5-hydroxytryptamine (50 μ g/min) induced a contraction of the rat stomach strip in venous blood which was intermediate to those induced by 10 μ g and 20 μ g/min intravenously. The rate of disappearance was remarkably constant from dog to dog. Up to the highest rates of infusion tested (400 μ g/min) the same percentage of the infusion disappeared, showing that the mechanism responsible for removing the amine from the blood was not easily saturated.

Disappearance of 5-Hydroxytryptamine in the Pulmonary Circulation

In these experiments, the assay organs were bathed in arterial blood taken from a catheter in the femoral artery. The effects of intravenous infusions were compared with those of infusions into a catheter in the ascending aorta. In eleven experiments, some of them in the same animals used for estimation of the disappearance of 5-hydroxy-tryptamine from the portal circulation, more than 90 per cent disappeared in one passage through the pulmonary circulation. The amount varied from 80 per cent to 98 per cent with a mean of 92-0 per cent, standard error ± 1.59 . For example, from an infusion of 5-hydroxytryptamine (10 µg/min) intravenously (Fig. 1), so much was removed in the pulmonary circulation that none could be detected in the arterial blood. From an infusion of 20 µg/min intravenously, less than 1 µg/min (<5 per cent) reached the arterial circulation.

Inhibition of Monoamineoxidase Activity

In three experiments, a monoamineoxidase inhibitor (nialamide, 20 mg/kg) was injected intravenously, after the disappearance of 5-hydroxytryptamine in the portal and pulmonary circulations had already been estimated. After 1 h, the removal of 5-hydroxytryptamine by the portal (one experiment) and pulmonary (three experiments) vascular beds was re-estimated. There was no change in the percentage removal of 5-hydroxytryptamine either in the liver or in the lungs (Table 2). To make sure that the enzyme inhibitor had sufficient time to have a maximum effect, three other dogs were treated with a long-lasting inhibitor, mebanazine, in a dose (7 mg/kg subcutaneously) which gave more than 95 per cent inhibition of rat liver and rat brain monoamineoxidase¹⁸.

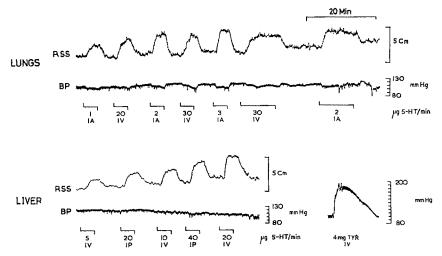


Fig. 2. The dog (12.5 kg male) was pretreated with mebanazine, as described in the text. First (upper tracing) the rat stomach strip (RSS) was superfused with arterial blood to estimate removal of 5-hydroxytryptamine (5-HT) by the lungs. From intravenous infusions (IV) of 20-30 ug/min, more than 1 μ_B and less than 3 μ_B /min were detected in the arterial blood. Later, an infusion of 80 μ_B /min was given intravenously for 10 min. From this, about 2 μ_B /min was detected in the arterial circulation. The RSS was then superfused with mixed venous blood (lower tracing) and infusions of 5-HT into the portal vein (IP) compared with intravenous infusions (IV). From 20 μ_B /min (IP) about 17 μ_B /min survived and from 40 μ_B /min (IP) about 15 μ_B /min came through the portal circulation. The last tracing shows the blood pressure response to tyramine (4 mg TYR IV). Compared with untreated dogs, the rise in blood pressure was greatly prolonged, showing that the amine oxidase inhibitor had been effective. Time 20 min; vertical scales in 5 cm and mm of mercury.

The injection was repeated on the second day and the animals taken for experiment on the third. The disappearance of 5-hydroxytryptamine in the liver and the lungs of these three animals was in the same range as in the untreated animals (Table 2).

An example is shown in Fig. 2, which is taken from an experiment in which the dog had been pretreated with mebanazine for 2 days. The assay organs were first superfused with arterial blood to estimate the disappearance of 5-hydroxytryptamine in the pulmonary circulation. During an intravenous infusion of 20 µg/min. only 1.5 µg/min was detected in the arterial circulation, and during an infusion of 30 μ g/min only about 2 μ g/min survived. This tracing also shows that the removal of 5-hydroxytryptamine was constant, even when the infusion was maintained for 10 min. The assay tissues were then bathed in mixed venous blood so that the disappearance of 5-hydroxytryptamine in the portal circulation could be determined. During an infusion of 20 µg/min into the portal vein, only about 7 µg/min was detected in the mixed venous blood and, during an infusion of 40 µg/min was detected in the mixed venous blood and, during an infusion of 40 µg/min was detected in the mixed venous blood and, during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected in the mixed venous blood and during an infusion of 40 µg/min was detected min, about 15 µg/min. Finally, an injection of tyramine (4 mg) was given intravenously. The effect on the blood pressure was greatly prolonged, taking more than 10 min to return to baseline, compared with a return within 3-5 min in untreated dogs. This prolongation of the action of tyramine shows that the amine oxidase had been effectively inhibited by pretreatment of the dog with me banazine.

Disappearance of 5-Hydroxytryptamine in Peripheral Vascular Beds

For these experiments the assay organs were bathed in mixed venous blood taken from the right ventricle. A catheter was also inserted into the exposed right carotid artery and advanced retrogradely until its tip was in the left ventricle, as shown by the pulse pressure recorded through it. The catheter was then withdrawn until the tip was in the ascending aorta, just above the aortic valves. Infusions through this catheter into the total cardiac output were compared for their effects on the assay organs with intravenous infusions. Thus, the 5-hydroxytryptamine which disappeared in one passage through the peripheral vascular beds was estimated. In one experiment 30 per cent disappeared, and in another 60 per cent.

Several conclusions can be drawn from these experiments. First, removal of 5-hydroxytryptamine by enzymes or cells in the blood is not sufficiently fast to account for its almost immediate disappearance from the circulation. In the few seconds that it takes blood to pass through the portal circulation, more than 70 per cent of the 5-hydroxytryptamine disappeared and, in the pulmonary circulation, more than 90 per cent. The pulmonary circulation takes the whole of the cardiac output, and so the lungs are presumably the chief site for the clearance of 5-hydroxytryptamine, and are much more effective than the peripheral vascular beds. The fact that we found a greater rate of removal of 5-hydroxytryptamine in the lungs than reported previously8,9 may be because injections temporarily swamp the removal mechanism, allowing more to escape. We used infusions and waited for equilibrium conditions to be established.

5-Hydroxytryptamine is released into the circulation in the carcinoid syndrome¹⁹ and has also been implicated in the dumping syndrome²⁰. 5-Hydroxytryptamine is released from the isolated perfused dog intestine by various drugs and by muscular contraction21,22. the intestine is probably the most important site of release of 5-hydroxytryptamine into the blood stream. The fact that the portal and pulmonary circulations together diminish the concentration of any 5-hydroxytryptamine in the portal blood by more than 99 per cent suggests a protective mechanism which prevents the amine from reaching the arterial circulation where it may have dramatic cardiovascular effects. It may also explain why increased concentrations of 5-hydroxytryptamine in peripheral blood during dumping in dogs have not been observed despite increased 5-hydroxytryptamine concentrations in venous blood draining the small intestine23,24. The specificity of the mechanism is shown by the much lower disappearance in the peripheral vascular beds and by the fact that other amines, such as adrenaline, pass through the pulmonary circulation without loss (unpublished results of Ginn and Vane).

What process is responsible for the disappearance of 5-hydroxytryptamine in the liver and the lungs? The amine is not lipid soluble and does not easily penetrate cell membranes²⁵, unless by a special transport mechanism. If it is entering cells, they probably do not contain amine oxidase, because inhibition of the enzyme did not change the amount of 5-hydroxytryptamine disappearing.

Radioactive techniques have shown that 5-hydroxytryptamine is specifically taken up into reticular endothelial cells in the liver and into septal cells in the lungs26, neither type of cell containing monoamineoxidase(personal communication from M. D. Gershon). It is possible that 5-hydroxytryptamine is metabolized by some other enzyme, or that it is stored, to be metabolized later at a slower rate. If storage is involved, the capacity of the stores must be enormous, for in some experiments the dog's lungs were removing about 100 μ g/min or 2 mg in a 20 min infusion. Experiments with labelled 5-hydroxytryptamine, used in conjunction with the present technique, should answer this point.

It is possible that 5-hydroxytryptamine is immediately taken up by, or gradually transferred to, platelets trapped in the lungs. Virtually all the 5-hydroxytryptamine in the blood is normally contained within the platelets. It has been calculated that from 20 to 50 per cent of the mature megakaryocyte population ultimately reaches the lungs, and that in man 7-17 per cent of the platelets are released in the pulmonary capillaries27. The lung capillaries are therefore a rich source of platelets, although it appears unlikely that they alone are sufficient to account for the very rapid disappearance of circulating 5-hydroxy-

tryptamine.

In many species, 5-hydroxytryptamine causes constriction of the airways when injected intravenously. 5-Hydroxytryptamine released from platelets has also been implicated in the airway constriction that develops in dogs after either pulmonary emboli²⁸ or administration of bacterial endotoxin²⁹. Little or no 5-hydroxytryptamine survives passage through the pulmonary circulation, and so airway constriction after embolization must be caused primarily by changes in the peripheral airways, which are perfused by the pulmonary circulation, and not the conducting airways, which are perfused by the bronchial circulation³⁰.

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"Pre-imaginal Conditioning" in Drosophila

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Drosophila reared on a medium containing geraniol showed reduced aversion to its odour when adult. Experiments show that this change is caused by a form of habituation and is not associative learning.

ATTEMPTS to demonstrate associative learning of any sort in the Diptera have met with little success and the only convincing report is that of Murphey1. However, flies and particularly *Drosophila* are very convenient experimental animals and any demonstration of conditioning opens up exciting prospects for the study of genetic effects on learning. Accordingly the recent claim of Hershberger and Smith to have demonstrated classical conditioning in *Drosophila* is of considerable importance². Their work makes use of the remarkable phenomenon first described by Thorpe³ and which he called "preimaginal conditioning".

Drosophila which have been reared normally are aversive to the smell of peppermint, but flies which have been reared in a medium containing 0.5 per cent peppermint oil show greatly reduced aversion. Thorpe showed conclusively that exposure to peppermint during the larval period alone was sufficient to change the behaviour of adults. While Thorpe originally interpreted these results in terms of conditioning, he later suggested that the change is due to a form of habituation which persists through the pupal stage.

Hershberger and Smith using a similar technique have been able to confirm almost all of Thorpe's results, but they disagree with his interpretation. They point out that in every case where a population of flies shows reduced aversiveness to peppermint, this smell had previously been associated with food, and they conclude that the changed behaviour of flies reared in peppermint medium is caused by positive conditioning to the smell, not to habituation.

One may reasonably question whether the evidence presented by Hershberger and Smith or by Thorpe is adequate to prove their conclusions. In neither case were the responses of individual flies tested on a second or subsequent occasion to obtain some measure of the consistency of their response, and this is crucial for deciding between the two hypotheses.

The experiments reported here were begun in an attempt to change the levels of pre-imaginal conditioning by selection and to look for genetic assimilation of the acquired trait, along the lines explored by Moray and Connolly. The results will be published in detail elsewhere, but as far as the general nature of the phenomenon is concerned,

they confirm those of Thorpe. Geraniol was used as a conditioning scent in preference to peppermint oil because it is a pure substance, not a mixture. It has a strong, sweet smell which when dilute is mildly attractive to the flies, but at the concentrations used in the tests some 80-90 per cent of normally reared flies show aversion.

A simple Y tube olfactometer was used, very similar to that used by Hershberger and Smith, and the general procedure was also similar. Groups of between ten and thirty flies of one sex were let into the Y tube through which a slow current of air was drawn. Flies could be collected from a small chamber attached to each arm of the Y after having made their "choice". One arm was "baited" with two small drops of geraniol placed upstream of the air intake to the collecting chamber; the scented arm changed sides at random between tests.

Flies were reared on a normal medium of agar, corn meal and molasses or on this medium with 0.5 per cent geraniol by volume added just before pouring into culture bottles. Adult flies were collected within a few hours of hatching and kept in groups of twenty to twentyfive in 3×1 in. vials containing normal, unscented medium. Before every test they were starved for 5-6 h in vials providing only water on cotton wool.

Table 1. Numbers of flies going to geraniol and blank arms of olyactometer after normal rearing (A) and rearing in geraniol medium (B), together with second run of geraniol-reared flies that went to GERANIOL ARM ON FIRST RUN (C)

		A			В		. σ				
	Flies r	eared no	rmally		rnes rea First ru		geraniol-medium Second run of flies going to geraniol on first run				
Gener-	Ger-		%	Ger-		%	Ger-		%		
ation	aniol	Blank	blank	aniol	Blank	blank	aniol	Blank	blank		
1	4	48	92.3	30	26	46.4	14	9	39.1		
3	_	_	_	120	103	46.2	58	55	48.7		
3				118	108	47.8	58	48	45.8		
4 5	5	53	91.4	64	123	65.8	25	39	60.9		
5	11	51	82.3	70	42	37.5	30	30	50.0		
6	8	32	80.0	57	96	62.7	27	30	52.6		
7	3	47	94.0	43	77	64.2	20	23	53-5		
8	7	45	86.5	109	123	53.0	53	49	48.0		
Total	38	276	87.9	611	698	53.3	285	283	49.8		

Table 1 (columns A and B) gives the results of a series of tests made on successive generations of flies bred normally and on geraniol medium. Although there is some variability—and olfactometers of this type are far from exact instruments—the difference between the two groups is consistent and striking. The mean "aversiveness" of the former is 87.9 per cent, but rearing in the presence of geraniol reduces this to 53.3 per cent.

Each test provided a group of flies which, having been reared in the presence of geraniol, "chose" to move toward this scent when moderately food deprived. The most critical test of positive conditioning versus habituation is to run these flies through the olfactometer a second time. Ignoring "accidental choices", on the habituation hypothesis one supposes that turning towards geraniol on the first trial represents only random choice and that such flies will segregate at random between geraniol and blank on the second run also. According to the conditioning hypothesis most flies choosing geraniol on the first run do so because they associate its smell with food and there should be an increased proportion of choices to geraniol on the second run.

The results of the second runs are also shown in Table 1 (column C) and they strongly support the habituation hypothesis, for in no case did the proportion of flies

Table 2. First and second runs of geraniol-reared flies "rewarded" for going to geraniol on first run

	First	t run			run of file	s going to Arst run
Group	Geraniol	Blank	% blank	Geraniol	Blank	% blank
1	31	38	55.1	13	16	55.1
2	64	100	60-9	30	29	49-1
3	60	102	62-9	18	34	65.4
Total	155	240	60-7	61	79	58-4

Table 3. Segregation in olfactometer of flies reared normally or on geraniol medium kept for 18 h before testing in scented or unscented vials

	Normal	ly reared	Geraniol reared		
	Scented vials	Unscented vials	Scented vials	Unscented vials	
Going to geraniol	81	45	55	38	
Going to blank	34	58	33	38	
Per cent blank	29.5	56-3	37.5	50∙0	

going to each arm differ significantly from random choice, using the x2 test.

"Rewarding" flies for choosing geraniol has no effect on their subsequent choice. Table 2 gives the results from three groups of flies which were allowed to feed on sucrose in the collecting chamber if they chose the geraniol arm on their first run. On the second run these flies also segregated at random in two of the groups; in the third run, segregation is significantly different from random, but in the opposite direction to that predicted by the conditioning hypothesis, that is, fewer flies "chose" geraniol. Possibly the concentration of geraniol was higher on the second run than on the first.

The results of two more experiments serve to reinforce the habituation hypothesis. First, an attempt was made to "counter-condition" adults by associating scent with food deprivation. Normal and geraniol-reared flies were confined on hatching in vials containing water soaked cotton wool. Half the vials in each group were scented with geraniol. When tested in the olfactometer 18 h later, both groups which had been starved in the presence of geraniol showed reduced aversiveness (Table 3), although only in the case of normal reared flies was this reduction significant ($\chi^2 = 15.93$). This result disagrees with that obtained by Hershberger and Smith2, but suggests that habituation also occurs in adult flies as well as larvae.

Second, one may consider the behaviour of flies which go to the blank arm of the olfactometer on the first run. The habituation hypothesis supposes that those flies which go to the scented arm represent approximately 50 per cent of that proportion from the total population which has been rendered insensitive to geraniol, the other 50 per cent will go to the blank arm. The proportion of habituated flies will vary for a number of reasons, but if we consider those cases in which less than half the flies go to geraniol on the first run, then, ignoring "accidental" choices, the preponderance of flies choosing the blank arm should represent that proportion of the population which has not become habituated and remains aversive to geraniol. If the number of flies going to the scented arm on trial one is x and that to the blank y, then y-x flies are aversive and should continue to avoid the scent on the second run. If the flies choosing the blank arm are given a second trial they should segregate with x/2 + (y-x)going to the blank arm.

The population recorded in Table 1 was not tested in this way, but data are available from another population being selectively bred for aversiveness, that is, for a reduced response to rearing in geraniol medium. In most generations less than 40 per cent of flies from this line go to the scented arm on the first run. Table 4 shows the results of tests on several successive generations and compares the actual segregation observed on the second run of flies "choosing" the blank arm on the first run with that which would be predicted using the formula given

Table 4. SEGREGATION ON FIRST RUN OF GERANIOL REARED FLICS AND SECOND RUN OF FLIES GOING TO BLANK ON FIRST RUN

DATON OF THE									
First run				Second	Second run of flies going to blank on first run				
Genera- tion	Ger- aniol	Blank	% blank	Ger- aniol	Blank	% blank	Predicted % blank		
1 2 3 4 5 6 Total	31 19 26 51 63 60 250	73 55 45 92 141 112 518	70·2 74·3 63·4 64·3 69·1 65·1 67·4	17 14 11 16 31 31 120	56 36 29 35 93 84 333	76·7 72·0 72·5 68·6 75·0 73·0 73·5	79·1 83·3 71·1 72·8 77·8 73·2 75·9		

The predicted segregation on the second run, based on the habituation hypothesis, is also shown.

above. In no case does the observed result differ significantly from the predicted one.

These experiments all suggest that Thorpe's interpretation is correct and that "pre-imaginal conditioning" is a form of habituation. Crombie's thorough work with blowflies, although somewhat different in procedure, also supports this conclusion. The physiological basis for this habituation remains quite obscure, although it seems unlikely to be caused by peripheral changes alone. The pupal stage in Diptera involves a great deal of breakdown and reconstruction of the body and sense organs, but the central nervous system is less affected.

Because most flies become habituated when reared in geraniol medium it is difficult to "improve" this response by selection. In most cases, however, a small proportion of flies seems to be unaffected by exposure and remains genuinely aversive. Preliminary results suggest that the difference between habituated and aversive flies is partly genetic and that the proportion of aversive flies can be increased by selection. Further tests on selected lines should help to clarify the basis of habituation.

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Establishment of a Lunar Unipolar Generator and Associated Shock and Wake by the Solar Wind

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The impact of the solar wind on a dynamo- and atmosphere-free body such as the Moon generates two distinguishable current systems denoted as Cowling $(\dot{B}\neq 0)$ and unipolar $(\dot{B}=0)$. The unipolar system representing the steady state must close through the neighbouring plasma and thus has the form of a simple linear unipolar generator. The relationship of the current to the conductivity of the body is investigated for the homogeneous and the radially inhomogeneous Moon. The current density is determined by the power flow in the solar wind and the conductivity of the body. The fundamental equation for the interior potential associated with the unipolar generator is solved for a special case.

Gold's suggested that the impact of the solar wind on the Moon would cause a captured magnetosphere and an accompanying shock wave. An additional consequence of the model was the formation of a poloidal magnetic field, which might masquerade as a dynamo field. In Gold's model the plasma contacted the surface and was wholly absorbed, neutralized, and re-emitted at the characteristic thermal state of the surface matter. The electrodynamics of interaction was described by a diffusion time required for field lines to transit the interior and emerge on the antisolar side where pinching off with an x-null geometry could take place. The characteristic time was given by

$$\tau = \mu \sigma L^2 \tag{1}$$

where τ is the Cowling² decay time, μ and σ the magnetic permeability and bulk electrical conductivity, respectively, and L the scale size (lunar diameter). More recently, Tozer and Wilson³ considered a similar model (compare Neugebauer⁴).

From examination of interplanetary monitoring platform I data, Ness⁵ suggested the formation of a lunar wake. Greenstadt⁶ and Hirshberg⁷, criticizing this suggestion, pointed out that the interplanetary medium was disturbed at the time of the observations and the certainty of the observations remains unclear (compare Ness⁸, Michel⁹). Gringauz et al.¹⁰ and Dolginov et al.¹¹ reported the existence of a sunward zone of interaction before the Moon. Ness suggested that the Moon was immersed in the Earth's magnetic tail during these observations. Finally, at least some of Gold's conclusions can be shown to arise from an excessive decay time attributable to a product, μσ, about 10⁶ larger than probable estimates for a lunar interior.

Additional work on this problem has been carried out by Dessler¹², who investigated the interaction with an atmospheric planet (Mars) with an ionosphere generated partly by the interaction. Michel¹³ has discussed the lunar atmosphere with magneto-hydrodynamic interaction. His calculations are approximations, for the supersonic case is not explicitly formulated and the motional electric field used does not account for a deviation of the flow to the limbs of the Moon.

Our purpose is to point out that the relative motion of the Moon with respect to the interplanetary plasma fulfils the essential requirements for a simple unipolar generator of linear geometry where the Moon is envisaged as a bar moving along a set of rails threaded by magnetic flux. This is the simplest type of unipolar generator (compare Panofsky and Phillips¹⁴; Cullwick¹⁵). Rotation of the Moon with respect to the Sun during its lunation is ignored. Application of Maxwell's equations permits certain very general statements to be made regarding the details of the interaction problem. The magnetohydrodynamic flow field cannot influence these results fundamentally. The plasma flow does modify the symmetry of the problem, and the flow deviation for strong interaction causes a decrease in the net induction in the Moon. In a very approximate way, to demonstrate the saturation process, we employ a coefficient of loss, k, for the plasma swept to the limbs of the Moon which also carries with it magnetic field. Thus the net unipolar electric field in the interior is reduced by the factor (1-k). This is discussed later to account for lessened induction.

For non-homogeneous bodies such as a hot Moon, it is probable that the electrical conductivity of the interior is a strong function of radius. In this case the

electric potential in the interior is radically altered from the homogeneous case and therefore the most resistive layer will dominate the current system. This means that a thin high impedance surface layer can quench the unipolar induction currents.

The principle of unipolar induction permits the electric field in the interior to be calculated. An equivalence to magneto-hydrodynamic diffusion has been shown by Sonett and Colburn in unpublished work. The diffusion of field in the Moon is not magneto-hydrodynamic, because it does not involve a fluid or deformable conductor. The process might be thought of as being caused by eddy currents, but this is conceptually misleading because, in the steady state, Maxwell's equations imply deformable conductors for induction fields. This difficulty is eliminated when the model using a unipolar generator is employed, with the current paths steady and closing in the plasma. For the steady state magnetic field, and consequently curl-free electric field, it is not possible for current streamlines to close in the Moon.

There are two current systems resulting from the interaction of the Moon with the solar wind. Consequently, all local perturbation fields have two distinguishable sources. In our model, the electric field excited in the Moon, which drives the current system, is either curl-free when the relevant solar-wind-driving parameters are time stationary or results from induction when $\dot{B}\neq 0$. We shall deal only with the steady state $(\dot{B}=0)$, and demonstrate that the mechanism is that of a unipolar generator. For the Moon, the diffusion time is sufficiently short compared with observed interplanetary sector structure to allow steady state conditions to be significant.

In the following the Gold mechanism is assumed to be applicable—that is, any plasma coming into contact with the surface of the Moon is adsorbed in the surface matter, electrically neutralized by electron pick-up, and eventually released into the lunar atmosphere thermally. Consider a solar wind with velocity, v, and a magnetic field, B, both independent of time. Then, in a frame of reference at rest with respect to the Moon, the motional electric field,

$$\mathbf{E}_m = \mathbf{v} \times \mathbf{B} \tag{2}$$

and everywhere $\dot{\mathbf{B}} = -\nabla \times \mathbf{E} = 0$. A body such as the Moon, when subjected to this field, becomes electrically polarized where $\mathbf{E}_p(t)$ is the field at time, t, that is,

$$\mathbf{E}_{p}(t) = [1 - e^{-(\sigma/\varepsilon)t}] \mathbf{E}_{p_{0}}$$
(3)

where \mathbf{E}_{p_0} is the final polarization field, ϵ , the specific inductive capacity, and

$$\tau = \epsilon/\sigma$$
 (4

the time constant for the placement of polarization charges. When conductive electrical contact between the Moon and the plasma is forbidden, for example when the Moon possesses an outer shell of extreme resistivity

$$\mathbf{E}_m + \mathbf{E}_p = 0 \tag{5}$$

the charges have a dipolar distribution and, for a homogeneous Moon, are all deposited on the surface. The field is electrostatically identical to that of a sphere in a uniform external electric field. In this limit there can be no interior current system, field lines are severed at the surface, and, other than the formation of a plasma cavity in the neighbourhood of the antisolar hemisphere, no interaction is observed. This statement implies the validity of the Gold mechanism and in the context of magneto-hydrodynamics is equivalent to the loss of individual identity of field lines in the interior. An alternative statement would be that field still diffuses through the Moon, but there is no way to identify the motion in the interior.

When the Moon is in conductive contact with the solar wind, polarization charges leak into the plasma and establish an externally closed current system and a perturbation magnetic field. The system constitutes a linear unipolar generator; the solar wind supplies the brushes. The charge carriers required in the plasma can easily be supplied by photoelectrons (unpublished work of the Project Tycho Study Group of the University of Minnesota), but they destroy the cylindrical symmetry.

The process is naturally limited by the tendency for plasma and field to slip around the flanks of the Moon when the unipolar magnetic field grows large. An approximate idea of this limiting process is given by balancing the solar wind pressure in the plane normal to the ecliptic and containing the Moon-Sun line against the unipolar field pressure. Both pressures will vary over the face of the Moon. That part of the deviated flow caused by the formation of back pressure from the unipolar field must be excluded in the calculation of the interior electric field. The fraction, k, of plasma carried to the sides has frozen in field which is also carried to the limbs. Thus the interior electric field must be decreased by the factor (1-k). Then the interior current density, $\mathbf{j} = (1-k)\sigma \mathbf{E}_m$ where \mathbf{E}_m is the electric field which would develop for no interaction. The total current depends on latitude. The total current at latitude, θ , is given by

$$I = jA(\theta) = j\pi r^2 \cos^2 \theta = (1 - k)\sigma E_m \pi r^2 \cos^2 \theta$$

where r is the lunar radius. The unipolar field at latitude θ is calculated from the circuital theorem

$$B = \frac{M_0 I}{2\pi r \cos \theta} = \frac{M_0 (1 - k) \sigma E_m r \cos \theta}{2}$$

and the pressure of the unipolar field is

$$p_{B} = \frac{B^{2}}{2M_{0}} = \frac{M_{0}(1-k)^{2}\sigma^{2}|E_{m}|^{2}r^{2}\cos^{2}\theta}{8}$$

This is balanced against the plasma pressure so that

$$\frac{k}{(1-k)^2} = \frac{\mu \sigma^2 |E_m|^2 r^2}{8nmv^2} \tag{6}$$

where k is the loss factor of electric field; E_m is the freestream motion field; $M = M_0$ and σ is the constitutive parameters of the Moon, as before; r is the lunar radius, and n, m and v are the parameters of the free-stream solar wind. The assumption is made that the unipolar field pressure at latitude 0 depends on the current flowing through the cross-sectional area for that latitude as if it were a cylindrical conductor of infinite length. introduces some error into the approximation. there is no account taken of the decrease in plasma pressure with longitude. In the present calculation of the unipolar field the pressure does not decrease with longitude. Thus there is here involved an additional approximation from the exact case. The values of k shown in Fig. 1 are reasonable approximations in the solar meridian plane for a homogeneous Moon. In equation (6) the solar wind pressure varies as cos² θ in the meridian plane where 0 is the latitude, while the current density and, consequently, the unipolar fields vary as $\cos \theta$. Consequently, polar dependence disappears from equation (6).

Values of k shown in Fig. 1 indicate that, for typical interplanetary magnetic fields, $10^{-6} \le \sigma \le 10^{-3}$ mho/m represents the transitional values of conductivity in going from weak to strong interaction, with the formation of a magnetosheath and the development of a shock wave. The ultimate source of work to drive the dynamo is determined by the solar wind power flow, also indicated in Fig. 1.

There must exist in addition the effect of terminating the plasma flow against the surface of the Moon. Because of the intrinsic diamagnetism of the plasma, a field jump can be expected at the surface, but the amount of the jump depends on the net surface currents. If the Moon were a perfect reflecting wall the field jump would not appear because the net surface current due to gyrating protons and electrons would be balanced by an equal

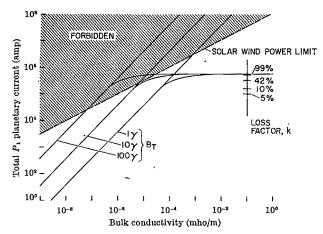


Fig. 1. Approximate total P_1 unipolar planetary current shown as a function of bulk conductivity for a homogeneous Moon. The straight lines are the free-stream tangential magnetic field. They can also represent the motional electric field in the solar wind and the electric field within the Moon in the limit of small interaction and constant v. The saturation caused by a unipolar field comparable with the solar wind field is shown to take place in the range $10^{-6} \leqslant o \leqslant 10^{-3}$ mho/m for typical values of the interplanetary magnetic field. The solar wind power flux, including the particle contribution for v = 400 km/sec and n = 5 cm⁻³ over a target area of lunar radius, determines the ultimate limit of available work for driving the unipolar current system. The loss factor, k, is shown on the right ordinate. The expectation that the Moon has a relatively cool insulating outer layer reduces the current by at least an order of magnitude from the homogeneous case.

current created by reflected particles¹⁶. For subsonic flow against the surface the Gold mechanism, which inhibits reflexion, would allow the field jump to appear, but for supersonic impact, only the electrons will have fast enough orbital velocity to contribute to the surface currents. We have not included the diamagnetic effect in the calculations, but it should be included in a more exact model.

We need here to clarify an important conceptual point. The transformation from the moving frame of the solar wind, where no electric fields are permitted because of the high conductivity, to another frame must be generally accompanied by the placement of polarization charge layers if there is a conducting body at rest in the new frame. E_m is strictly a property of the Galilean transformation and by itself cannot determine the electric fields in a configuration. Polarization charges are consequently found on the surface of the Moon in the present model. The assumption in the present model is that the plasma conductivity is always significantly greater than that of the lunar interior or surface layer, so that polarization fields are absent for the homogeneous Moon or present only in the interior when the low surface conductivity limits the current flow. An additional limitation on the current flow is approximated by the k factor, however, for, if plasma and the accompanying frozen-in field lines are deflected around the Moon, the E_m itself is reduced in the lunar interior.

These arguments, developed for a homogeneous Moon, can be generalized to one having a steep thermal and conductivity gradient. The unipolar system is characterized by curl-free electric fields and therefore in the Moon $\nabla \cdot \mathbf{j} = 0$. Because $\mathbf{E} = -\nabla \varphi$ and $\mathbf{j} = \sigma \mathbf{E}$, it follows that $\nabla \cdot (-\sigma \nabla \varphi) = 0$, so that the fundamental equation for the unipolar electric potential in the Moon becomes

$$\sigma \nabla^2 \varphi + \nabla \sigma \cdot \nabla \varphi = 0 \tag{7}$$

Under the constraint that $\sigma = \sigma(r)$ alone, that is, no angular dependence, equation (7) is separable. We define the potential as $\varphi(r,\theta) = R(r)\Theta(\theta)$ so that equation (7) becomes

$$\frac{\mathrm{d}}{\mathrm{d}r}\left(r^2 \frac{\mathrm{d}R}{\mathrm{d}r}\right) + \frac{r^2}{\sigma} \frac{\mathrm{d}R}{\mathrm{d}r} \frac{\mathrm{d}\sigma}{\mathrm{d}r} - n(n+1)R = 0 \qquad (8a)$$

$$\frac{\mathrm{d}}{\mathrm{d}\mu} \left[(1 - \mu^2) \frac{\mathrm{d}\Theta}{\mathrm{d}\mu} \right] + n(n+1)\Theta = 0 \tag{8b}$$

where $\mu = \cos \theta$. Solutions to equations (8) are given by

$$\varphi(r,\theta) = \sum_{n=1}^{\infty} A_n R_n(r) P_n(\cos \theta)$$
 (9) where P_n is the Legendre polynomial of order $n = 1, 3, 5 \dots$

where P_n is the Legendre polynomial of order $n=1,3,5\ldots(2n+1)\ldots$ (The existence of only odd order solutions is a consequence of the intrinsic antisymmetry of the electric field for which only odd order angular solutions are valid.) The boundary conditions are imposed at the surface, that is, $R_n(r)=1$ and $\sigma(r)=\sigma(r_1)$ at $r=r_1$. For the case of uniform lunar conductivity and negligible plasma resistivity the boundary conditions are satisfied by the P_1 current system alone, but other profiles introduce higher orders. The electric field for the P_1 current system is shown in Fig. 2 for a nominal radiogenic, convective lunar interior with a boundary condition imposed on temperature. The bulk conductivity through the interior is calculated from the thermal dependence of olivine P_1 .

The P_1 current system solution of Fig. 2 is given in order to demonstrate that a strong angular dependence exists and that the largest potential drop is restricted to the cool manticular mantle. These conclusions are expected to hold for the complete solution. For $\sigma=$ constant, equation (7) reduces to Laplace's equation with uniform current density. The result that the potential appears primarily across the most resistive part of the unipolar circuit is consistent with circuit theory. The assumption that the solar wind conductivity is higher by orders than that of the Moon means that the magnitude of the electric current is determined by the latter.

The more general case with axial dependence has not been solved because of complications with the current carrier system. For example, if photoelectrons are the primary carriers, then currents can flow only on the sunlit hemisphere. A current model of this type will introduce zonal harmonic dependence, but equation (7) is still separable, and therefore the zonal dependence is multiplicative and the radial and polar dependences of equations (8b) and (9) are valid. The primary limit to

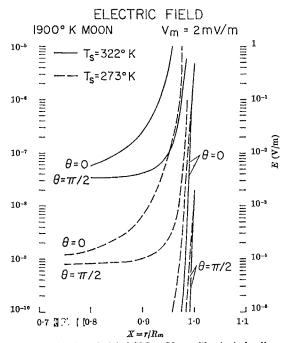


Fig. 2. The planetary electric field for a Moon with a typical radiogenic thermal profile with convection (Model 7 of Fricker et al. ''). The customary thermal profile is modified by specifying the surface temperature, which defines the surface conductivity, one of the boundary conditions for equations (8). The distance scale is normalized to the lunar radius. A strong polar dependence of the electric field appears, and the radial dependence shows that the bulk of the potential drop is in the first few hundred kilometres beneath the surface. The motional field assumed is 2 mV/m. Other values yield similar results by scaling.

our solution will arise from deviations from the assumption as to the introduction of the interplanetary field into the Moon. On the limbs the supersonic flow will still imbed the field, but the motion is mostly along the direction of the field lines and therefore the induced electric field is reduced.

The curves of Fig. 1 can be reinterpreted for the case of the inhomogeneous Moon with a low conductivity outer layer. The relation of total current to the k factor shown in the figure applies to the inhomogeneous case because the pressure balance is determined primarily by the total current crossing the lunar equator. Similarly, because the potential drop is determined by the k factor, the total planetary joule loss for a given current is nearly unchanged by the inhomogeneity. When the loss is concentrated in the outer layers, however, the accumulated effect will be diminished because more heat will be lost in radiation through the surface, leaving less to be retained

Under the assumptions of our model, the density of field lines in the interior will drop away to the free-stream value at the centre modified appropriately by the kfactor. The approximation of the circuital theorem used to calculate the induction field implies a magnetic field that increases outward to the surface and then falls away as the reciprocal distance. The vector addition of the free stream and perturbation fields leads to a configuration which agrees substantially with that of Gold regarding the upwind enhancement and shock and a downwind null or quasi-null, provided the mantle conductivity is sufficient. The simplest source for large o is a high temperature—that is, T is about 1,500°-2,000° K.

The conclusions can be applied to any planet. For the Earth $k \sim 1$ and little, if any, field enters the magnetosphere. Unipolar currents would be sheetlike and confined to the surface of the magnetopause. Planets substantially free of dynamo fields but containing an insulating mantle could not support an interior unipolar mechanism, nor could Mars or Venus if the atmosphere insulates. In these cases a degenerate unipolar generator might be imagined where the current closes in the ionosphere and returns to the solar wind12 but does not engage the solid body of the planet. Any unipolar system closing through the planet must be bounded in value by the highest series circuit impedance, here the lower atmosphere.

Finally, plasma must contact the lunar surface in order to drive the unipolar generator. The efficiency is given by the factor (1-k). When $k\rightarrow 1$, all plasma and field are swept to the limbs. This condition, however, is a reductio ad absurdum because it depends on a strong perturbation field. The condition $k\equiv 1$ can therefore never exist and some plasma and field must be decoupled at the surface. The details are complicated by the form of the sheath, and our model does not explore this. For the Moon more detailed calculations to be reported later will be used to refine our expression for the degree of saturation of the unipolar system and the limits on the formation of a shock wave and wake. Future experimental measurements can be employed by an inversion of our deductions to determine the mean mantle conductivity and thus shed light on the thermal profile.

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Sex Pili and the Classification of Sex Factors in the Enterobacteriaceae

by

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The properties of sex pili determined by different plasmids of the Enterobacteriaceae suggest that there may be only two major classes of sex factor, typified by F and the factor of colicin factor lb.

THE study of conjugation promoted by F and other sex factors of the Enterobacteriaceae has been stimulated by the identification on F+ and Hfr bacteria of specialized filaments to which F-specific phages adsorb¹-4. filaments, which are examples of the appendages named "fimbriae" (refs. 5, 6) or "pili" (ref. 7), may indeed be the organs of conjugation^{3,4}. Many other transmissible plasmids have since been shown to determine the synthesis

of pili (refs. 8-12 and an unpublished work) and, to distinguish these pili as a class, we have suggested11 that they, together with the F pilus, should be called "sex pili" as opposed to the various types of "common pili" found on many enterobacteria4-7.

The synthesis of sex pili is determined by the bacterial sex factor, and so the pili can be used to classify the sex factors of plasmids such as R factors or col factors. By this means, it has already been shown that the fi⁺ class of R factors (which repress the function of F in F+R+ cultures¹³) has a sex factor related to F, because F- cells carrying an fi⁺ factor are sensitive to phages that adsorb specifically to F pili^{8,9,14}; whereas the presence of an fi-R factor (one not inhibiting F in an F+R+ strain) does not render cultures susceptible to F phages¹⁴. In many cases^{12a}, however, they are attacked by a newly isolated filamentous phage which attacks cells carrying the pilus¹¹ determined by colI. The sex pili determined by wild-type conjugation factors, other than F and colV (ref. 15), are formed for only a few generations after the factor enters a new host^{11,16,17} and thereafter only 0·01-1 per cent of bacteria are piliated at any one time. The morphology of sex pili must therefore be studied either in a host newly infected with wild-type factors before repression occurs (a "high frequency transfer" or HFT preparation¹⁷) or with mutant factors which do not become repressed¹⁰.

In this article, we describe the morphology of the sex pili determined by eleven conjugation factors, and their interaction with antibody and donor-specific phages as seen in the electron microscope. It can be said at once that all the sex pili thus observed resemble either F pili or the I pili of collb-P9. In addition, sixty transmissible plasmids have been examined by the susceptibility of their hosts to F and I phages, and most were found to confer sensitivity to one or other, but not both, phages.

Morphology of Sex Pili

Cultures of Escherichia coli or Salmonella typhimurium were examined by electron microscopy after negative staining with uranyl acetate. Their plasmids fell into two groups: the first, related to F, included F itself (both autonomous in an F+ strain, or as F'13 (ref. 18) or F-lac (ref. 19); and integrated in the strains HfrH and HfrC), colV-K94 (ref. 15) F₀-lac (ref. 20) and the fi+ R factors, R1 (both wild type and depressed), R100-1 (a de-repressed mutant of R100; refs. 12b and 21) and HFT cultures of R124 and R237 (ref. 8). The second group was related to colI and included de-repressed mutants of the fi- factors, R64 and R144 (ref. 10), as well as HFT cultures of colIb (ref. 11) and colEIa-16 (ref. 12a).

Table 1. CHARACTERISTICS OF SEX AND COMMON PILI

			Clusters				
Pili	Maximum	Axial		of pili	Phage adsorption		
	length (μ)	"canal"	knobs	occur	F	I	
F-like	20.0	Often seen	+	+	+		
I-like	2.0	Rarely seen	+	+		+	
Common	1.5	Prominent	****		-		
(type I)							

The sex pili of members of the first, F-like, group, whether determined by F, $col\ V$ or F₀-lac, or by R1, R100–1, R124 or R237, could not be distinguished from one another by appearance. Those of the second, I-like, group, comprising collb-P9, colEIa-16, R64 and R144, were also indistinguishable from one another, except for the occasional presence of unusually thin filaments associated with collb (ref. 11). There were, however, certain definite differences between the sex pili of the two groups, as shown in Table 1, despite the fact that the appearances varied so much that it was impossible to decide by morphological criteria alone to which group a particular sample belonged. Although I-like pili were usually shorter than F-like pili, their length was in part dependent on the bacterial strain used as host. Thus the pili formed by de-repressed R144 were longer in strain M396 than in strain M385, although both strains are Salmonella typhimurium. The knobs found on the ends of sex pili often have a distinct substructure, and those of F-like pili tended to be larger and more complex than those of I-like pili, which were usually small and undifferentiated. There were usually between one and ten sex pili to a cell,

but occasionally between fifty and a hundred were seen (Fig. 1). In some cultures, sex pili were not randomly distributed on the cell, but tended to arise in clusters from one or a small number of points¹¹.

Host Range of I and F Phages

The first of the two groups of phages used comprised two F phages, MS2 (ref. 22), an isometric RNA phage attaching along the length of the F pilus, and M13 (ref. 23), a filamentous DNA phage attaching to the tip of the pilus. The second group of phages consisted of two isolates of a filamentous phage with a new host range^{12a}. Both were isolated from sewage by enrichment on Salmonella typhimurium carrying a de-repressed ft-R factor, followed by plating on Escherichia coli K12 carrying the same factor, so that unwanted salmonella phages propagated during enrichment could be disregarded. The two isolates may well be the same phage, for their antisera cross-neutralize and neither is affected by high titre antiserum to the filamentous F phage, M13. This new phage we provisionally name an "I phage" to conform with "I pilus", the prototype of the second morphological class of sex pili.

The I phage lysed strains carrying de-repressed mutants of the fi-R factors, R64, R342, R144 and R163, but did not attack those with F, colV or de-repressed mutants of the fi+ factors, R1, R192 or R100-1; thus, its host range evidently comprised strains forming I-like sex pili. It was then tested for its ability to replicate in strains carrying a variety of wild-type plasmids isolated, so far as is known, from independent natural sources. They included colB and Hly, the haemolysin factor of E. coli, which are related to F (refs. 24-27), and colEIa (ref. 28), which is fi-and determines an I-like pilus^{12a}. Results are shown in Table 2, which includes tests on de-repressed mutants¹⁰ and those previously reported^{12b,14} using the F phage, MS2. Of the sixty strains, forty-seven fell into the first two groups.

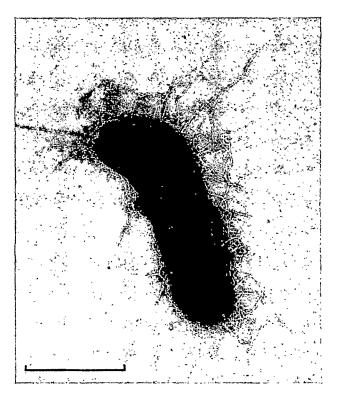


Fig. 1. A cell with an exceptionally large number of I-like sex pili from a culture of *Escherichia coli* K12 carrying a de-repressed mutant of the fi^- factor, R64. Calibration bar, $1\cdot 0\mu$.

The first, which propagated F but not I phage, contained most of the fi+ R factors, no fi- factors and plasmids like F_n -lac and colB. In contrast, the second group which propagated I but not F phage, contained only one strain with an ft^+ factor, twenty with ft^- factors and six carrying plasmids like colI and colEIa. The third group, containing five strains, propagated both phages. These strains and the single fi+ strain of the last group we suspect to carry two plasmids, one F-like, the other I-like. Such strains can easily be prepared in the laboratory; and the factors have already been separated in two of these five strains. The fourth class contained eight strains in which no replication of either F or I phage was detected, and these may contain a third kind of factor. This form of test, however, can give false negative results because the phage did not invariably increase in cultures of wild-type R64 unless HFT preparations were used. It was impossible to obtain HFT cultures with the six cultures which gave negative results, and independent experiments showed that the proportion of cells capable of donation was usually small $(10^{-4} - 10^{-5})$.

Table 2. Transmissible plasmids classified by their association with susceptibility to F and I phages

Susceptibility to*		R factors		Col factors and	
F phage	I phage	fi+ '	fi-	other plasmids	
+	****	11a	0	. 90	
	. +	1 ^b	20*	6h	
+	+	5¢ ,	0	0	
-	-	2d ;	61	0	

* Wild-type plasmids tested by measuring increase of phage in broth ltures. De-repressed (dr) mutants scored by presence or absence of visible cultures. De-relysis in lawns.

Key to plasmids: aR1, 28, 36, 51, 52, 82, 100-1dr, 124, 136, 192dr, 312

R1, 28, 36, 51, 52, 82, 100-1ar, 124, 150, 152ar, ...

R62

R2, 73, 77, 114, 196

R2, 7, 128

R3, 56, 64dr, 92, 342dr, 143, 144dr, 145, 163dr, 183, 293, 296, 297, 298, 299, 302, 306, 307, 308, 313

R45, 46, 199, 300, 305, 310

FF, F_r-lac, Hly, WG3, WG4, col V-K94, col V-K30, col B-K77, col B-K166

hcolla-CA53, colla-CT2, collb-P9, collb-CT4, col Ela-16, col Ela-18

Phage Adsorption

The mode of adsorption of F and I phages was examined by electron microscopy, using broth cultures fixed by addition of about 1 per cent (v/v) formalin. Filamentous phages of each group were almost indistinguishable in appearance from the pili to which they attached, and the junction between them had therefore to be revealed by labelling either pilus or phage. With filamentous F phages, an isometric F phage adsorbing along the pilus could be used2, but because no such phage is available for the I pilus, the pilus or the phage was labelled with specific antibody29.

All the phages attached only to sex pili. The two F phages, MS2 and M13, attached to the sides and tips, respectively, of pili of the F-like group but not to pili formed by the I-like group. The filamentous I phages, on the other hand, attached to the tips of pili of the I-like group but not to F-like pili (Table 3). Furthermore, every strain able to adsorb a given phage could propagate it. F₀-lac differed from the other members of the F-related group, for although it does not confer sensitivity to isometric F phages²⁰, Sanderson (personal communication) has shown that it confers sensitivity to the filamentous F phage, M13; as expected, only M13 was seen to adsorb to the Fo-lac pilus (unpublished work of Sanderson and

Table 3. REACTIONS OF SEX PILI WITH DONOR-SPECIFIC PHAGES AND WITH

,	Phages			Antiserum to		
Pili	F	I	F	col V	R1	R144
F	+	_	+	+	+	****
col V F-lac	+	_	+	+		
F-lac	+	-	+		+	
\mathbf{F}_{o} -lac	+	_	+			
R1 (fi+) colIb-P9	+	-	+	+	+	****
colIb-P9	_	+			_	+
R64 (fi-)	_	+	_			+
R144 (fi~)	_	+	-		_	+

-, No antibody seen on pili when serum used at a concentration considerably higher than that giving a + reaction with homologous pili.

Serology

The combination of antibody with sex pili can be studied fairly easily by electron microscopy because the filaments lie free of the bacterial body against the background provided by the specimen grid²⁹. Table 3 gives the reactions observed, and Fig. 2 shows the appearance of R1 pili treated with anti-F serum and of Ib pili with anti- \tilde{R} 144 serum. Antisera were prepared by immunizing rabbits with formolized suspensions of $E.\ coli\ K12$ carrying the various plasmids, while the pili were usually examined with the plasmids in Salmonella to avoid reactions with other bacterial antigens. It is clear from Table 3 that the pattern of positive and negative reactions was that to be expected from the morphological type and phage adsorptions of the sex pili. That is, pili determined by F, col V, Fo-lac and R1 showed cross reactions with each other but not with pili determined by collb or fi-R factors, while the latter showed cross reactions with each other

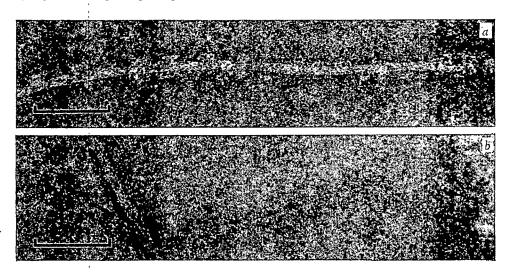


Fig. 2. Sex pill with antibody attached. a, Pilus determined by the f_i^+ factor, R1-16, with anti-F serum. b, I pilus with anti-R144 serum. Note that no antibody is seen on the flagellum. Calibration bars, 0.1μ .

and not with the first group. F_c-lac (ref. 20) and R100-1 (ref. 30) are known from agglutination tests to be antigenically related to F.

Cross reactions with unabsorbed sera demonstrated a relationship within each of the two groups, but to determine the degree of identity cross absorption tests were required. Table 3 shows that anti-F serum combined with R1 pili as well as with F pili. The two types of pili were nevertheless not identical antigenically, for absorption of anti-F serum by bacteria carrying de-repressed R1 considerably diminished the reaction with R1 pili without affecting that with F. In the reciprocal test, where anti-R1 serum was adsorbed with F+ bacteria, the reactions with both R and F pili seemed to be equally diminished. Thus it appears that there is an antigen common to both types of pilus, but that F pili have an extra antigenic component, not represented in R1.

Conclusions

Our principal conclusion is that most sex pili fall into two groups, typified by F and I pili, respectively, whether they are classified by morphology, phage adsorption or antigenic structure. This is so, although the pili are determined by a variety of different plasmids. Thus the same class of sex factor appears to be present in F-lac and in fi+ R factors, although the former is detected by ability to ferment lactose and the latter by drug resistance. The implication is that, although plasmids may manifest themselves in different ways according to the functions they determine and by whether or not their conjugation function is repressed, their sex factors are generally related to F or to the sex factor of colicin factor Ib.

Many plasmids, like colEI-30 (ref. 31), the salmonellin factor³², P-lac of Proteus³³ and the conjugation factor of Serratia³⁴ have still to be examined, as have those of the unrelated genera, Vibrio³⁵ and Pseudomonas³⁶. particularly important that more fi-R factors be tested, because these are defined solely by the negative criterion of a failure to repress F. When classified by other criteria, this group may prove to contain a number of unrelated sex factors with only this negative characteristic in common. Table 2 summarizes the tests so far made on sixty transmissible plasmids of various kinds which show that at least fifty-two are related to F or to the sex factor of collb. The pili determined by two related factors like F and R1 show slight differences in antigenic structure, but long-established clones of a given factor may well come to vary slightly with the passage of time, and relatively minor differences of this kind do not nullify an otherwise

strong taxonomic relationship. Even if further sex factors come to be identified in Enterobacteriaceae, it seems probable that F and the factor of collb-P9 will still be seen to predominate within the group.

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Lethal Unbalanced Growth in Bacteria

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The lethal effect of unbalanced growth following damage by ionizing radiation or thymidine starvation is attributed to loss of ability to synthesize an outer cell wall that is strong enough for its essential role in cell division.

TREATMENTS which stop DNA replication but not RNA and protein synthesis—such as ultraviolet or X-irradiation or thymine starvation—often lead to the formation of filamentous cells which can grow by elongation but cannot divide. Filaments can also be induced by some agents which do not directly affect DNA synthesis—for example, penicillin1, crystal violet2, D-amino-acids3 or

magnesium deficiency4. I describe here a hypothesis which ascribes the cause of death in these conditions to inadequate cell wall synthesis resulting from unbalanced

The cell wall in Bacillus subtilis is seen in electron micrographs to consist of three parts: a relatively thick outer wall, an inner wall and membranous structures

continuous with the inner wall (mesosomes). Ryter and Jacob' have shown that the nuclei are often (possibly always) attached to mesosomes at one point. In addition, mesosomes appear to be attached to the septum.

The outer wall is composed of a polymer of three or four amino-acids, acetyl glucosamine and acetyl muramic acid. It is not an osmotic barrier. The inner wall (cytoplasmic membrane) has a high lipid content, is flexible and is an osmotic barrier. The cytoplasmic membrane alone is too weak to resist the osmotic pressure of the cell contents which is of the order of six atmospheres or more, but it is normally supported by the outer wall, which must therefore be very strong and rigid to withstand the high pressure. B. subtilis is typical of Gram positive bacteria. Gram negative bacteria such as E. coli have a rather different cell wall composition. Nevertheless, an outer wall, a cytoplasmic membrane and mesosomes can be seen^{6,7}.

Wall synthesis occurs in two situations: (a) when the cell elongates and the nuclei segregate and (b) when the septum and cross-wall form. Jacob, Brenner and Cuzin⁸ suggested that wall synthesis is co-ordinated with chromosome replication so that, on completion of a cycle of DNA replication, growth of the wall between the points of attachment of the new pair of chromosomes is triggered, thus segregating the nuclei. In the simplest case, DNA replication takes place at the equator of the cell and is followed about one generation later by division at the equator; so an attractive idea in considering what determines the site of septum formation is that one and the same wall priming site may serve first to extend the wall, segregating the nuclei, and then later to form the septum.

The cycle of division and replication can then be visualized as in Fig. 1. The replicative apparatus of the chromosome occupies a site on the inner wall, which is potentially a "primer region" for wall synthesis. At the end of a cycle or replication, the wall primer site is uncovered and synthesis of both inner and outer walls begins. This results at first in elongation; later, the rate of outer wall synthesis (surface area/sec) fails to keep pace with the rate of inner wall synthesis and the inner wall invaginates. This is the essential first step in septum and crosswall formation. Finally, the cross-wall forms and the cell divides.

The role of the outer wall in creating conditions permitting septum formation can be assessed in *B. subtilis* because it is possible to remove the outer wall completely by digestion with lysozyme. In an osmotically stabilized medium, protoplasts are formed which retain all the properties of normal cells, with two exceptions: in liquid media they cannot divide and they cannot regenerate their outer wall.

These considerations suggest the following about the relation of DNA damage to filament formation.

- (1) Failure of invagination of the inner wall leads to filament formation and is caused by mechanical weakness of the outer wall resulting from inadequate synthesis of the outer cell wall material.
- (2) Inadequate synthesis of the outer cell wall is a consequence of blocked DNA replication. Hypothesis (1) is based on work which has shown that although B. subtilis protoplasts cannot divide in liquid media, they can divide in solid media. Division in moderately soft agar was inefficient; the DNA and cytoplasm were shared very erratically between the daughter "cells" and mesosomes were not visible in the dividing protoplasts. On hard agar or gelatine, however, protoplasts were able to revert to the bacillary form, complete with outer wall and mesosomes.

That hard agar can successfully reverse the inhibition of division caused by loss of the outer wall implies a role for the outer wall which hinges upon the only common property of the outer wall and the agar—their rigi-

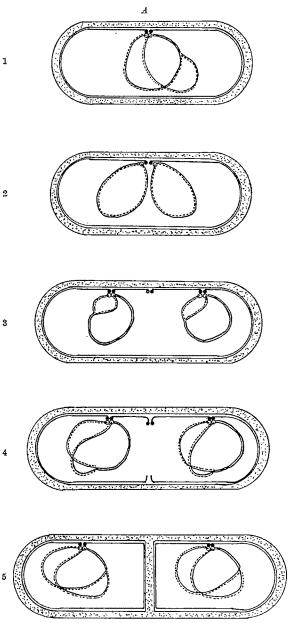


Fig. 1. ○○, DNA polymerase; ●●, wall primer site. For clarity the mesosome is not shown. (1) DNA replication in progress. (2) Replication cycle ended. Wall primer sites ●● exposed. Wall synthesis begins by sequential addition of units at A. The locus of the growth points relative to the cell is a helix of low pitch. (3) Wall continues to grow at A. Elongation of cell and segregation of nuclei. Meanwhile a new round of replication is initiated. (4) Rate of outer wall synthesis fails to keep pace with that of inner wall. Result is infolding of the membrane at A—the first step in septum formation. The mode of growth of the septum is a flat concentric spiral. (5) Septum and cross-wall completed.

dity. This was suggested by Necas^{11,12} and is illustrated clearly in his experiments on yeast protoplasts, which would divide and form colonies when suspended in 15 per cent gelatine but not when placed on it. Similarly, Lederberg and St. Clair¹³ obtained L colonies of E. coli spheroplasts only in submerged culture in solid medium. As discussed by Landman et al.^{9,10}, the stimulus for division of E. subtilis protoplasts seems to be the invagination of the protoplast membrane and it follows from their experiments that invagination is not only necessary but sufficient to trigger septum and cross-wall formation. (No doubt intermediate steps could occur, for example: invagination—produces mesosome—wall primer site—wall extension.)

Invagination of the inner membrane can only occur if the rate of extension of the outer wall (surface area/sec) is less than that of the inner wall. If the outer wall is absent (protoplast) or weak (as in a filament) the inner wall will be permanently distended by the osmotic pressure of the cytoplasm, so that no invagination and thus no division will be possible.

A filament, according to this interpretation, is in a state very similar to that of a protoplast in liquid medium in that it can carry out all the processes of cell metabolism but cannot divide. There is experimental evidence to support this view. Grula and Grula¹⁴ noted that a strain of *Erwinia* formed filaments and spheroplasts simultaneously in some conditions and they concluded that filaments are partial spheroplasts. Chemical analysis of the cell walls of these filaments showed that they contained 30–40 per cent less acetyl glucosamine and muramic acid for each unit of cell mass than normal cells. Further evidence about the failure of filamentous cells to form rigid cell walls has been discussed by Howard Flanders et al.¹⁵.

As presented, hypothesis (1) rests on one interpretation of Landman's protoplast reversion experiments: that the mechanical resistance of the agar or gelatine facilitates membrane invagination and leads eventually to cell division. There is another tenable hypothesis, considered by the same authors: that the hard agar prevents cell wall enzymes and precursors from diffusing away from where they are needed at the exterior surface of the membrane. The model for filament formation described here can readily be translated into these terms (invagination = prevention of diffusion; cell wall weakness = cell wall porosity).

Hypothesis (2) implies a direct relationship between DNA synthesis and outer wall synthesis for which evidence is so far lacking and which requires investigation. There are, a priori, two ways in which DNA synthesis could regulate cell wall synthesis: (a) by controlling the availability of cell wall precursors; and (b) by controlling the availability of wall primer sites.

Regulation of type (a) could be exerted through feedback mechanisms linking the concentration of DNA precursors with the concentration of conjugated uridine and thymidine nucleotides available for wall synthesis. Regulation of type (b) is implied in the scheme for cell division previously outlined (Fig. 1) in which a block in DNA synthesis would prevent the initiation of new sites of cell wall synthesis. Wall synthesis may then either diminish (which will happen if the synthesis occurs in cycles) or remain constant; meanwhile the capacity for RNA and protein synthesis may actually increase as the cell's complement of ribosomes increases to the outer wall and inhibited division.

Weakness or porosity of the outer wall and consequent inhibited division could be caused by any agent (for example penicillin) which selectively inhibits outer wall synthesis. Filaments produced in this way, however, differ from those produced by specific inhibition of DNA synthesis in an important way: the ratio of DNA to cell volume is not changed by selective inhibition of outer wall synthesis. In such filaments the capacity to make a normal cell wall is not lost but merely suppressed, and if the agent is removed division may resume if conditions are favourable. It has been suggested here, on the other hand, that a block in DNA synthesis leads to a reduced capacity for wall synthesis compared with the capacity for other macromolecular synthesis; and removal of the block will not immediately restore this imbalance. It may therefore be useful to distinguish two types of inhibited division: one reversible, in which the ratio of DNA to cell volume is ndar normal and one irreversible, in which the ratio of DNA/cell volume has fallenbelow a critical level.

Some bacterial strains with a particularly strong tendency to form filaments (E. coli B (refs. 1, 17, 18), E. coli B₁₁₁ fil⁺²,

E. coli K12 lon⁻¹⁵) have been studied because they are also unusually sensitive to ultraviolet and X-irradiation. Many of the properties of these strains can be accounted for according to the scheme presented here if it is supposed that the fil⁺ gene is concerned with some aspect of synthesis of the outer wall and that fil⁺ strains even when growing normally make a cell wall the strength and rigidity of which are near the minimum needed for cell division. Being already rather weak walled, such cells would be especially vulnerable to any agent which restricts outer wall synthesis either directly by interfering with the supply or utilization of precursors or indirectly by inhibiting DNA replication. They would pass very easily to the "defective outer wall" state with inhibited division.

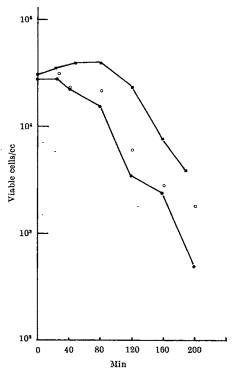


Fig. 2. Comparison of the sensitivity to thymineless death of *E. coli* Bs thy- (fil-, her-, radiation sensitive) and *E. coli* B3r thy- (fil-, her-, radiation resistant). The bacteria were grown in Spizizen minimal medium with 20 µg of thymidine/ml. to a density of 5 × 10⁸ ml., then diluted 10,000 times into minimal medium with no thymidine and plated at intervals on 'Oxoid' nutrient broth with or without 1-2 per cent pantoyl lactone. • • • E. coli Bs thy-; OO, *E. coli* Bs thy, pantoyl lactone plates; • • E. coli B3r thy-.

On this basis, the somewhat paradoxical properties of fil⁺ strains summarized by Kneser¹⁸ can be explained as follows. (a) Filaments are formed which are unable to divide^{1,17,19}. (b) The difference in sensitivity to ultraviolet light between growing and resting bacteria is much more pronounced in fil⁺ than in fil⁻ strains¹⁸. (c) Fil⁺ strains are very sensitive to agents which specifically attack DNA. This sensitivity seems to have nothing to do with the excision repair enzymes, because E. coli B excises thymine dimers as efficiently as E. coli Br (ref. 20). (d) The recovery of fil⁺ strains can be enhanced by a variety of post-irradiation treatments which either slow down growth or speed up excision repair (this process was termed K reactivation by Kneser). Such "rescue" conditions affect only lethal lesions. Neither ultraviolet mutagenesis, phage inactivation, prophage induction nor other macromolecular synthesis is affected. (e) Non-specific treatments can prevent inhibition of division caused by agents which specifically attack DNA^{17,21}. (f) The same genetic locus can control simultaneously radiation sensitivity and filament forming ability^{1,15,22}. (g) The fil gene in E. coli

K12 (where it is termed lon) is involved in polysaccharide synthesis or is closely linked to another gene that is 15,22. (h) Filaments can readily be induced in fil+, radiation sensitive strains by agents affecting not DNA synthesis but cell wall synthesis (penicillin, crystal violet^{1,2}). (i) In some radiation resistant revertants of E. coli B obtained by Witkin, the acquisition of resistance to radiation was accompanied by the acquisition of resistance to penicillin, which causes filament formation in E. coli B1.

It has been suggested (hypothesis (2)) that there is a direct relationship between DNA synthesis and cell wall synthesis; also, that fil+ strains are especially sensitive to inhibition of cell wall synthesis. It follows that fil+ strains should exhibit enhanced sensitivity to anything which blocks DNA synthesis; in particular, they should be more sensitive to ultraviolet irradiation and to thymine starvation than fil-strains. Furthermore, if lethal damage in thymineless death affects the cell wall, not DNA, excision repair enzymes should not play any part and rec+ or rec-strains should be equally sensitive to thymineless death but not to ultraviolet irradiation.

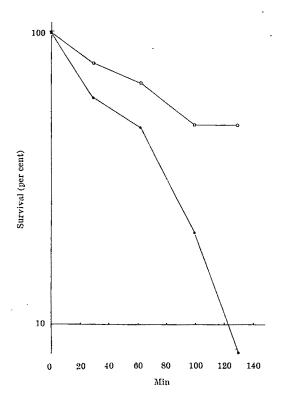


Fig. 3. Protection by pantoyl lactone against thymineless death. After growth in Spizizen minimal medium with 20 μg of thymidine/ml., log phase E. coti Bs thy- were rapidly filtered, washed and transferred at a density of about 10' cells/ml. to: (1) minimal medium without thymidine (••); (2) minimal medium without thymidine but with 1.2 per cent pantoyl lactone (ΟΟ). Suitable dilutions were plated on 'Oxoid' nutrient broth plates supplemented with 10 μg/ml. of thymidine with or without pantoyl lactone (1.2 per cent).

These predictions are in agreement with the results of Cummings and Taylor²³ in their study of the comparative sensitivities of various E. coli mutants to ultraviolet light, mitomycin C and thymine starvation. Also, as expected, E. coli B, thy-(fil+) is more sensitive to thymineless death than the fil-strain B3r thy- (Fig. 2). E. coli B_s thy, however, does not seem to be as sensitive to thymine starvation as the thymineless mutant of E. coli B isolated by Cummings and Taylor.

If the foregoing interpretation of the pattern of thymineless death and ultraviolet sensitivity in fil+ and filstrains is correct, thymine starvation of a fil-strain should reduce its reserve capacity for cell wall synthesis and so induce a state of instability similar to that which has been supposed to be always present in fil+ strains. Thus the model predicts that the curve of ultraviolet dose against survival of a fil-strain irradiated after a period of thymine deprivation should be of the form normally encountered in fil+ strains. This is exactly what was found by Gallant and Suskind²⁴.

Similarly, Fuerst and Stent²⁵ found that thymine starvation sensitized E. coli 15T- to the effects of the decay of phosphorus-32 incorporated in DNA. They inferred from this that thymineless death was a "nuclear inactivation". The suggestion here is rather that damage to the DNA caused by phosphorus-32 tipped the scales toward lethal unbalanced growth and cell wall weakness when the bacteria had already been sensitized (that is partly unbalanced) by previous thymine starvation.

X-ray and ultraviolet damage to fil+ strains can be reduced by plating with pantoyl lactone 18,28. If the relation between the effects of thymine deficiency and ultraviolet damage is as has been suggested, it might be thought that cells suffering thymineless death should also be protected by pantoyl lactone. When added to the culture medium, pantoyl lactone can protect against thymineless death (Fig. 3). When added only to the plating medium, however, there is less protection (Fig. 2). This is consistent with the idea that pantoyl lactone added to plating media protects chiefly by preventing the development of a state of imbalance rather than by reversing a pre-existent state.

In this discussion evidence has been put forward to support the notion that for some cell strains defective cell wall synthesis may be a common factor in death by ultraviolet irradiation and thymine starvation. I think that sufficient evidence exists to make it a useful working hypothesis; nevertheless, some complicating factors have been ignored, notably the possible role of prophage induction, and the premature initiation of a cycle of DNA replication after a block has been imposed27-29. To this extent the model, like most models, is oversimplified.

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Dispersal of Tropical Marine Shore Animals: Coriolis Parameters or Competition?

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Contrary to recent supposition, species gradients cannot be relied on to indicate the direction of successful migrations by tropical shore animals or the direction of the principal ocean currents.

In the low latitude regions of both the Atlantic and Pacific Oceans, the North and South Equatorial Currents move the surface waters in a westerly direction. By comparison, the equatorial countercurrents are weakly developed and move only small amounts of water. The question is whether the predominantly westward movement of tropical waters means that a successful dispersal of shelf animals takes place in the same direction. Theoretically, it may seem logical, and this is the assumption made by Professor H. B. Fell in his article¹ relating both present and fossil distributions to Coriolis (major oceanic current) effects.

As support for his assumption, Fell noted that the molluscan genus Voluta and other groups demonstrated speciation gradients in which the number of species gradually diminished around the world in a westward direction. For the purpose of comparison, I shall examine the distribution of the tropical shore fish genus Entomacrodus. Here, there is also a diminution in the number of species in the same direction: the western Pacific has sixteen or seventeen species; the Indian Ocean has six or seven; the eastern Atlantic has two; the western Atlantic has two, and the eastern Pacific has one. Entomacrodus, however, has just been subjected to a careful systematic study². The relationship of the species indicated that the genus originated in the western Pacific but migrated from there eastward across the Pacific to the New World and then eastward across the Atlantic to West Africa.

Is there any other evidence of successful eastward migrations across the Atlantic and Pacific? Studies on the shore fish fauna in general's have shown that the East Pacific Barrier, the extensive deep-water area that lies between Polynesia and America, has been successfully crossed (migration followed by apparent colonization) by about sixty-two species. Circumstantial evidence was given to show that this was entirely an eastward colonization movement. Apparently, species belonging to typical New World genera have been completely unable to colonize in the opposite direction. At least some of the shore invertebrate groups apparently behave in the same way.

The Mid-Atlantic Barrier is the deep-water area separating the western Atlantic tropics from those of the West African coast. Apart from a group of about twenty-four shore fishes which apparently make their way from the Indian Ocean around the Cape of Good Hope and then westward across the Atlantic, the predominant migratory movement seems to be from west to east4. Many of the transatlantic species range broadly along the western Atlantic shelf but have attained only limited purchase in the east. Others that have achieved broad distributions in the east are clearly representatives of American genera. None of the transatlantic species belongs to genera which are typically eastern Atlantic. There are about one hundred and eighteen such species and they comprise about 30 per cent of the shore fish fauna of tropical West Recent works on West African invertebrate groups also show that an appreciable percentage of the species is transatlantic.

It may be concluded, on the basis of strong evidence for the shore fishes and at least some similar indications for the shore invertebrates, that successful migrations across the Atlantic and Pacific Oceans usually take place in an eastward direction opposite to the flow of the North and South Equatorial Currents. How can this apparently anomalous occurrence be explained? It has been observed

that the region of the Indian and West Pacific oceans has served as the evolutionary and distributional centre for the entire marine tropics4. It seems clear that the unusually stable ecosystems and high level of competition provide the proper environment for the evolution of dominant species that can successfully invade the other regions.

From the Indo-West Pacific, dominant species migrate across the open ocean to America, westward around the Cape of Good Hope into the Atlantic, and northward through the Suez Canal into the Mediterranean. Successful reciprocal migrations are, at least, very rare and may be completely lacking. To judge from the general indications of relationship among the four great tropical marine faunas (Indo-West Pacific, eastern Pacific, eastern Atlantic, western Atlantic), this process has been going on for many millions of years. In the shore fishes, for example, virtually all of the tropical families and most of the genera are probably of Indo-West Pacific origin. Some of the dominant species are so successful that they have been able to establish and maintain circumtropical distributions.

The western Atlantic tropics may be considered a secondary centre of evolutionary radiation. Many species produced in this area have proved capable of migrating eastward to colonize the eastern Atlantic region. Species originating in the eastern Atlantic, however, are apparently incapable of successfully invading the western side. Again, the advantage seems to lie with the area that possesses the richer fauna and higher level of competition. Such movements seem to illustrate well a basic zoogeographic concept which received its modern emphasis from Matthew6—that dominant species arise in certain important centres and gradually become dispersed into the peripheral areas.

The existence of gradients in numbers of species (or genera) across the major barriers may mean only that certain areas have offered more opportunity for evolutionary radiation than others. In the tropical Atlantic and Pacific there is certainly not a positive correlation between the direction of the major currents and the successful migration of shore species across the midoceanic barriers. The Indian Ocean is not separated from the western Pacific by a major zoogeographic barrier and the fauna of the East African coast may be considered an attenuation from that of the Indo-Australian Archipelago. For most groups, there is a decreasing species gradient extending from the latter area westward across the Indian Ocean as well as eastward into the island groups of Polynesia.

In conclusion, I wish to observe that in the tropical oceans the degree of biological competition is probably a more important factor in the dispersal of shore animals than the direction of the major currents. As the direction of the species gradient in recent, tropical animal groups does not necessarily indicate the direction of successful migrations or major currents, there is no reason to believe that fossil gradients can be relied on to give us such information for past epochs.

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LETTERS TO THE EDITOR

ASTRONOMY

On the Space Distribution of Identified Quasi-stellar Objects and Radiogalaxies

The ratio of identified quasi-stellar radio sources to identified radiogalaxies in the 3CR catalogue is about 1:2. As the identified sources constitute a large fraction of all the extragalactic sources in the catalogue, and redshifts have been obtained for a considerable fraction, it is clear that the complete identification of all such sources could not change this ratio in a very significant way. It follows therefore that, independent of any optical selection effect, the number of quasi-stellar radio sources is approximately comparable with the number of radiogalaxies down to a radio flux of 9 units at 178 Mc/s.

The redshifts of the quasi-stellar radio sources extend up to about 2 where a limiting value seems to exist. The redshifts of the radiogalaxies in question extend only up to about 0.25, however. Of the sixty-five 3CR radiogalaxies for which redshifts are known, only two, 3C 109 and 3C 295, have shifts greater than this. This means that if we accept a cosmological interpretation of all such redshifts, the quasi-stellar radio sources occupy a spatial volume about a thousand times greater than that occupied by the radiogalaxies. The approximate equality of numbers has then to be explained on the basis that the density of radiogalaxies is about a thousand times greater than the density of the quasi-stellar radio sources. It then appears fortuitous that these two large factors should compensate each other. The situation appears particularly odd in view of the similarities that seem to exist between quasistellar objects and the nuclei of Seyfert galaxies (for example, the high-frequency radio properties of NGC 1275 and 3C 279 and the infrared flux in NGC 1068 and 3C $(273B)^{2.3}$ and between quasi-stellar objects and N-type galaxies (ref. 4; also Oke, J. B., private communication).

The cosmological interpretation of the redshifts of quasi-stellar objects is not supported by the form of the redshift-magnitude plot, at optical and at radio frequencies⁵. The redshift-magnitude plot at optical frequencies becomes a scatter diagram when a $(1+z)^3$ correction for redshift effects is applied⁶, whereas at radio frequencies the plot is already a scatter diagram before such a correction⁵. This is consistent with a gravitational rather than a cosmological interpretation of the redshifts, provided that the low frequency radio emission is considered to arise in a more extended region than the optical emission? It is important that these features appear in the identified sources in the 3CR catalogue, so that the optical features can hardly be said to arise from an optical selection effect.

For sources of very small angular size there does appear to be a correlation between radio flux and redshift⁶; this is consistent with what is found at optical wavelengths for if the radio emission arises in very small volumes, the intrinsic redshift corrections should be put in as for optical emission, and they will tend to give a scatter diagram again.

All these considerations suggest the hypothesis that the quasi-stellar objects and the radiogalaxies are members of the same family of phenomena, and that they occupy similar volumes of space. On this hypothesis the total redshift, z, of a quasi-stellar object would be given by

$$1 + z = (1 + z_c)(1 + z_g) \tag{1}$$

where z_c and z_g are shifts due to the expansion of the universe and to local gravitation, respectively. A similar equation applies in principle for the radiogalaxies. There is, however, no evidence to suggest that z_g for radiogalaxies is much different from zero. So z_c for the radiogalaxies in question extends up to about 0.25. In accordance therefore with our hypothesis, z_c for the quasi-stellar objects extends up to about 0.25. Quasi-stellar objects with small redshifts could be at distances comparable with those required by a cosmological interpretation of the total redshift z_c , but quasi-stellar objects of large z must have $z_g \approx z$.

If one were to survey all sources within z_c about 0.25, most sources would be expected to have z_c near the maximum of 0.25, simply because of volume availability. There would be occasional sources however, at comparatively close distances. If for the moment we assume that all the sources are intrinsically similar, the occasional nearby sources would appear anomalously bright. In Fig. 1 we give the plot of visual magnitude against z for 104 quasistellar objects. Evidently 3C 273 is an exceptional case. On our hypothesis this can be understood if 3C 273 is exceptionally close so that $z \approx z_g$ for this object.

Consider now two quasi-stellar objects with the same z_c , that is to say at the same distance but with different z_g . We expect the object with the larger z_g to appear fainter, in part because of the factor $(1+z_g)^{-2}$ and in part because larger z_g implies a more compact structure with less volume available for a central emission cloud. The assumption that all quasi-stellar objects are intrinsically similar is therefore unlikely to be true. It seems likely that quasi-stellar objects of large z_g will be intrinsically fainter than those of small z_g . This leads to the curious situation that quasi-stellar objects of large z_g , that is, large z_g , may well be systematically closer than quasi-stellar objects of small z.

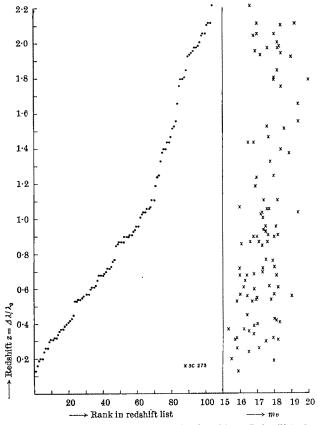


Fig. 1. Distribution of redshifts of quasi-stellar objects. Left: Plot of redshift against rank of quasi-stellar objects in redshift list in order of increasing redshift (1 to 104); right: plot of redshift against apparent V magnitude. Note that 3 $\mathcal O$ 273 at $m_v = 12.8$ belongs to right-hand side of diagram.)

We now arrive at a model in which radiogalaxies and the quasi-stellar objects behave in opposite senses with respect to z. The distances of radiogalaxies increase with z. For the sample for which redshifts are currently available the most distant radiogalaxies so far identified are very frequently N-type and z is about 0.25. The quasi-stellar objects, on the other hand, have distances that decrease with increasing z. The most distant are the ones of small z, while those with z about 2 are comparatively close by. In this picture the greatest distances involved are about 700 Mparsec. The quasi-stellar objects of largest redshift could be at distances of about 50 Mparsec or less. Indeed, the discovery that all the quasi-stellar objects which have absorption lines in their spectra have absorption line redshifts either very close to 1.95 or approximately equal to the emission line redshifts has led to the conclusion that for the objects with z = 1.95 the contribution due to z_c must be exceedingly small, so that a limit to the distances of these objects of about 40 Mparsec is found. Only where a standard spectrum at z = 1.95 is clearly visible can we be sure that this limit is present, but it may be that all of the quasi-stellar objects with large redshifts are comparatively close by.

This model is consistent with there being fewer quasistellar objects of large z than small z. The volume effects are in the correct sense to agree with the distribution shown in Fig. 1, where the usual relation of z to distance would lead us to expect the opposite behaviour.

What is new in the present model compared with previous concepts of a local theory of quasi-stellar objects is that z instead of being uncorrelated with distance is correlated, but in an opposite sense to what is usual.

What will such a model predict for the sources which have not been identified? It is possible that some quasistellar objects have such large intrinsic redshifts that they are not visible optically at all, but because the low frequency radio sources which they have ejected arise in an extended volume, their emissivity in these frequencies is not cut down. These may be the "empty field" or "optic-ally quiet" quasi-stellar objects. Some of the fainter radio sources in the 4C or Parkes catalogues may be quasi-stellar objects at distances corresponding to values of z_c significantly greater than 0.3. Except in special cases, however, we can only expect to detect such sources if they have modest values of z_g , because for values of z_g about 2 they will be too faint to be seen. Thus objects with $(1+z)\gg 3$ will be exceedingly rare.

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PLANETARY SCIENCE

Polonium-210 in Marine Plankton

THE occurrence of polonium-210 and its precursor lead-210 in the natural radiation environment has been studied increasingly during the last decade. Radon emanating

from materials in the Earth's crust introduces lead-210 into the atmosphere and there is then a natural fallout of lead-210 and its daughters. These nuclides are then incorporated into natural samples in the hydrosphere and lithosphere. Papers by Burton and Stewart¹, Patterson and Lockhart² and Peirson et al.³ provide data for lead-210 and polonium-210 in air and in rainwater. Hill4 has discussed in detail the concentrations of polonium-210 and lead-210 in foodstuffs, tobaccos and human tissues from various environments, and additional data have been provided by Hill⁵, Holtzmann⁶, Little and McGandy⁷ and others.

Very little work appears to have been done on polonium-210 or lead-210 in the marine environment. Rama et al.8 reported lead-210 values in seawater samples from the Pacific, and Goldberg⁹ extended these data. Cherry¹⁰ reported unsupported polonium-210 at a level of 17 pc./g of dried material in a single sample of marine zooplankton, and Beasley and Palmer¹¹ studied polonium-210 in a few marine samples from Alaska. The primary purpose of the present work was to establish the general level of polonium-210 in marine plankton samples and to this end we made alpha counting measurements on ninety-eight plankton samples obtained from the seas around the Cape of Good Hope during 1965 and 1966. Full details of the sample localities and further particulars will be available elsewhere12.

The experimental techniques were essentially the same as those used previously in this laboratory10; the total alpha activity of a sealed sample and the thorium-series contribution to this were measured and, by subtraction, the uranium-series activity was obtained. The variation with time of this activity was studied. Seventy-three of the seventy-eight zooplankton samples and all twenty of the phytoplankton samples showed a decrease in uranium-series activity on a time-scale of the order of several months. We were consistently able to interpret this decrease as a result of unsupported 138-day polonium-210, and the amount of unsupported polonium-210 present in the samples at the time of collection was calculated by extrapolation.

The results can be summarized as follows. shows, in histogram form, the unsupported polonium-210 content of the ninety-eight samples measured. A modal value of about 3 pc./g of dry plankton is seen to result. Dividing this by an approximate concentration factor of 15 gives an in vivo (wet plankton) concentration of about 200 pc./kg. The unsupported polonium-210 activity in our plankton samples is thus more than an order of magnitude greater than the total polonium-210 reported

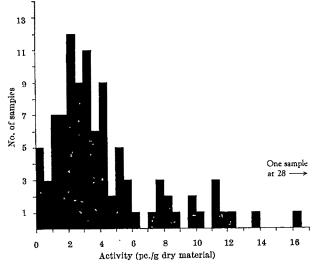


Fig. 1. Frequency distribution of unsupported polonium-210 activity for ninety-eight plankton samples at the time of collection.

in human soft tissues. It is worth noting that the radiation dose to plankton from unsupported polonium-210 at the level quoted will be about 200 mrem/yr. This is to be compared with a total background dose to man from all sources of about 130 mrem/yr. We have, in addition, determined unsupported polonium-210 in four samples of (whole) pelagic fish and find a range of 0.7-3.3 pc./g of dry weight, with a mean of 2.0 pc./g. The wet/dry weight concentration factor is now only 3.3, however, which gives a wet fish tissue concentration of about three times the plankton figure and more than an order of magnitude higher than the values quoted by Beasley and Palmer¹¹ for polonium-210 in salmon, butterfish and whitefish.

The ninety-eight plankton samples showed in addition a time-independent alpha-activity. This activity, which showed a mean value of 2.3 pc./g of dry plankton, is principally due to uranium, radium and daughters (excluding unsupported polonium-210), and, in some cases, thorium-series contributions. The mean value is in general agreement with previous data¹⁰; the contribution of supported polonium-210 to this mean is uncertain but is probably not more than 0.2 pc./g. It thus seems clear that the bulk of the polonium-210 activity, in the marine plankton samples measured, is unsupported by its parent, lead-210, and it follows that the lead-210 to polonium-210 activity ratio must be less than unity in plankton. We are not aware of any published data giving both lead-210 and polonium-210 activities in seawater, but it would be surprising if the lead-210 to polonium-210 activity ratio was not greater than unity in accordance with the general fallout pattern¹⁻³. We conclude that the enrichment factor from seawater to plankton must be larger for polonium-210 than it is for lead-210. Using the values of Rama et al.8 for lead-210 in seawater, namely about 5×10^{-5} pc./g, and combining this with the data given here, we see that an enrichment factor greater than 4,000 results for polonium-210; for lead-210 the enrichment factor must be less than this figure.

The mean unsupported polonium-210 activity in the seventy-eight zooplankton samples was 4.7 pc./g of dry weight, while in the twenty phytoplankton samples it was 2.8 pc./g.

Reference to Fig. 1 shows that eleven samples had values greater than 9 pc./g. It is interesting to note that six of these samples were obtained from localities more than 70 miles away from the coast. As eighty-two of the ninety-eight samples measured were from localities less than 70 miles offshore, it would seem that there is a tendency for offshore samples to contain higher unsupported polonium-210 activity. A possible explanation could lie in the fact that much of the inshore surface water off the west coast of the Cape of Good Hope is upwelled water of Atlantic central water origin¹³. Such water has, in general, less time in contact with the atmosphere than the subtropical surface water found further offshore; because the polonium-210 is presumably derived chiefly from atmospheric fallout a lower polonium-210 content in the inshore waters seems reasonable. An attempt to investigate this correlation in terms of the water masses involved has so far yielded inconclusive results, but it is still worthwhile to draw attention to the possible use of polonium-210 as a natural oceanographic tracer.

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Lead and Lead-210 in Rainwater

THERE is a continuing interest in the possible toxicity to man and animals from the present and future concentrations of lead in the environment. The importance of environmental lead was emphasized by Monier-Williams in 1938 (ref. 1), and has since resulted in numerous studies2-4.

Lead exists in the atmosphere as an aerosol which contributes to the body content of lead in man4. Knowing the mean residence time of the lead aerosol in the atmosphere is important, because for any given set of meteorological conditions and a fixed rate of injection, the atmospheric concentration will be an inverse function of the residence time.

Lead-210 has a half-life of 21.4 yr and would be an ideal tracer for stable lead if lead and lead-210 were acted on by the same forces. Because the mean residence time of the lead-210 can be estimated from the ratio of its specific activity to that of one of its decay products, bismuth-210 or polonium-210⁵⁻⁸, this residence time would also apply to stable lead. There are both differences and similarities in the mode of injection of lead and lead-210 into the atmosphere: lead-210 is derived from the decay of radon-222 (a noble gas) which escapes from the soil and is dispersed throughout the atmosphere by winds and convection, while lead aerosols originate from automobile exhaust, in smelter and other industrial operations. The source of both stable lead and lead-210 is at, or very near, the Earth's surface. Except for regional differences in the rates of injection of lead and lead-210 the sites of injection seem very similar, so that both may experience the same meteorological conditions. Both are returned to Earth chiefly by the cleansing action of rainfall from altitudes between 1 and 10 km (ref. 10).

Although fairly extensive measurements of the concentrations of lead-210 in rainwater have been reported5-8, little is known of the concentrations of stable lead11. Furthermore, the concentrations of lead and lead-210 have not been reported from the same samples.

The aim of this study was to evaluate the relationship between lead and lead-210 in the atmosphere. Rainwater was selected because it samples a large volume of air at high altitudes and thereby minimizes effects from local sources. Samples of rainwater were collected on the roof of the physics building at Argonne National Laboratory, and on a farm in Ottawa County, Michigan. The first site is located in a semi-rural area 25 miles west of Chicago and the second is located in an area of farms 125 miles north-east of the Argonne site. This latter site was located 1,200 ft. from the nearest roadway and 15 miles from the nearest city (pop. 25,000). Consecutive samples of rainwater were collected at Argonne from July 1 to September 14, 1966. All samples were collected in porcelain coated steel pans which had been cleaned of dirt and dust immediately before collection of the rainwater. The samples, of between 1 and 6 l., were filtered through a fritted glass filter before analysis. Analyses of both portions of the sample showed that less than 10 per cent of the lead was in the solids retained on the filter. The data in Tables 1 and 2 are for the materials in the soluble portion of the rainwater. The samples were stored in

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Table 1. LEAD AND LEAD-210 IN RAINWATER AT ARGONNE NATIONAL LABORATORY DURING THE SUMMER OF 1966

Date	Precipita- tion (mm)	Pb (μg/l.)	²¹⁰ Pb (pc./l.)	Specific activity $(pc./g \times 10^{-4})$
July 1	18-S	10.6	1.08	10.2
July 11	15-8	9.2	0.63	6.8
July 13	10-S	4.6	0.28	6.1
July 26–27	106-S	4.0	0.51	12.8
August 2	6-S	6.3	0.10	1.6
August 8	9–8	13.2	0.62	4.7
August 10	9-R	44.3	3.10	7.0
August 21	11-TS	3.8	0.28	7.5
September 14 (total)	9	71.1	4.68	6.6
September 14				
(early interval)	7-TS	87.4	5.84	6.7
September 14				
(last interval)	2-TS	20.6	1.05	, 5.1

R signifies a rain of an even, slow character; S signifies a shower (rapid, brief rainfall varying in intensity); TS signifies a thunderstorm.

Table 2. Lead and lead-210 in rainwater in Western michigan during the summer of 1966

Date	Precipitation (mm)	Pb (μg/l.)	210Pb (pc./l.)	Specific activity (pc./g \times 10 ⁻⁴)
August 1	13–R	29·2	2·90	9·9
August 10	8–S	6·2	0·33	5·3
August 15	25–TS	6·5	0·93	14·3
August 21	51–R	27·5	2·96	10·8

acid-cleaned polyethylene bottles for shipment. Loss of lead to the walls was negligible in these conditions.

Lead analysis and reagent purification were by the standard AOAC spectrophotometric dithizone method¹². Reagent blanks were less than 0.4 µg. Triplicate analyses were performed with each sample containing at least 4 μg of lead. Under these conditions, the standard deviation of a single determination was \pm 5 per cent.

The lead-210 was determined from the beta count rate of its bismuth-210 daughter. These samples were prepared by evaporating the dithizone solution on a 1.5 in. diameter stainless steel planchet, covering it with a 5 mg/cm² aluminium foil and allowing the sample to age for 30 days to attain radioactive equilibrium. The aluminium foil prevented the variable amounts of the alpha emitting activity of polonium-210 which accompanied the lead-210 from being counted. The samples were counted in a foursection, low-background, end-window, gas-flow Geiger counter¹³. Background counting rates were about 0.25 c.p.m. and the counting efficiency for these samples was 50 per cent. Samples of known lead-210 concentrations showed that the procedure gave a chemical yield of 93 ± 5 per cent. Samples were counted for sufficient time to give a statistical counting error (o) of less than 5 per cent.

The concentrations of the stable lead and lead-210 and the amount and type of rainfall in the various samples of rainwater for the Argonne and Michigan sites are summarized in Tables 1 and 2, respectively. The arithmetic mean lead concentration of 18·9 μg/l. at Argonne is essentially identical to the 17.4 $\mu g/l$. in Michigan. Similarly, the mean concentrations of the lead-210 were 1.26 and 1.78 pc./l. at the Argonne and Michigan sites, respectively. These values for lead-210 are a little lower than the average of 2.3 pc./l. found by King et al. and by Glöbel et al. in Germany.

Although the number of data is limited, there seems to be a correlation between the type of rainfall and the concentrations; a shower (rapid, brief rainfall varying in intensity) had lower concentrations than a slow, even rainfall. Thundershowers usually had lower concentrations, but they could also be very high. There does not seem to be a correlation between lead concentration and the length of time between rainfalls. Weather preceding a rainfall, however, may affect the lead concentrations. Thus the September 14 rainfall, with its high values, was preceded by a thermal inversion for the 7 days prior to precipitation. In this case the lead and lead-210 aerosols appear to have been formed at the usual rates, but they were confined to a relatively small atmospheric volume over this long period of time.

The rates of deposition at Argonne are estimated at about 1 µg cm⁻² yr⁻¹ for the lead and 0.08 pc. cm⁻² yr⁻¹ for the lead-210. These estimates are based on this series representing 20 per cent of the measured rainfall at this site during 1966, a mean weighted lead content of 10.6 µg/l. (each result multiplied by its respective depth of rainfall) and mean weighted lead-210 content of 0.83 pc./l. The contribution of these materials to those already present in the soil is therefore small. A brief study near Argonne showed that the soil contained about 20 µg of lead and 1.5 pc. of lead-210 per g of dry material. This lead concentration is similar to Swaine's estimate of 16 $\mu g/g$ world wide¹⁴. The lead-210 concentration is probably also of the same order as the world average. With these values for lead and lead-210 and a soil density of 2 g/cm³, the contribution of both lead and lead-210 to the top 15 cm (6 in.) of soil would be 0.2 per cent per year. This neglects leaching of the lead from the soil or run-off of the rainfall before it can enter the soil.

The specific activities of the lead (pc. 210Pb/g Pb) are also shown in Tables 1 and 2. These values for the two series are much more nearly constant and give an average value of 6.7 and 10.4×10^4 pc./g at the Argonne and Michigan sites, respectively. Student's t test indicates the specific activities to be significantly different (P < 0.01). The correlation coefficients for the concentrations of lead and lead-210 in the two series of data were 0.98 (P < 0.0001) for the Argonne and 0.63 (P < 0.1) for the Michigan samples. The lower level of significance of the latter is probably because of the small number of samples.

These data show that there is good mixing of lead and lead-210 in the atmosphere before they are returned to the Earth in rainfall. Thus lead-210 will be a valid tracer for stable lead in the atmosphere, and it would appear that the meteorological conditions which affect the concentrations of lead and lead-210 do not appear to substantially affect their relative mean residence times in the atmosphere. A similar result was noted by Kuroda¹⁵ in an analysis of fallout from weapons testing, that is, the concentrations of several radioisotopes in rainwater varied over a wide range, but the ratios of their concentrations were very uniform, varying only as a function of decay rates. The mean residence times established from lead-210 daughter assay may also apply to the total lead in these same samples. Burton and Stewart⁵ found a mean residence time of 29 days for lead-210 in the total atmosphere and 22 days for the troposphere. Lehmann and Sittkus reported 33 and 14 days from rain and air samples, respectively. Fry and Mennon⁸ estimated the mean residence time to be about I week. This lower value may be caused by the high frequency of rainfall in the area studied. From these data and from the correlation between lead and lead-210 established in this study, a mean residence time for stable lead of about I month is indicated.

The lack of correlation between lead and lead-210 in air samples from about 1 m above the ground 16 suggests that atmospheric mixing at low altitudes does not eliminate local variations in the rate of injection of one or the other of these materials. Consequently, it appears that the radioactive isotope is an adequate tracer for estimation of the residence time of stable lead only in rainwater or perhaps in air from higher altitudes.

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Response of Soil Bacteria to High Temperatures and Diurnal Freezing and Thawing

Previous experiments1,2, both with known organisms and unidentified aerobic organisms isolated as airborne contaminants, indicated that spore forming organisms did not readily grow or survive in vegetative form in freeze-thaw conditions. Severe freeze-thaw conditions are thought to exist on Mars and spore forming organisms are most likely to survive attempts at sterilization, and so we speculated that spores remaining on spacecraft which have been subjected to sterilization procedures would not grow on Mars even if nutrients and water were present. Detailed experimentation with several different strains of Bacillus subtilis variety niger, however, revealed that certain strains could grow, at least marginally, in freezethaw conditions (+25°-70° C) where thaw periods were as short as 5 h, and growth was good when thaw periods were longer. This result was obtained only when a particularly favourable medium, heart infusion broth, was used. Nevertheless, this indicated that spore formers could survive and grow vegetatively in freeze-thaw conditions, if provided with an otherwise sufficiently favourable environment.

In order to further test the ability of potential spacecraft contaminants to survive and grow in freeze-thaw conditions it was decided to work directly with soil samples. Soil was obtained from a flower bed outside the laboratory, air dried and screened to remove bulk materials. Samples (100 mg) in 5 ml. vials were heated at 135° C for various times between 16 and 144 h and at 145° C and 200° C for 24 h. Sterile soil extract or heart infusion broth was then pipetted into the vials which were sealed and incubated at room temperature (25° C). (Soil extract was prepared by autoclaving 1,000 g of soil with 1,000 ml. of water for 30 min and filtering the subsequent suspension. The resulting clear fluid was sterilized by autoclaving for 15 min and used directly in the experiments.) In each case, between twenty-four and fortyeight samples were assayed by microscopic examination and the percentage of vials showing growth of microorganisms was calculated. The results are shown in Table 1.

Table 1. Percentage soil samples having viable organisms after varying time at 135° c. 145° c and 200° c dry heat

Time at 135° C	Percentage samples viab Soil extract	le at room temperat Heart infusion
16 h	100	
24 h	100	100
48 h	100	100
72 h	100	9
96 h		0
120 h		0
144 h		. 0
Time at 145° C		
24 h		26
Time at 200° C		
24 h		n

Some of the 16 h and 24 h vials were also subjected to several cycles of freezing and thawing after heating and were shown to have survivors.

Because contaminating micro-organisms on board a spacecraft would probably be low in total numbers, an experiment was conducted to determine the quantity of soil which, when inoculated into glucose broth, would yield a population of micro-organisms when incubated in freeze-thaw conditions.

A glucose mineral salts medium (0.2 per cent magnesium chloride, 0.5 per cent sodium chloride and I per cent potassium nitrate) buffered at pH 7.0 with phosphate was inoculated with dried screened soil to give 100, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 mg/ml. The suspension was stirred vigorously and distributed in each case in 2 ml. quantities to each of twenty-five 10 mm tubes, which were plugged with cotton. A baseline plating on glucose agar was made, and the tubes frozen at -75° C. They were then cycled daily between +25° C and -75° C with 4-1.5 h allowed at $+25^{\circ}$ C. The results are shown in Table 2.

Table 2. RESULTS OF SOIL EXPERIMENT USING VARIOUS AMOUNTS OF UNSTERILE SOIL

Amount of soil (mg/ml.)	Colony counts (ml. initial)*	Colony counts (ml. after 11 cycles of freeze-thaw)
100	1.0×10^{8}	1·4×10°
10	1.0×10^{5}	3.4×10^{7}
	3.0×10^{4}	1.7×10^{8}
3	1.0×10^{4}	4.7×10^{8}
Ō•3	3.0×10^{3}	7.3×10^{7}
0.1	1.0×10^{3}	1·8×10 ³
0.03	3.0×10^{2}	7·6 × 10°
0.01	1.0×10^{2}	5.6×10^5

* Assay was made by serial dilutions on highest soil concentration and extrapolations made for lower concentrations.

It is readily apparent that at the lowest concentrations used the available micro-organisms can grow in freezethaw conditions, even after only about 48 h total growth time. No effort was made to determine the nature of the organisms producing the growth in this experiment. It may therefore involve either spore formers or non-spore formers, or both.

To determine the effect of freezing and thawing on micro-organisms which survived heating in soil, 100 mg amounts of dried screened soil were distributed to each of 1,320 10 mm tubes. These were divided into sixty sets of twenty-two tubes each. Twelve sets were subjected to each of five different sterilization periods as follows: 2, 4, 8, 16 and 24 h at 135° C dry heat. Each group of twelve sets was further sub-divided into couples, and the tubes in each couple were incubated with 2 ml. of the following sterile media.

- (1) Difco heart infusion broth
- (2) Mineral salts—glucose
- (3) Mineral salts—fructose
- (4) Mineral salts—maltose
- (5) Mineral salts—lactose
- (6) Mineral salts—glucose+fructose+maltose+lactose

The sugars, when used individually, were at a concentration of 0.2 per cent. When used in the mixture each sugar was at 0.05 per cent, yielding a total concentration of 0.2 per cent.

Media containing sugar, in addition to heart infusion broth, were used in this experiment because we thought that photochemically produced sugars might be available on Mars. These sugars could serve as carbon sources for microbial growth.

Baseline data were obtained after heating by plating on each of the media, supplemented with agar, and counting the resulting colonies. After 2 h heating the population density was reduced to approximately 10⁴ organisms/g of soil. Four hours or more heating reduced this density to less than 103 organisms/g of soil. In no case were baseline populations sufficient to be detectable by microscopic examination. One set (twenty-two tubes) from each couple (two sets) was incubated at room temperature for 8 days and assayed by microscopic examination. In all cases, every tube revealed the presence of significant growth. The other set from each couple was subjected to freeze-thaw using a thaw period of approximately 6 h. After 25 days of such cycling, half of the tubes from each set were assayed by microscopic examination, or by making serial dilutions and plating in duplicate on the same medium as was used for the original incubation, but with the addition of 2 per cent agar. Positive growth was recorded for any tube in which the organisms were microscopically apparent or in which ten or more colonies developed from plates prepared from second dilution tubes, after at least 7 days' incubation. Dilutions were in steps of 1:10. The results are shown in Table 3.

Table 3. No. of soil samples showing growth during freeze-thaw cycling after various times at 135° c $\,$

No. of		Trank				
hours at 135° C	Fructose	Lactose	Maltese	Glucose	Mixture	Heart infusion
2	10†	11	11	11	11	10†
4	8	11	11	6†	10	11
8	1	1	3	6	2	9†
16	0	1	1	2	0	6
24	0	7	0	1†	0	1†

Eleven samples for each treatment except those marked † which had ten.

As noted here, all the tubes have micro-organisms capable of growth even after 24 h of heating when incubation is at room temperature. In contrast, the introduction of freeze-thaw conditions greatly reduces this capability in tubes heated for 8 h or longer. In some tubes of each medium organisms are unable to grow even after 4 h of heating when incubated in a freeze-thaw environment. After 24 h the organisms in nearly all the tubes are unable to grow in the freeze-thaw environment in all media except lactose. In this case a definite anomaly exists, which, while it has not yet been explained, is probably an artefact. The result is apparently very much dependent on the medium used, heart infusion broth sustaining superior growth. In heart infusion, a population of microorganisms grows up readily in nearly all tubes heated for 8 h and then subjected to freeze-thaw. Even here, however, only one of the ten tubes tested which had been heated for 24 h revealed the presence of detectable micro-This appears to indicate that freeze-thaw organisms. conditions, following heat treatment, impede the growth of organisms placed in an environment where they would otherwise grow.

It should be noted that all growth experiments were done in conditions of ample water, a situation not likely to be encountered on Mars. The likelihood of the contamination of spacecraft by even small samples of dirt may also be questioned. It seems clear that the survival of bacteria in even small amounts of heat treated soil is striking and the inclusion of such dirt must be rigorously guarded against in planetary exploration in order to exclude contamination of experiments designed to analyse planetary surfaces for evidence of life and life related compounds. The conditions required for growth on the planetary surface, however, are unlikely to be met on Mars and explosive bacterial growth is not to be expected according to prevailing notions of the Martian environment.

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Mantle Convection and Sea-floor Spreading in the South-west Pacific

THE interpretation of south-west Pacific structure recently proposed by Summerhayes1 is, in many respects, in agreement with suggestions previously made by myself2. Both emphasize the importance of sub-crustal convection and sea-floor spreading in determining the structure of the region, and both recognize the development of island arc structures to the north and south of New Zealand.

On one significant issue, however, the nature of the Antipodes Fracture Zone, east of New Zealand (Fig. 1), there is a basic difference of opinion which has wide implications in the elucidation of the regional structure and the mechanism of mantle convection and sea-floor spreading. While the original description of the Antipodes Fracture³ presented evidence for transcurrent displacements and suggested comparison with the parallel Alpine Fault⁴ of New Zealand, Summerhayes¹ regards the Antipodes Fracture as a rift margin fracture produced by movement of the crust away from the crest of the East Pacific Rise.

Summerhayes's hypothesis suffers from oversimpli-It is concerned primarily with the role of sub-crustal convection currents emanating from the East Pacific Rise, and fails to take fully into account the effects of similar currents rising beneath the Indian-Antarctic and Pacific-Antarctic ridges, south-west and south of New Zealand. This oceanic ridge system forms a broad are, concave northward, within which northmigrating convection currents tend to converge. resultant movement of crustal blocks expected from such a convective system would be in a general northward direction, and I (ref. 2) envisage a mobile belt extending from the region south of New Zealand to Fiji and Samoa, within which essentially northward migration of the crust has been helped by a series of parallel N.E.-S.W. fracture zones.

The distance separating the Antipodes Fracture Zone from the crest of the East Pacific Rise increases northeastward from about 1,750 km to 2,500 km, a divergence

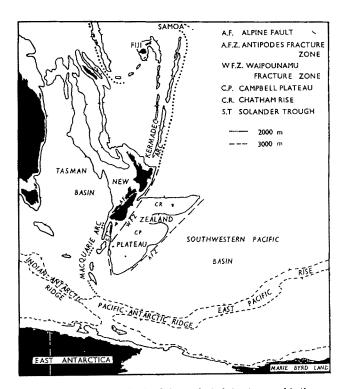


Fig. 1. Sketch map showing localities, geological structures and bathymetric features mentioned in the text.

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of 20°-25° of arc. Summerhayes's hypothesis of the rifting apart of these two features requires therefore a rotational movement of the New Zealand Plateau over distances up to 2,500 km in a north-westward direction. Movement of this magnitude and trend cannot be accommodated by the Alpine Fault; neither is there evidence of displacement or foreshortening on a comparable scale of the perpendicularly aligned Mesozoic ridge and basin structures north-west of New Zealand. A logical corollary of the hypothesis—concurrent rotation of northern New Zealand and the structurally continuous regions further north-involves movements too excessive to be seriously considered.

Menard⁵ notes significant differences in the morphologies of the East Pacific Rise and the Pacific-Antarctic Ridge, comparing the latter with the Mid-Atlantic and Mid-Indian ridges, which are commonly accepted as being of rift origin. Thus there is evidence of rifting between the southern end of the New Zealand Plateau and the Pacific-Antarctic Ridge, complementary to the northward movement of the Plateau. No such morphological evidence supports the hypothesis of rifting associated with the East Pacific Rise. The morphological differences also indicate that, contrary to Summerhayes's1 suggestion, the supposed south-westward axial spread of the East Pacific Rise stopped well short of the Macquarie Arc. and could have had little direct influence on its evolution.

The E.-W. strike of both Tertiary and pre-Tertiary formations on the Campbell Plateau¹ and Chatham Rise^{3,6,7} confirms the existence of crustal stresses compatible with northward movement, but is difficult to reconcile with north-westward migration. The E.-W. dilatational rifting of the Solander Trough and Waiau Depression¹, south of New Zealand, would have been opposed by crustal movements having a westward component, although not at variance with the local E.-W. tension implicit in northward movement of the New Zealand Plateau.

Summerhayes's1 designation of the Alpine Fault as a transform displacement separating oppositely directed island ares is again misleading. The Alpine Fault has a complex history, dating back at least to mid-Mesozoic times—long before the inception of the Tertiary island arcs north and south of New Zealand. Moreover, the extension of the Alpine Fault north of Cook Strait is questionable, and the Kermadec Arc is associated, not with the Alpine Fault, but with the Waipounamu Fracture³ east of New Zealand.

Summerhayes1 cites the existence of parallel linear magnetic anomalies in the Southwestern Pacific Basin as evidence of rifting apart of sialic blocks on either side of the East Pacific Rise. In view of the geological evidence against rifting in this region and the present incomplete state of knowledge of the geomagnetic circumstances of the south-west Pacific, such an inference is not permissible.

The former close proximity of New Zealand and Antarctica has long been contemplated because of the occurrence of similar rocks-greywackes, schists and granites—on the Chatham Rise^{6,7} and Campbell Plateau⁸ and in Marie Byrd Land9. The separation, however, of New Zealand from Antarctica cannot be explained by simple rifting away from the East Pacific Rise as Summerhayes suggests. Even if allowance is made for rotation of the New Zealand Plateau, the discordance between the E.-W. regional strike of the Chatham Rise and Campbell Plateau and the N.W. to N.N.E. strike of formations in Marie Byrd Lando is excessive. Furthermore, the intensity of folding in West Antarctica is considerably greater than on the Campbell Plateau and Chatham Rise.

The opening of the Australian-Antarctic and Atlantic-Indian-Antarctic basins, following the disruption of Gondwanaland, implies eastward migration of Antarctica with respect to the present position of New Zealand.

Reassembly of the fragments of Gondwanaland suggests that western West Antarctica originally lay close to the south or south-west of New Zealand 10, and nowhere in the vicinity of the developing East Pacific Rise. Separation of New Zealand and Antarctica is here attributed to rifting apart along the Pacific-Antarctic and Indian-Antarctic ridges, followed by simultaneous relative northward movement of the New Zealand Plateau and south-east migration of Antarctica. The virtual absence of Tertiary sediments in Antarctica¹¹ contrasts with their wide occurrence on the Chatham Rise6,7 and Campbell Plateaus, and may indicate that separation began in late Mesozoic or early Tertiary times. This hypothesis does not deny emanation of sub-crustal convection currents from the East Pacific Rise; it recognizes them as competitors with the N.E.-directed currents in maintaining northward migration of the New Zealand Plateau.

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Palaeocene Core from the Norwegian Basin

DURING the second cruise of the Atlantic Seal, the Texas Instruments, Inc., contract oceanographic research vessel, a Palaeocene core was taken from the Norwegian basin at 66° 21' N. and 00° 18' E. (Fig. 1). The collection and analysis of the core were part of the US Naval Oceanographic Office, Marine Geophysical Survey Project. The occurrence of this core is important for several reasons: it provides knowledge of the diversity of Palaeocene planktonic organisms in a high latitude; and it establishes the existence of a seaway in this part of the North Atlantic in Palaeocene times.

The core (AS 1-9) is 332 cm long and was recovered from a gentle slope in 3,017 m of water. It consists of two distinct lithological units (Fig. 2) separated by a disconformity. The top 60 cm accumulated during Late Pleistocene and Recent times while the underlying 272 cm represents deposition during the Palaeocene.

The upper unit contains three poorly defined layers of soupy sediments which are, from top to bottom, sandy mud, mud and sandy silt. They are the products of sedimentation in deep marine waters.

A disconformity marks the boundary between younger sediments and the Palaeocene beds below. The Palaeocene sediments are clay, mud and sandy mud and are dark greenish grey and dark brown in colour. They consist of partially indurated (Fig. 2) volcanic lutites, very poorly sorted, strongly coarse-skewed and mesokurtic to very platykurtic. All sand and silt-sized materials are angular scoria fragments and appear to be the products of submarine erosion of basaltic scoria. Although it is impossible to locate the source of this material from the analysis of a single core, it is suggested that basaltic extrusives of the Brito-Arctic province1 at one time

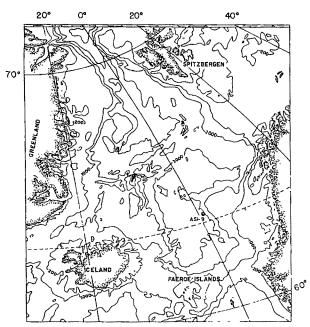


Fig. 1. Bathymetric map of the Norwegian basin showing the location of Palaeocene core AS 1-9.

cropped out nearby. The parent rock has broken down to predominantly clay-sized material and subordinate amounts of silt and sand during submarine weathering and transportation. This combination of size grades produces the strongly coarse-skewed character of the size distributions.

In addition to sediment analysis, palaeomagnetic measurements were also made on this core and interpreted by Dr Opdyke of this observatory. These show a normally magnetized section from 100 to 216 cm overlying a reversely magnetized section from 216 to 316 cm. Seismic profile records taken in conjunction with this core site show a gently sloping bottom topography. Beyond this, the resolution is generally poor but some discontinuous, horizontal layering is discernible.

Foraminifera in the top 60 cm of the core are predominantly planktonic and represent a typical modern subarctic assemblage², characteristic of the latitude at which the core was taken. Globigerina pachyderma (Ehrenberg) and Globigerina bulloides d'Orbigny are the most dominant, while Globigerina quinqueloba Natland is common and Globorotalia scitula (Brady) and Globorotalia inflata (d'Orbigny) are rarely present. The overall aspect of the fauna suggests a late Pleistocene-Recent age.

Benthonic foraminifers, constituting as much as 10 per cent of the total population, are largely calcareous. Common species include *Planulina wuellerstorfi* (Schwager), *Pyrgolaevis* Defrance, *Cassidulina translucens* Cushman and Hughes and *Cassidulina norcrossi* Cushman. The predominance of planktonic species and the presence of bathyal type benthonic species and the presence of bathyal type benthonic species such as *Planulina wuellerstorfi* suggest that a water depth similar to that of the present prevailed around the core site during the deposition of the top 60 cm of the core.

As described earlier, the layer between 60 cm and 100 em is similar in lithology to the layer below. Except for a few Pleistocene-Recent species, typical Palaeocene calcareous foraminifers are absent in this interval, although a few arenaceous species such as Bathysiphon nodosariaformis and Rhabdammina cylindrica which are diagnostic of the layer below occur. The layer below 100 cm is characterized by common occurrences of Palaeocene foraminifers (Table 1). A lower Landenian (lower Upper Palaeocene) age for this layer is established on the basis of four planktonic species. Globigerina triloculinoides, the most abundant planktonic species in the Palaeocene section of the core, is known from sediments ranging in age from Danian (earliest Palaeocene) to early Landenian (late Palaeocene), at which time Globigerina triangularis evolutionarily branches off from G. triloculinoides³. Globorotalia varianta is reported from Danian to lower Eccene of Europe and northern Africa⁴ and from Dano-Montian (lower Palaeocene) to lower Upper Landenian in the US Gulf Coast⁵. Globorotalia imitata is recorded from Dano-Montian to lower Upper Landenian5.

The benthonic species, which represent nearly half of the total population, further support a Palaeocene age for the fauna. Ceratobulimina perplexa, Pullenia quinqueloba angusta, Pulsiphonina prima, Anomalinoides midwayensis and Pseudoparella madrugaensis are all characteristic of the US Gulf Coast Palaeocene. Glomospira iranensis, originally described from the Palaeocene of Iran, was reported to be extremely rare in the Danian but common in Palaeocene sediments about 15 feet above the top of the Danian.

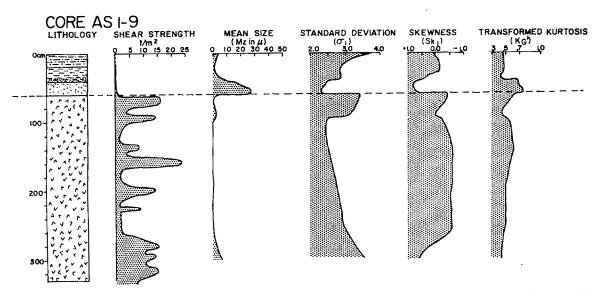


Fig. 2. Lithology, shear strength and grain-size measures of core AS 1-9. Pleistocene-Tertiary boundary is indicated by a dashed line. Note abrupt increase in shear strength below the boundary which reflects change from unconsolidated Pleistocene sediments to partially indurated, Tertiary volcanic lutites.

Table 1. PALAEOCENE FORAMINIFERAL SPECIES FROM THE LOWER SECTION

	(60-332 cm) of core AS 1-9
Family	Species
Astrorhizidae	Bathysiphon nodosariaformis Subbotina Rhabdammina cylindrica Glaessuer
Ammodiscidae	Ammodiscus glabratus Cushman and Jarvis Glomospira iranensis Kayary
Texturalidae	Siphotextularia sp.
Trochamminidae	Trochammina quadriloba Högland
Nodosariidae	Dentalina nana Reuss
	Dentalina pseudoobliquestriata (Plummer)
	Dentalina tenuissima Franke
Dalum ambinidas	Lagena sulcata semiinterrupta Berry
Polymorphinidae Glandulinidae	Pyrulinoides acuminatus (d'Orbigny) Glandulina sp.
Giandunnidae	Seabrookia lagenoides Ten Dam
Turrilinidae	Buliminella parvula Brotzen
Buliminidae	Bulimina denticulata Cushman and Parker
Dumminidae	Bulimina sp.
Discorbidae	Pseudoparella madrugaensis Cushman and Bermudez
Eponididae	Eponides plummerae Cushman
•	Eponides toulmini Brotzen
	Eponides spp.
Cibicididae	Cibicides simplex Brotzen
	Cibicides umbilicata Brotzen
Cassidulinidae	Globocassidulina sp.
Nonionidae	Allomorphina halli Jennings
	Chilostomella trinitatensis Cushman and Todd
	Nonionella sp.
	Pullenia jarvisi Cushman Pullenia quinqueloba angusta Cushman and Todd
	Quadrimorphina allomorphinoides (Reuss)
Osangulariidae	Osangularia convexa (Olsson)
Osangaiarridae	Pulsiphonina prima (Plummer)
	Gyroidinoides octocameratus (Cushman and Hanna)
Pleurostomellidae	Ellipsonodosaria cf. paleocenica Cushman and Todd
	Pleurostomella app.
Anomalinidae	Anomalinoides longi (McLean)
	Anomalinoides midwayensis (Plummer)
	Anomalinoides umboniferus (Schwager)
	Cibicidoides proprius Brotzen
Ceratobuliminidae	Ceratobulimina perplexa Plummer
Globorotaliidae	Ceratobulimina tuberculata Brotzen
Gronotoranidae	Globorotalia imitata Subbotina
Globigerinidae	Globorotalia varianta (Subbotina) Globigerina triangularis White
Groniger tutting	Globigerina triloculinoides Plummer
	CHORNEL FURT OF WAT WELLING TO THE HITTER

The affinities of the Palaeocene fauna in AS 1-9 seem to be with the circum-Atlantic and Tethys region such as the US Atlantic Coast⁷, the Gulf Coast and Caribbean region⁸, northern Sinai⁹, Belgium¹⁰ and the northern Alps¹¹. There are particular affinities with the Swedish Palaeocene¹². The present fauna, however, is distinct in that it has a larger variety of species of the family Pleurostomellidae and Nodosariidae and lacks species of Nonion, Elphidiella, and Astegerina. Differences in faunal composition between these two areas may be due to a shallower water environment for the Swedish Palaeocene, as modern representatives of species related to this Palaeocene assemblage seem to support a shelf depth for the Swedish Palaeocene and an upper bathyal depth for AS 1-9. The upper bathyal depth establishes the presence of a seaway in the Norwegian basin during the

Sediment samples studied from different levels in the Palaeocene section of the core seem to contain essentially the same fauna. It is noteworthy, however, that in the normally magnetized Palaeocene section of the core (100-210 cm), species of Globorotalia are absent. As both G. varianta and G. imitata range higher up in the Palaeocene section elsewhere, the absence of Globorotalia in this upper section of the core may be attributed to some changes in the local environment.

In lower latitudes the Landenian is the time when an explosive evolution of marine planktonic biota took place following the drastic decline of marine planktonic life at the end of the Cretaceous¹³. The upper half of the Palaeocene section in lower latitudes is generally characterized by diversified species of Globorotalia, many of which possess a distinct peripheral keel. The impoverished planktonic foraminiferal population in the Norwegian Sea near 66° 30' N. and the lack of any keeled Globorotalia suggest that climatic zones existed in middle Palaeocene seas.

To our knowledge this is the most northerly Palaeocene deep sea sediment recovered from the North Atlantic. Further coring in this area is indicated and should help resolve such problems as faunal diversity in high latitudes, palaeo-oceanography and the various geological and geophysical problems related to ocean floor spreading and continental drift.

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Palaeomagnetic Result from the Hook Intrusives of Zambia

THE Hook batholith is a composite granite mass intruding meta-sediments of the Katanga system in Zambia and situated at about 15° S., 26.5° E. The chief part of the batholith is syntectonic, but associated with it are numerous post-tectonic satellite bodies of diorite, syenite and gabbro¹. An age of $500 \pm 17 \times 10^6$ yr has been reported by Snelling et al.2 using the Rb-Sr method on a specimen of red granite which is also believed to be post-tectonic.

In September 1964, oriented cores were drilled at six sites in the satellite bodies: four in gabbro and one each in syenite and diorite. The natural remanent magnetization of specimens cut from the cores was measured with a spinner magnetometer3, and alternating field demagnetization techniques4 were employed in an attempt to isolate a primary component of magnetization. In only three of the six sites did the initially scattered directions respond to treatment, showing a grouping of directions after treatment in fields between 10 and 20 mwb.m-2: higher fields led to the onset of instability and the directions became more scattered again. The mean directions obtained using the closest grouping at each site are themselves fairly well grouped, and are believed to represent the direction of thermoremanence acquired when the rocks cooled. The site means are shown in Table 1.

Palaeomagnetic poles for the three sites were combined yielding a mean North Pole position as follows: latitude 14° N., longitude 23.5° W., K = 13, $A_{95} = 36$, where K is the precision parameter, and A_{95} the semi-angle of the cone of confidence.

It is unlikely that results from three sites are sufficient to average secular variation, and the result should therefore be treated with some caution. But the mean pole is close to that obtained from a much more detailed study of syenitic rocks in Malawi which are of a similar age3. The Malawi results give those reported here greater

Table 1								
Site	Latitude	Longitude	Rock type	\boldsymbol{D}	I	N	k	
ZA	15° 18′ S.	27° 08′ E.	Gabbro	274	+58	4	2.6	
$\mathbf{z}\mathbf{E}$	14° 49′ S.	26° 56′ E.	Diorite	320	+33	2	43	
\mathbf{ZF}	15° 09′ S.	27° 39′ E.	Gabbro	295	+55	4	6.8	

D, Declination; I, inclination; N, No. of samples; k, precision parameter.

significance than if they stood alone, and they jointly suggest that the extension of the African polar wander path into the Palaeozoic need no longer depend on a single result from the Siluriane.

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Current Density Measurements in Atmospheric Electricity

In 1929, Watson¹ pointed out that very close to the surface of the Earth the atmospheric electric current, in normal fine weather field, is carried only by positive ions, so that the current density can be computed from

$$J = E_s \lambda_{+s} \tag{1}$$

where J is the density of the current from ionosphere to Earth, E_s is the electric field at the surface, and λ_{+s} is the conductivity of the air at the surface due to positive ions. The electric field and the conductivity, however, are measured usually at a height of approximately 1 metre. The density of the current due to conductivity, $J_c(1)$, at this height is

$$J_c(1) = E(1)\{\lambda_+(1) + \lambda_-(1)\}$$
 (2)

where E(1) is the electric field at 1 metre and $\lambda_{+}(1)$ is the conductivity due to positive and negative ions at 1 metre. The diffusion current is usually neglected and it is assumed that the current density is approximately equal to the density of the conduction current at 1 metre, so that it can be computed from equation (2). This assumption is supported by the observations of Nolan^{2,3} and Hogg⁴. It will be shown here that this assumption is not correct and that the current density should be estimated from

$$J = E(1).\lambda_{+}(1) \tag{3}$$

This statement can be proved by three arguments which will be discussed here shortly.

In the first place, the observations of Hogg and Nolan do not justify the use of equation (2) to compute the current density. Measurements show that E_s and E(1) are nearly equal^{1,2}. In quasi-static conditions the current density is independent of height, so that it can only be computed from equation (2), if $\lambda_{+\epsilon} = \lambda_{+}(1) + \lambda_{-}(1)$, that is if the conductivity due to positive ions close to the surface is approximately twice that at a height of 1 metre. Observations of Hogg and Nolan seem to prove this, but, referring to their papers, some objections can be made. Nolan performed his observations at a place which is not representative of most atmospheric electric observatories: "... the station is exposed at a place between two houses, with high ground on a third side...." Objections can also be made to the methods used by Hogg. Observations at various heights were performed in different seasons of the year and the apparatus was changed during the measurements. Although large corrections were applied to make the observations comparable, results obtained in this way cannot be very reliable.

Moreover, the results of Hogg and Nolan are in contradiction with the observations of Watson¹ and O'Donnell5, who observed $\lambda_{+s} \approx 1.15 \lambda_{+}(1)$. We may therefore conclude that the observations of Hogg and Nolan do not prove the validity of equation (2) for estimating the current density.

Second, from theoretical arguments it can be shown that equation (3) is a better approximation of the current density. In 1929 Watson pointed out that space charge, transported by atmospheric turbulence, contributes to the current density. He supposed that I metre above the surface, transport of space charge by eddies cancels the conduction current due to negative ions, so that the current density could be estimated with equation (3). As Watson did not give the cause of this space charge, his ideas were abandoned in favour of the results of Hogg and Nolan. In my opinion, however, Watson's idea was right: negative space charge, in the form of negative ions, is transported downward neutralizing almost completely the conduction current due to negative ions. The explanation is as follows. The conditions for inactivation of ions below the level of 1 metre are about the same as the conditions above this level, if there is some atmospheric turbulence. Above the height of 1 metre the number of ions created is equal to the number of ions that is inactivated, so the net production of ions below 1 metre is equal to the additional ionization close to the surface. It can be shown that the net production below 1 metre is less than 10⁶ pairs m⁻² s⁻¹. When we assume that no negative ions are emitted by the surface, then the net production below 1 metre must be equal to the net flux of negative ions that passes this level. The flux of negative ions that drift upwards under the influence of the atmospheric electric field is of the order of 107 ions m⁻² s⁻¹.

The total net production is much smaller, 10° ions m⁻² s⁻¹, so that negative ions must be transported downward by eddies, almost neutralizing the conduction current due to these ions. The approximations are not valid any more when, during strong temperature inversions close to the surface, the eddy diffusivity becomes very weak. Because such strong temperature inversions do not often occur, monthly and yearly mean current densities can be computed with equation (3).

Third, the best proof of the validity of equation (3) can be found in the results of the application of this formula. The density of the current from the ionosphere to the Earth can be obtained in four different manners. (1) From the columnar resistance, r, and the potential difference between the Earth and the ionosphere, V, J = V/r. According to recent observations the mean potential difference is 260 kV. The columnar resistance, r, ranges from $8 \times 10^{16} \Omega$ m² in polar regions to $24 \times 10^{16} \Omega$ m² in polluted regions. (2) From upper air observations of the electric field and the conductivity. Above the exchange layer the current density $J = E(\lambda_+ + \lambda_-)$. (3) From direct current measurements performed with an isolated net at surface level. (4) From the conductivity and electric field observations at a height of approximately 1 metre: $J = E(1) \cdot \lambda_{+}(1)$.

In order to compare the observations of various stations the Earth was divided into three regions. I, Polar regions (Antarctic, Arctic, Greenland). II, Oceanic and continental regions above which the air is little polluted (Alaska, parts of Australia). III, Continental regions above which the air is polluted (Europe, USA).

Over fifty current density measurements at various places were compared, some of them extending over many years. The mean current densities for each group are

J in pA/m ²	I	\mathbf{m}	III
(1) Resistance: $J = V/r$	3.4	2.4	1.1
(2) Upper air: $J = E(\lambda_+ + \lambda)$	3.3	1.8	1.3
(3) Surface: direct observations	3.0		1.2
(4) Surface: $J = E(1), \lambda_{\star}(1)$	3.4	1.9	1.4

given here. Observations in mountain areas are excluded because they depend too much on local conditions.

If we should have computed the current densities at the surface from equation (2), the values would have been doubled. It is clear that this is not in agreement with the other results, so that we may conclude that equation (3) is a better approximation.

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THE SOLID STATE

Chemically Stimulated Exo-emission from a Silver Catalyst

Exo-electron emission, the vaporization of very low energy electrons from solids, can be produced in a variety of ways, mechanical deformation, X-ray irradiation and chemical reaction. The emission usually decays in an exponential fashion, but, provided the excitation is not great enough to produce normal electron emission, steady exo-electron emission can be produced by steady

Chemically stimulated exo-electron emission has been found by Lohff¹ with the oxidation (or oxygen absorption) of zinc, and by Seidl² with the oxidation of copper. Exoelectron emission by some solid catalysts has also been Nassenstein and Menold³, when studying a silver catalyst used for partial oxidation of ethylene, found that exo-electron emission occurred temporarily with no additional exitation in a temperature range between 300° and 400° C. This result was confirmed by Ohashi et al.4 and they also found that the emission activity was proportional to the catalytic activity. In our investigations, we wished to see whether the exoelectrons were emitted by the silver catalyst on which

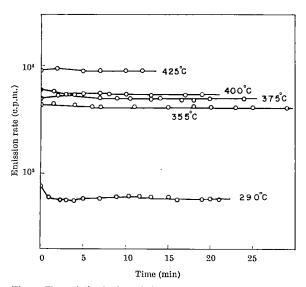


Fig. 1. The variation in the emission rate of chemically stimulated exoelectrons plotted against time. All count values plotted are the actual count minus the background count. The time scale for each curve starts at some time after the silver catalyst has reached a constant temperature.

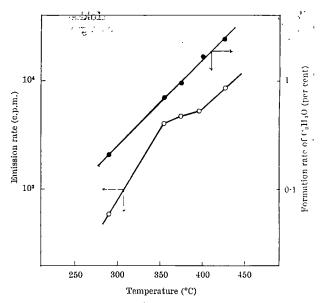


Fig. 2. The chemically stimulated exo-electron emission plotted against the rate of formation of ethylene oxide on the silver catalyst at a constant temperature in the range 250° to 450° C. The formation rate of ethylene oxide is represented as a percentage of ethylene oxide in the product gas to the percentage of ethylene in the reaction gas. The results are reproducible and stable with respect to time, provided the reaction gas flows steadily at constant speed.

partial oxidation of ethylene was taking place and to compare the emission with the formation of ethylene

The apparatus used was essentially the same as that previously reported^{4,5}. It consisted of a Geiger-Müller counter with a stainless steel cathode. Stainless steel was used, because it is catalytically inert to ethylene oxidation. An aperture at one end of the counter tube opened into a reaction compartment in which a small piece of silver wire could be heated by a small external furnace. G-M tube was kept at room temperature with a cooling jacket. The gas mixture, 91.8 per cent argon, 3.4 per cent ethylene and 4.8 per cent oxygen at 1 atm, was fairly stable as a counting gas in the temperature range examined and at an anode voltage of 2,100 V, provided the decrease in ethylene concentration due to oxidation was small. The gas mixture flowed at constant speed through the G-M tube and the reaction compartment, and simultaneous measurements of the exo-electron emission and ethylene oxide formation were made using a counting recorder and a high-sensitivity gas chromatograph.

The results are shown in Figs. 1 and 2. Fig. 1 shows the variation with time of chemically stimulated exoelectron emission at five different temperatures. In Fig. 2, the steady exo-electron emission rate has been compared with the rate of formation of ethylene oxide and demonstrates a relationship between electron emission and catalytic oxidation. It can be seen that electrons vaporize at a constant rate from the catalyst.

Blank experiments have also been carried out without the silver catalyst. In these experiments, there were hardly any counts and little or no ethylene oxide was formed in the temperature range under consideration. This excludes the possibility of ethylene oxide formation and electron liberation in the G-M tube. This leads us to believe that the electrons vaporize from the catalyst and diffuse from the reaction chamber into the G-M tube.

Our findings may provide an easy way of investigating quantitatively exo-electron emission and may also give an effective tool for the study of solid catalysts. An investigation is now in progress⁶ at this laboratory into a complete method for studying solid catalysts by chemically stimulated exo-electron emission.

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Observations of a Metal-Oxide Interface

THE partial spalling that occurs when a metal is cooled after high temperature oxidation has made it possible to make direct observations of the metal—oxide interface. Scanning electron microscopy, with its large depth of focus and perspective view, has permitted this interface to be studied at high magnification in the centre of specimens away from the edges.

A high purity iron-chromium binary alloy (Cr 28 per cent) was oxidized in pure oxygen at atmospheric pressure for 6 h at 950° C. The surface was prepared before oxidation by mechanical polishing, electropolishing and brief cathodic etching. The oxide formed was chromic oxide with a small percentage of iron present, as reported by Wood and Whittle¹. On cooling after oxidation, spalling was first observed at temperatures less than 500° C, occurring violently at alloy grain boundaries, as reported previously by Howes². More extensive spalling occurred as room temperature was approached. The spalling is due to the differential contraction between the oxide and the alloy, and the broken oxide faces examined indicated brittle fracture rather than plastic deformation.

Fig. 1 shows the structure of the oxide surface, the structure of the oxide through its thickness, the face of the alloy as it existed under the oxide and the structure of the interface between the alloy and oxide. The presence of



Fig. 1. The broken face of the oxide and the alloy-oxide interface.

voids at the interface is clearly seen, and they explain the corrugated nature of the alloy face—the rough upper levels are regions where contact had been maintained between the oxide and alloy and the smooth depressions are where voids have developed.

This direct evidence of void formation at the interface verifies the outward diffusion of chromium involved in the oxidation mechanism. The considerable reduction, however, in the contact area between the oxide and alloy raises questions of how the chromium then enters the oxide, and the significance of weight gain kinetic data. It seems probable that as contact diminishes, chromium transport across the voids after evaporation, or by surface diffusion around the voids, becomes appreciable.

This investigation is continuing and will be reported more fully elsewhere.

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Tetragonal and Hexagonal Iron-Manganese Carbides

During recent studies of the iron-manganese-carbon system, specimens, each weighing approximately 5 g, were carefully prepared according to predetermined compositions. The components were 500-mesh powders of 99·995 per cent pure iron and spectroscopically pure carbon and a 200-mesh powder of 99·995 per cent pure manganese. The component powders were intimately mixed by shaking, then each specimen was packed into a pure alumina crucible the end of which was closed, but not sealed, with high purity alumina cement. Each specimen was then sintered at 960° C for 110 h in a silica capsule containing helium at approximately 0·25 at m. (such time having been previously found sufficient for equilibrium to be attained¹).

Debye-Scherrer X-ray photographs were taken of at least two samples from different parts of each specimen using a 114.6 mm diam. camera. The exposure time was two hours using filtered chromium radiation, at a tube voltage of 30 kV and a tube current of 10 m.amp. For several of the specimens, it was found that photographs of samples taken from near the end of the specimen were somewhat different from those taken from the main body of the specimen, indicating the presence of composition gradients in the specimen. The presence of such gradients was confirmed by chemical analysis of two different portions of the sample.

Two specimens yielded unusual X-ray powder patterns from the main body of the specimen. The powder patterns did not indicate the presence of free iron or manganese and were not compatible with a pattern which was taken of a specimen the nominal composition by weight of which was 10 per cent iron, 90 per cent manganese (the nominal ratio of iron to manganese in all the specimens), which was prepared under identical conditions to all the other specimens. The patterns could be indexed with the aid of Bunn charts, and the crystal classes of the structures present are shown in Table 1 together with the nominal

			Tab	le 1		
Speci- men No.	 by weig 	ercentage ht from lysis Determi- nation 2	Nominal carbon per- centage by weight	Nominal iron per- centage by weight	Nominal manganese per- centage by weight	Crystal class of structures present
3 4	4·90 5·73	5·2 4·4	6·0 6·3	9·4 ₀ 9·3 ₇	84-6 ₀ 84-3 ₃	Tetragonal Tetragonal + hexagonal

chemical compositions of the specimens and the results of duplicate chemical analyses for carbon performed on two separate portions of the specimen. Analysis showed that no graphitic carbon was present so that the figures refer only to dissolved carbon. The d-spacings, intensities and indices of the observed tetragonal and hexagonal patterns are shown in Tables 2 and 3, respectively. Powder photographs taken near the ends of the specimens indicated the presence of the monoclinic x-carbide.

Table 2.	TETRAGONAL CA	RBIDE
Miller indices	Interplanar spacings Å	Observed intensities*
113	2.63,	W –
212	2.52	vw
004	2.36	M-S
104	2.23	vw
213	2.15_{5}	M +
311	2.08	vw
302	2.03_{s}	vs
214	1.86,	$\mathbf{M} - \mathbf{S}$
313	1.78,	S
322	1.75,	M+
323	1.610	vw
314	1.59_{a}	M
511	1.32	M
512	1.28_{3}	M+
503	1.24_{7}	S+
433		
217	1.23.	W
335	1.22_{2}	S
425	1.18_{s}	M
108	1.162	M

* VS, very strong; S, strong; M, medium; W, weak; VW, very weak.

Table 3. HEXAGONAL CARBIDE

Miller indices	Interplanar spacings Å	Observed intensities*
003	2.31.	VVW
112	2.21	VW broad
103	2.10	VS
210	1.90	M —
211	1.82	W
312	1.29_{a}	W
115	1.26,	W +
205	1.22	VS
304	1.20	W
313	1.19°	W broad
402	1.178	VW broad

*VS, very strong; S, strong; M, medium; W, weak; VW, very weak; VVW, very, very weak.

The lattice parameters of the carbides were investigated by the use of a least squares computer programme developed by Barnet of Sydney University, and the resulting unit cell parameters are shown in Table 4. Because, in the case of the hexagonal carbide, there are only eleven observed reflexions and because three of these are broadened while another is very weak, it is felt that the quoted standard deviation may be a little optimistic in this case. Comparison, however, with the unit cell parameters obtained by Picon and Flahaut² for the tetragonal carbide Mn₄C and for the hexagonal carbide Mn₆C₂ shows that the axial ratios of Mn₄C and the tetragonal iron-manganese carbide are very similar as are the axial ratios of hexagonal $\rm Mn_6C_2$ and the hexagonal ironmanganese carbide. Furthermore, the theoretical carbon content of Fe0.4Mn3.6C is 5.17 per cent by weight, while the theoretical carbon content of Fe_{0.6}Mn_{5.4}C₂ is 6.78 per cent by weight. It is likely that a carbide of composition Fe_{0.4}Mn_{3.6}C could exist in specimen No. 3, which was observed to contain a tetragonal carbide, and that a mixture of carbides of compositions Feo. 4Mn3.6C and Fe_{0.6}Mn_{5.4}C₂ could exist in specimen No. 4, which was observed to contain a mixture of hexagonal and tetragonal carbides, by virtue of the variation in the compositions of the specimens shown in Table 1. If the carbides examined here are isomorphous with the tetragonal carbide

Table 4									
Crystal		This i	nvestig	ation Stan-		Pic	on an	d Flaha	ut"
class of carbide	a	dard devi- ation	c	dard devi- ation	Axial ratio c/a	For- mula	as	Ch	Axial ratio ah/ch
Tetragonal Hexagonal	6·772 5·77	0.004 0.01	9·427 6·98	0.005 0.01	1·38, 1·21	${f M_4C} {f M_5C_2}$	7·66 5·48	10·57 6·71	$1.38_{0} \\ 1.22$

Mn₄C and the hexagonal carbide Mn₆C₂, then it appears that the substitution of iron atoms for approximately 10 per cent of the manganese atoms causes a decrease of approximately 30.3 per cent in the cell volume of the tetragonal carbide and an increase of approximately 15.5 per cent in the cell volume of the hexagonal carbide. No explanation for such a difference in behaviour is apparent at this time.

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PHYSICS

Measurement of Electron Temperatures produced by Collisionless Shock Waves in a Magnetized Plasma

WE report measurements of the shock heating of electrons resulting from the propagation of collisionless shock fronts through a highly ionized plasma in a direction perpendicular to an initial magnetic field. The structure of the magnetic field (B_z) and the radial electric potential (V_R) within these shock fronts was reported previously and their collisionless nature deduced indirectly.

The direct measurements of electron temperature, made by Thomson scattering of laser light, were aimed at verifying two previous inferences: (a) that these shocks cannot be explained in terms of the binary transport coefficients: and (b) that the electron heating, with negligible ion heating, satisfies the conservation relations across the shock for Alfvén Mach number $M_A \leq 3.7$.

In the experiments, the shock front, driven by the fast compression of a linear Z-pinch², propagates radially inwards with velocity $V_S=2.5\times 10^7$ cm/sec through an initial hydrogen plasma, with electron density $n_{eo} \sim 7 \times$ 10^{14} cm⁻³ and temperatures $T_{eo} = T_{to} \sim 1$ eV, in various initial axial magnetic fields. A 400 MW, 20 nsec pulse from a ruby laser is passed across the diameter (50 cm) of the discharge tube at the midplane. The laser pulse is timed relative to the shock structure using an electric probe at 9 cm radius but moved azimuthally out of the optical paths. The light, scattered into the axial direction from a 1 cm length of the laser beam centred at 9 cm radius, is detected by a photomultiplier. The spectral profile of the scattered light is obtained by using interference filters with narrow pass-bands (3 to 35 Å) and high rejection ratios ($\sim 10^{-4}$).

The spectral profile, which results from Doppler broadening by the electrons, will be Gaussian if the electrons are The experimental results, covering just over a half width of the profile, fit a Gaussian curve to within ± 15 per cent and yield an electron temperature with a standard deviation of \pm 10 per cent. Systematic errors increase the possible error to \pm 15 per cent. Calculations, using these measured temperatures, show that there should be sufficient time for the electrons to thermalize before the measurements.

Three different types of shock structure arise as the Alfvén Mach number is increased $(M_A = 2.5, 3.7, 6.3)$ by decreasing the initial magnetic field ($B_{zo} = 1.2, 0.75, 0.43$ kG). At low M_A (< 3) the shock transition is sharp (width ~ $7c/\omega_{pe}$), while at high M_A (> 6) it is more than ten times broader. At intermediate M_A (~ 3.7) a double structure exists with the sharp preceded by the broad transition.

The measured electron temperatures, T_e , for $M_A = 2.5$ and 3.7 are shown in Fig. 1 as a function of time, τ , relative to the sharp transition. For $M_A=3.7$, the change in magnetic field, ΔB_z , is plotted to show the measurement of T_e within the broad transition.

The electron temperatures in Fig. 1 are compared with two theoretical predictions of the total shock heating, $T_e + T_i$, derived independently of the shock mechanism (assuming $\gamma = 5/3$). These predictions are from (i) plane geometry steady state conservation relations4 using the measured V_s , n_{so} and B_{zo} , (ii) the cylindrical collapse MHD computations⁵ (private communication by K. Hain, K. V. Roberts and D. L. Fisher) based only on the initial plasma and circuit conditions. This collapse computation has been shown previously to give good agreement with the measured dynamics of the experiments.

There is good agreement between the measured T_e and the predicted $(T_e + T_i)$ immediately behind the shock for $\bar{M}_A = 2.5$. This verifies the previous inference that the ions are not appreciably heated in this case. For $M_A = 3.7$, however, the measured Te is less than the predicted

 $(T_e + T_i)$, allowing the possibility of some ion heating. The measured electron temperatures for $M_A = 6.3$, shown in Fig. 2 with ΔB_z , are within the broad shock structure which is neither well separated from the piston nor in steady state. Consequently, neithe predictions for $(T_e + T_i)$ appears appropriate. Consequently, neither of these

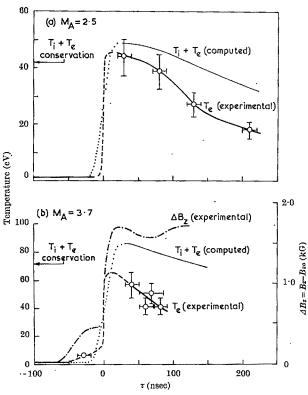


Fig. 1. Measured electron temperatures in hydrogen as a function of time and a comparison with predictions (a) $M_A = 2.5$, (b) $M_A = 3.7$, with the observed magnetic field plotted to show the slow feature.

The possibility of temperature limitation by end effects or impurities was investigated in an experiment performed in deuterium at half the number density. The dynamics were the same as for hydrogen $(M_A=2.5)$ and so the temperature should be doubled. The measured temperature, $T_e = 91$ eV for $\tau = 40$ to 60 nsec, confirmed this simple scaling and ruled out temperature limitation.

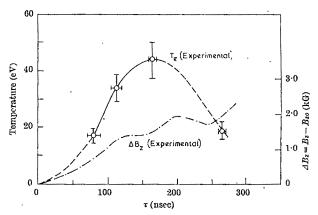


Fig. 2. Measured electron temperatures in hydrogen as a function of time for $M_A=6.3$ with the observed magnetic field plotted for comparison.

In this scaling experiment, when the electron temperature doubled, the shock width doubled while for binary resistivity it should have more than halved. Also in both these cases the binary electron-ion collision time, \(\tau_{et}\), derived from the measured T_e behind the shock, is longer than the measured shock rise time, τ_s , making such collisions ineffective; for example, in deuterium with

 $T_e = 91 \text{ eV}, \ \tau_{et} \sim 5 \tau_s.$ The measured electron temperatures are, in all cases, many times greater than those calculated from the observed structure assuming binary resistivity. Binary viscous effects are negligible. The ratios (R) of the observed to the calculated temperature rise in hydrogen are as follows: $M_A = 2.5$, $R \sim 6$; $M_A = 3.7$, broad transition $R \sim 5$, total structure $R \sim 8$; and $M_A = 6.3$, These figures demonstrate that appreciable collisionless electron heating occurs in all these shocks.

For the low M_A shock the most probable mechanism is the excitation of plasma waves by electron-ion streaming instabilities in the high current density of the sharp transition. This process provides a collective or anomalous resistance to the current. The subsequent interaction and damping of these waves can heat the electrons. A formula for the resistivity resulting from such a mechanism has been derived by Sagdeev7. Substitution of the appropriate values into this formula results in a resistivity which is more than adequate to account for the observed electron heating. Moreover, this analysis is in accordance with the absence of appreciable ion heating. The observed scaling of shock width between hydrogen and deuterium is also consistent with this theory. At present there is no satisfactory interpretation of the observed high M_A shocks.

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Effect of Compression upon the Magnetic Behaviour of Magnetite in Thermal Cycles across the Magnetocrystalline Isotropic Point

The value of the first magnetocrystalline isotropic constant, K_1 , of magnetite changes at low temperature. This results in an isotropic point at a temperature T_k of about -143° C^{2,3}, where there is effectively zero magnetocrystalline anisotropy. A transition of remanence and associated memory are observed in thermal cycles across $T_k^{4.5}$. We have observed that the memory and the low-temperature moment are increased when the thermal cycles are carried out under elastic compression.

The specimen used in the experiments was a [111] bar cut from a natural single crystal of magnetite. It was $1\cdot62$ cm long and had a square cross-sectional area of $0\cdot29$ cm². Its coercive force was 9 oersted, and the saturation isothermal remanence, $7\cdot1$ emu/cm². Uniaxial compression was applied using a plexiglass device which could be mounted on a vibration magnetometer³. It was compensated for differences in thermal expansion to give a constant stress throughout the experimental temperature range. The specimen was insulated to prevent large thermal gradients. The temperature was recorded with a copper-constantan thermocouple in contact with the specimen. All experiments were carried out under external field cancellation to ±25 gammas.

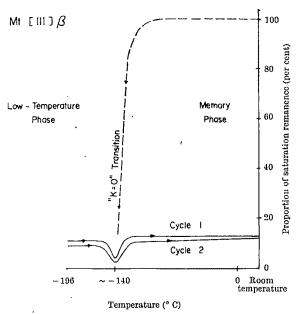


Fig. 1. Warming curves for magnetite single crystal. Sample initially saturated at room temperature.

The specimen was initially saturated at room temperature and cooled to -196° C. A control warming curve of remanence against temperature was then obtained (Fig. 1). A second cycle without re-saturation gave a slightly lower low temperature moment and memory. The experiment was then repeated after a compressive stress of 80 kg/cm² was applied at room temperature and maintained throughout the saturation process and the thermal cycles. The warming curve is shown as curve a in Fig. 2 and reveals an increase of 28 per cent and 38 per cent in the moments measured at -196° C and room temperature, respectively. The experiment was then repeated with the compressive stress applied after the saturation. This reduced the initial remanence by 14 per cent, and gave warming curve b. This procedure enhanced the low temperature moment by 41 per cent and the memory by 38 per cent.

The applied stress of 80 kg/cm² gives a small elastic strain. It is much less than the applied stress of over 500 kg/cm² required to give a stress control of magnetization in magnetite⁷⁻⁹. The experimental stress here is thus not sufficient to modify the bulk magnetization, in direction and intensity, by control of the domain pattern by bulk magnetoelastic anisotropy energy. Also, the positive bulk magnetostriction would encourage a decrease in the measured moment with compression, not the observed increase. Changes in the domain configuration, and the magnetization, should therefore be attributed to localized energy effects.

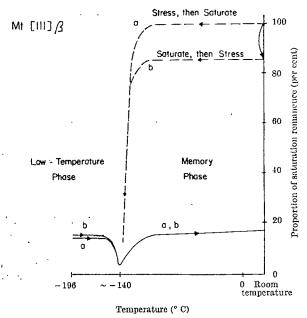


Fig. 2. Warming curves for crystal under compression of 80 kg/cm * .

a, Saturated after stressing; b, saturated before stressing.

The apparent coincidence of the transition point T_k on the temperature axis, for stressed and unstressed experiments, suggests that the magnetocrystalline anisotropy has not been measurably changed by the stress. The invariance of room temperature saturation remanence and coercive force at all stages in the stress history of the sample indicates that there has been no permanent irreversible structural change in the crystal. The applied stress, however, has increased the low temperature moment, and recovered memory, by an average of 35 per cent and 38 per cent, respectively. Thus the effectiveness of the mechanism for transferring the magnetization across the transition point has been enhanced by the stress.

These results are consistent with models of stress pinning of magnetization across isotropic points. It is suggested that the applied stress is sufficient to redistribute some existing dislocations elastically. These dislocations accumulate at local pinning points in the crystal, forming localized atmospheres, or pile-ups. These pile-ups, existing metastably under the influence of the resolved elastic shear stress, are larger than those present in the defect configuration when the crystal was not subjected to external stress. These larger pile-ups have greater stress accumulations and associated magnetoelastic energy. Our interpretation is that they then serve as more effective local pinning centres and hence nucleate a larger fraction of the moment across T_k .

The transfer of magnetization across T_k has been demonstrated to be stress-sensitive, and is interpreted as being caused by the effect of defect-associated magneto-

elastic control of the magnetization in multidomain materials.

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Flow of Elastic Liquids through **Curved Pipes**

THE purpose of this communication is to outline certain interesting features which have arisen from an experimental investigation into the behaviour of dilute polymer solutions as they are made to flow under a pressure gradient through curved pipes of circular cross section.

The experimental arrangement consisted essentially of a plastic tube of circular cross section which was coiled into an anchor-ring; see Fig. 1, where a denotes the radius of the cross section of the pipe and R the radius of the central line of the pipe. The observable quantities were the pressure gradient (measured by means of transducers)

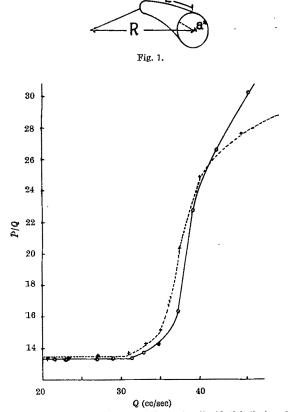


Fig. 2. Straight pipe data for a Newtonian liquid (full line) and a 0-1 per cent aqueous solution of polyacrylamide (broken line). a=0.5 cm.

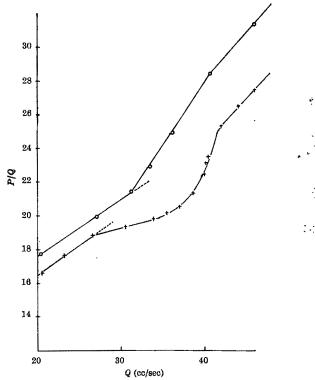


Fig. 3. Curved pipe data for a Newtonian liquid (full line) and a 0-1 per cent aqueous solution of polyacrylamide (broken line). a=0.5 cm. R=300 cm.

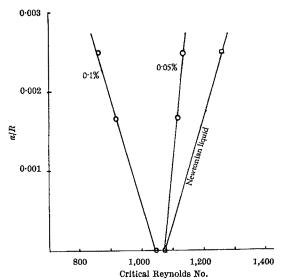


Fig. 4. Critical Reynolds number data for aqueous solutions of polyacrylamide. $a=0.5\,\mathrm{cm}$.

and the flow rate (obtainable by a conventional catchand-weigh technique). A peristaltic pump was used to create the flow and a reservoir was used to ensure that the flow was uniform and steady. All the experiments were carried out at 20° C.

Figs. 2 and 3 contain pressure gradient, P, against flow rate, Q, information for a 0.1 per cent aqueous solution of polyacrylamide and a glycerol-water mixture with a very similar viscosity. It will be observed from Fig. 2 that, in the case of the straight pipe, the data for the two liquids do not differ significantly in laminar flow, but that noticeable drag reduction occurs in turbulent flow. Of more interest in the present context is the laminar-flow behaviour of the two liquids in the curved pipe. Here the flow rate in the case of the polymer solution is noticeably higher than that for the Newtonian liquid under identical flow conditions. This is presumably because of normal-stress effects in the polymer solution, these being of no importance (so far as the flow rate is concerned) in the straight pipe. The observed trend is in agreement with the theoretical predictions of Thomas and Walters¹.

The transition region between laminar and fully developed turbulent flow is clearly discernible in Fig. 3. From this figure, it is apparent that the polymer solution is less stable than the Newtonian liquid. That the elasticity in the liquid is a destabilizing influence is further illustrated in Fig. 4, which contains "critical Reynolds number" R_{θ} data for different a/R ratios. It appears that elasticity has the greatest effect on the stability criterion when the bend in the pipe is sharpest. It will also be observed that, while curvature enhances stability in the case of Newtonian liquids^{2,3}, the opposite is possible in the case of elastic liquids.

These results are consistent with the findings of Jones and Maddock⁴ for flow in a straight pipe. We know of no theory to predict the effect of elasticity on the stability criterion in the case of curved pipes, but the observed trend is consistent with the theoretical predictions of Chan Man Fong and Walters⁵ for a straight channel

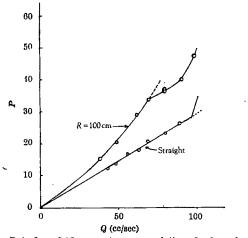


Fig. 5. Data for a 0.15 per cent aqueous solution of polyacrylamide. $a = 0.8 \text{ cm}_{\bullet}$

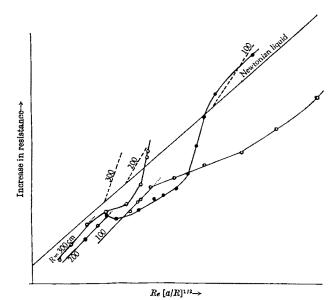


Fig. 6. Information for a 0-1 per cent aqueous solution of polyacrylamide compared with that for a Newtonian liquid. Broken lines indicate turbulent flow behaviour of Newtonian liquids. $a=0.5~\mathrm{cm}$.

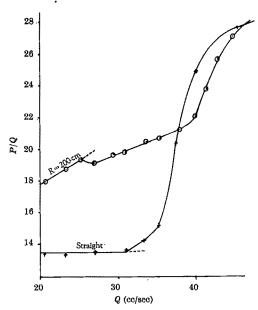


Fig. 7. Data for a 0·1 per cent aqueous solution of polyacrylamide. $a\!=\!0.5\,\mathrm{cm}.$

Fig. 5 contains pressure gradient versus flow-rate data for a 0·15 per cent aqueous solution of polyacrylamide. The interesting feature about this figure is that the drag associated with the turbulent flow of the polymer solution in the curved pipe is less than it would have been if the flow had remained laminar. This is in obvious contrast to the situation which exists in the straight pipe. Fig. 5 would seem to indicate that quite substantial drag reduction is sometimes possible in the turbulent flow of dilute polymer solutions in curved pipes. That this is so is clearly illustrated in Fig. 6, which shows that the resistance experienced by the polymer solution is always less than that experienced by a Newtonian liquid under the same flow conditions.

The substantial drag reduction associated with the flow of dilute polymer solutions through curved pipes is such as to make it easier under some flow conditions to pump these liquids through curved pipes rather than through straight pipes (see Fig. 7).

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Dimensionless Form of the Double Logarithmic Equation relating Shear Stress to Shear Rate as applied to Slowly Coagulating Milk

Ostwald and Auerbach¹ were perhaps the first to point out that, for many colloidal systems, the curve of shear stress (τ) plotted against shear rate $(\dot{\gamma})$ consists of four parts. At the very lowest shear rates, if there is no yield-value, flow is Newtonian, but this is quickly followed by a region in which $\log \tau$ is linear with $\log \dot{\gamma}$ (called "Strukturviskositāt"). At a certain shear rate all the structure

is broken down and we have a Newtonian region, rather misleadingly called the "Laminarast", finally followed by a condition of turbulence around the particles called "Strukturturbulenz".

The power equation

$$\log \tau = \log K + a \log \dot{\gamma} \tag{1}$$

is not dimensionally inhomogeneous2 but has the disadvantage that the dimensions of K depend on the magnitude of a.

In a recent paper, Nedonchelle and Schutz³ have shown that in the retrogradation of starch at a constant temperature, not only is a series of straight lines obtained when $\log \tau$ is plotted against $\log \dot{\gamma}$ during the course of time (we have changed the symbols slightly) but also that $\log K$ is linearly related to a, so that

$$\log K = \log \alpha - (\log \beta)a \tag{2}$$

Combining equations (1) and (2), they get

$$\log \tau - \log \alpha = a(\log \dot{\gamma} - \log \beta) \tag{3}$$

From this it follows that a must have the dimensions of a stress and β those of a shear rate or, substituting τ' for α and $\dot{\gamma}'$ for β

$$\frac{\tau}{\tau'} = \left(\frac{\dot{\gamma}}{\dot{\gamma}'}\right)^a \tag{4}$$

This means that there must be a single point having co-ordinates $\tau':\gamma'$ at which all the log-log curves would meet if extrapolated and also that the awkwardness of the dimensions of equation (1) is overcome.

Our intention has been to enquire whether the same equations would apply to the early stages of milk coagulation by rennet, before rigidity appears, and, if possible, to test the equations over a wider range than was possible for starch.

Berridge showed that the rennet action on milk is complete in 4-6 h in the cold (2°-4° C), whereas the subsequent process of coagulation would take hundreds of hours at this temperature. Our problem was to find the right temperature at which the coagulation would proceed

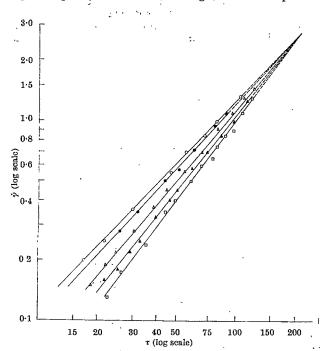


Fig. 1. Double log curves of stress and shear rates during early stages of coagulation. The five curves represent results 10 (\bigcirc), 30 (\bigoplus), 60 (\triangle), 90 (\triangle) and 120 (\square) min after reaching temperature equilibrium at 15° C.

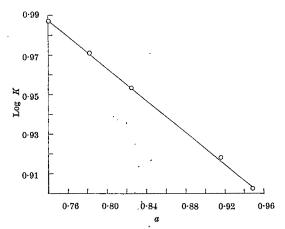


Fig. 2. Plot of $\log K$ against a for equation (2).

in such a way that there was no rigidity during the time needed to complete our experiments (about 2 h). Unfortunately, the appropriate temperature varies from one sample of milk to another. Nevertheless, we were able to carry out the experiment about four times and present here the results of one of these experiments. The τ:γ relation had to be measured within a very short space of time and this was done using a viscometer consisting of two fairly wide graduated vertical glass tubes, bent at right angles at the bottom to connect with a horizontal capillary tube, length about 16 cm, diam. 0·1 cm (see ref. 5). The viscometer was placed in a glass-sided tank full of water kept at the appropriate temperature: in the case quoted it was $15^{\circ} \pm 0.1^{\circ}$ C. One litre of skim milk was cooled to 6° C, and 20 ml. of commercial rennet at a concentration of 1:100 (final concentration 1:5,000) was The milk was stored at 2° C for 18 h. About 70 ml. of milk were then heated as quickly as possible to a temperature of 15° C and the viscometer was filled. The milk was sucked up into one of the side tubes and, on release, the head was recorded every 10 sec by means of a metronome (in another experiment, photographic recording was used which gave better data at high shear rates and showed signs of the "Laminarast", but it had no advantage for the work described here). The average head during each 10 sec interval was taken as a measure of τ and the fall in head as a measure of $\dot{\gamma}$. No attempt was made to convert to absolute units. A series of five experiments made during the course of 2 h (after which time gelation started) is shown plotted double-logarithmically in Fig. 1. It will be seen that these are good straight lines which, on extrapolation, would meet at a point. Experimentally, in fact, the "Laminarast" would be reached well below this point and at shear rates which are not independent of the age of the sample.

A plot of $\log K$ against a is shown in Fig. 2. This is reasonably linear.

We conclude that coagulating milk, like starch, follows the equations of Nedonchelle and Schutz and we suspect that they may well hold for other similar systems.

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RADIOBIOLOGY

Repair Replication in HeLa Cells after Large Doses of X-irradiation

We have observed "unscheduled DNA synthesis" and non-conservative synthesis of DNA after the irradiation of mammalian cells with ultraviolet light. We have presented autoradiographic evidence that unscheduled DNA synthesis occurred in HeLa cells after X-irradiation with 5,000 r.¹. Normal semi-conservative DNA replication is considerable at this dose, so neutral equilibrium density gradient experiments to examine the molecular nature of this synthesis were precluded. In this communication we report that, after very large doses of X-rays to HeLa cells, incorporation of precursors into DNA occurs as a result of a process which has the characteristics of "repair-replication", a phenomenon which has been correlated with enhanced survival in bacteria².

HeLa S3 cells were grown and lysates prepared for density gradient analysis as previously described¹, except that incubation with pronase (500 μg/ml.) for 1 h followed the freeze-thaw lysis and preceded deproteinization with chloroform-amyl alcohol (24:1). Density gradient analyses were carried out in a fixed-angle rotor (type 40) by centrifugation at 34,000 r.p.m. and 35° C for 36-60 h in the model L centrifuge. For neutral gradients the diluent was sodium chloride-sodium citrate (0·15 molar and 0·015 molar respectively) with a pH of 7·4; for alkaline gradients the diluent was Na₂HPO₄-NaOH (0·1 molar each) adjusted to a final pH of 12·5. In both cases, the volume was adjusted to 5·9 g of CsCl, for alkaline gradients to 6·5 g of CsCl. After centrifugation five-drop fractions were collected, diluted with 0·5 ml.

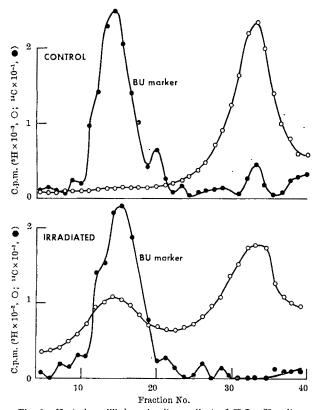


Fig. 1. Neutral equilibrium density gradient of HeLa S3 cultures labelled with BUdR (5 μ g./ml.) for 16 h, before growth for 2 h in normal medium, irradiation (or sham irradiation) with 100,000 r. and postirradiation labelling with *H-TdR (5 μ g./ml.) for 2 h. HeLa DNA labelled with *C-BUdR added as marker for hybrid DNA. O, *H counts; •, **C counts. Upper figure, controls; lower figure, irradiated.

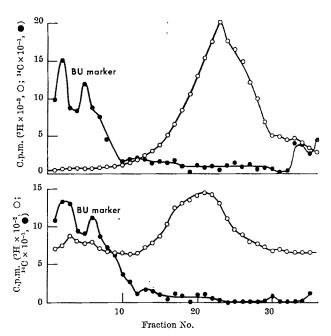


Fig. 2. Alkaline equilibrium density gradient from same cultures as Fig. 1. O, ³H counts; , ¹C counts. Upper figure, control; lower figure, irradiated.

citrate-saline and samples (0·1 or 0·2 ml.) removed for counting in a scintillation spectrometer.

In the first experiments, HeLa S3 cells were grown for 16 h in Eagle's medium containing 5 μg/ml. bromouracil deoxyriboside (BUdR). This was followed by incubation for 2 h in regular Eagle's medium before X-irradiation with 100,000 r. (300 kVp., 3,400 r./min, G. E 'Maxitron' no external filtration). Immediately after irradiation a medium containing 5 μc./ml. ³H-thymidine (³HTdR-11 c./mmole, New England Nuclear Corp.) was added and the cells incubated for 2–3 h before density gradient analysis. A ¹⁴C-BUdR-grown HeLa S3 DNA standard was added to define the position of BUdR-substituted DNA in the gradients.

Fig. 1 shows the results of a neutral density gradient experiment of this kind. In the control there is only one tritium peak, appearing at the density expected for unsubstituted DNA. In the gradient from irradiated cells, however, there is also a peak of tritium activity in the hybrid portion of the gradient at the position of the ¹⁴C-BUdR-DNA marker. That this ³H-TdR uptake is not the result of semi-conservative replication is confirmed by examination of the alkaline density gradients (Fig. 2). In these gradients the heavy, BUdR-substituted, single-stranded DNA is found in the first few fractions from the bottom of the gradient. A peak of tritium activity is present in this part of the gradient from irradiated, but not from control, cultures. Thus thymidine was incorporated into the DNA strands already formed which contained bromouracil. The appearance of tritium activity at the density of normal DNA in both neutral and alkaline gradients from irradiated cultures demonstrates that semi-conservative DNA replication also occurred in these cultures, even after this extremely high dose of radiation. The uptake of 3H-TdR into DNA of irradiated cells, however, was less than 10 per cent of that in controls.

Experiments were performed to determine whether the same process occurred in DNA of normal density. The medium on HeLa S3 cultures was replaced by one containing 5 μ g/ml. BUdR. After incubation for 1 h the medium was removed, citrate-saline added and the cultures irradiated. Incubation of the cultures with BUdR before irradiation initiates formation of hybrid

DNA and assures that subsequent semi-conservative replication in the presence of 3H-BUdR will result only in molecules which band as hybrid DNA. This eliminates the possibility of molecules with small amounts of terminal tritium that might band at or near the position of DNA of normal density. Immediately after the irradiation, a medium containing 20 μc./ml. ³H-BUdR (6 c./mmole, New England Nuclear Corp.), 5 μg/ml. unlabelled BUdR, and 10-3 molar hydroxyurea was added to the cultures. Hydroxyurea at this concentration suppresses normal DNA synthesis in HeLa S3 cells by about 98 per cent³ but has no effect on unscheduled DNA synthesis4. After incubation for 2 h, the medium was removed and the cells prepared for alkaline density gradient analysis (Fig. 3). The control shows a single tritium peak near the bottom of the gradient at the position expected for BU-substituted DNA, demonstrating that the residual DNA synthesis which occurs during hydroxyurea inhibition is of a semi-conservative kind. In the gradient from irradiated cells there is also a peak at the bottom of the gradient-again indicating a low level of semi-conservative replication occurring even during hydroxyurea treatment after 100,000 r. In this gradient, however, the most prominent tritium peak occurs at the position of normal density DNA. This demonstrates that BUdR has been incorporated into molecules of normal density at such low frequency per molecule that their density is not appreciably affected. Under the conditions of the experiment, any incorporation occurring as a consequence of semiconservative replication must result in tritium incorporation exclusively into heavy DNA strands. The observed incorporation could therefore only have resulted from insertion of ³H-BUdR into previously existing DNA molecules.

We have been unable to demonstrate unequivocally this "repair replication" at doses lower than 50,000 r., and it may be a pathological response of the cells caused by the high dose of radiation. It is more likely, however, that it occurs at low doses but is obscured by the relatively massive incorporation of tritium resulting from normal semi-conservative replication. This seems certain if one considers that unscheduled DNA synthesis, which probably results from the same primary process, has been observed at doses at least an order of magnitude lower1.

Lett et al. have recently demonstrated intracellular rejoining of single strands of mammalian DNA broken by

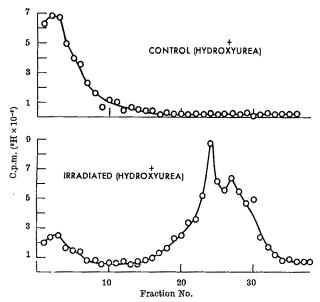


Fig. 3. Alkaline equilibrium density gradient of HeLa S3 cultures labelled with H-BUdR (20 \(\rho_c\)/ml.) in the presence of I mmolar hydroxyurea for 2 h after irradiation (or sham irradiation) with 100,000 r.

O, H counts. Upper figure, controls; lower figure, irradiated.

ionizing radiation⁵. This observation may have nothing to do with "repair replication" reported here, especially because the two phenomena probably involve different kinds of lesions (strand breakage and base damage). observations strongly suggest, however, that mammalian cells possess means by which they can repair damaged DNA. Further work to determine if "repair replication" is active and effective after low doses of ionizing radiation is in progress.

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Erythropoiesis in Lethally Irradiated Mice grafted with Bone Marrow or Spleen Cells

In a recent paper OKunewick et al. reported that splenic erythropoiesis, defined as the 24 h uptake of iron-59 into the spleen, in mice lethally irradiated and grafted, depends on the source of the grafted cells. They found that grafting 106 bone marrow cells gave a greater uptake of iron-59 into the recipient spleen than grafting 10° spleen cells. In a second series of experiments, they found that 4×10^6 bone marrow cells grafted gave higher values than 4×10^7 spleen cells. They concluded that the relative erythropoietic activity of the spleen derived from spleen or bone marrow grafts is not a function of the number of cells administered and that the spleens are more erythropoietically active if the animals have been grafted with bone marrow as opposed to spleen cells.

During experiments to estimate the influence of drugs on the colony forming capacity of different haemopoietic tissues, we investigated the erythropoiesis in irradiated mice grafted with spleen cells and bone marrow. Our results seem to disagree with the conclusions of OKunewick et al.

Adult C3H male mice (11-13 weeks old) received a total body irradiation of 850 rad. The irradiation was performed with a Resomax X-ray machine, operating at 300 kVp and a current of 19.5 m.amp. A half value layer of 2.0 mm of copper was used. The dose rate was 31 rad/ min. In each experiment, both the bone marrow and the spleen cell suspensions were derived from the same two donor animals. The recipient animals received 5×10^4 bone marrow cells or 5×10^5 spleen cells. The cell grafting was performed within 2-3 h of irradiation. Erythropoiesis was assessed by injection of 0.5 μc. of iron-59 on the seventh day and killing on the tenth day when iron-59 was measured in 0.2 ml. of blood. The incorporation of iron-59 in the blood after 72 h was calculated by assuming a total blood volume of 7 per cent of the body weight, and was expressed as a percentage of the injected dose. After fixing the spleens in Bouin's solution, the number of spleen colonies was counted under the dissection microscope.

The results of these experiments, given in Table 1, show the relationship between the number of spleen

Table 1. EFFECT OF THE SOURCE OF THE GRAFT ON THE 72 H IRON INCORPORATION INTO BLOOD OF MICE GRAFTED WITH BONE MARROW OR SPLEEN

Injected suspension	Color sple Mean No.			uptake on-59 S.E.	Significance level
A ₁ Bone marrow cells (1 Spleen cells (1	7) 12·3 0) 8·9	0·9 0·29	1·05 2·01	0·13 } 0·34 }	5 per cent
	5) 12·8 5) 11·8	1·53 1·6	2·40 3·22	$\left. \begin{smallmatrix} 0\cdot19 \\ 0\cdot21 \end{smallmatrix} \right\}$	5 per cent
	7) 10·1 9) 10·9	$\substack{2\cdot 1\\1\cdot 24}$	0·98 1·51	$0.135 \ 0.26$	>5 per cent
	4) 11 9) 11	1·78 1·24	1·53 3·63	0·305 } 0·49 }	1 per cent
	1) 8·54 8) 8·63		0·66 2·39	0·064 0·31	1 per cent

Figures in parentheses are the number of animals used.

colonies and the iron uptake in the peripheral blood. Group A were recipients grafted with bone marrow or spleen cells from normal donor animals, whereas the donor animals of groups B and C received 1 mg/kg of hydrocortisone, respectively, 4 and 24 h before killing and preparing of the suspensions.

These data indicate that by grafting 5×10^4 bone marrow cells or 5×10^5 spleen cells an equal number of haemopoietic colonies is found 10 days after irradiation. By comparing the iron utilization in both groups, however, a higher iron utilization always showed in the spleen cell grafted animals. The packed red cell mass was determined in groups A and B and no substantial difference could be seen. These observations seem to disagree with the conclusion of OKunewick et al., who claim that spleen colonies derived from bone marrow are erythropoietically more effective than those derived from spleen cells.

Some of the grafted animals $(A_2 \text{ and } \tilde{B}_2)$ were treated with antibiotics in the drinking water (terramycin and colomycin) and, as shown in Table 2, the treated recipients have a higher iron incorporation than the untreated animals, irrespective of whether they were grafted with bone marrow or spleen cells. Even in the animals treated with antibiotics, however, the spleen grafts are more erythropoietically potent.

Table 2. EFFECT OF ANTIBIOTIC TREATMENT ON THE 72 H IRON INCORPORATION INTO BLOOD OF MICE GRAFTED WITH BONE MARROW OR SPLEEN

Injected suspension	ns	Color sple Mean No.			uptake on-59 S.E.	Significance level
A_1 Bone marrow cells A_2 Bone marrow cells A_1 Spleen cells A_2 Spleen cells	(7) (5) (10) (5)	12·3 12·8 8·9 11·8	0·9 1·53 0·29 1·6	1·05 2·40 2·01 3·22	$\begin{array}{c} 0.13 \\ 0.19 \\ 0.34 \\ 0.21 \end{array} \right\}$	1 per cent 1 per cent
B_1 Bone marrow cells B_2 Bone marrow cells B_1 Spleen cells B_2 Spleen cells	(7) (4) (9) (9)	10·1 11 10·9 11	2·1 1·78 1·24 1·24	0·98 1·53 1·51 3·63	0·135 } 0·305 } 0·26 } 0·49 }	> 5 per cent 1 per cent

Figures in parentheses are number of animals used.

The difference in erythropoietic activity between the animals grafted with spleen cell and bone marrow is not understood. The fact that recipients treated with antibiotics show higher erythropoiesis may indicate that they are better protected. This may be the case in the mice grafted with spleen cells, which also received, apart from the colony forming units, some immuno-competent cells.

We can thus conclude that for an equal number of colonies in the spleen, the animals grafted with spleen cells will have higher erythropoietic activity than the mice grafted with bone marrow. The difference between these results and those of OKunewick et al. may have developed because different methods were used. A 24 h uptake of radio iron into the spleen is not as sensitive a measure of the erythropoiesis in the whole animal as the 72 h incorporation of iron into red cells.

It is likely that the radio iron content of the spleen 24 h after injection of iron-59 is a measure of iron in both erythropoietic precursor cells and storage sites.

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Cytological Analysis of Chimaerism in Rats irradiated during Foetal Life

WE have shown that adult rats irradiated during foetal life show specific tolerance of maternal skin graft1. Furthermore, we also showed that the degree of tolerance was proportional to the dose of irradiation. These results corroborate those of Lengerova² and were interpreted as being caused by the passage of maternal blood cells through the placenta into the foetus.

Ramseier and Brent³ have investigated the survival of parental skin grafts in F₂ hybrids of DA and Lewis strains of rats whose mother's placentae had been irradiated during pregnancy. A marked tolerance of DA skin graft was obtained. They also thought that the passage of maternal cells through the irradiated placenta was responsible for this tolerance.

It was desirable to find out whether maternal cells really proliferated in rats irradiated during foetal life. The sex of the rat karyotype is quite easily identified, and so this marker was used in male progenies for the identification of the mother's cells in bone marrow.



Fig. 1. Karyogram of a female cell found in a male rat irradiated during foetal life.

[•] Expressed as a percentage of the injected dose.

The Sukhatme test (Fisher and Yates) was used to test significance between the bone marrow and the spleen grafts.

The combined results, spleen cell versus bone marrow, were significant P < 0.001.

^{*} Uptake of iron-59 expressed as a percentage of the injected dose. Significance test was carried out in the same way as for Table 1.

Combined results $A_1 + B_1/A_2 + B_2$: significant, P < 0.001.

Pregnant females were irradiated using a Philips X-ray machine. Irradiation conditions were as follows: 220 kV, 15 m.amp, 0.5 mm copper and 1.0 mm aluminium filters, dose rate 80-85 r./min measured in air. Target distance was 43 cm. The doses applied were 100, 200 and 400 r:; respectively. Chromosome preparations of the bone marrow of 1-2 month old male rats irradiated at between 16 and 20 days of foetal life were made, as described by Fox and Zeiss. Two 'Colcemid' injections were given 90 min apart, amounting to 1 mg/100 g of body weight each. The animals were killed 90 min after the second injection. The arrangement of chromosome pairs and the subsequent determination of the sex of the individual karyogram was carried out by the method of Fitzgeralds. Each karyogram was determined by several workers, each one not knowing the result of the others, or by a single worker arranging one karyogram several times. The karyograms scored as male or female by all workers or by one worker at least three times were assumed to be correct.

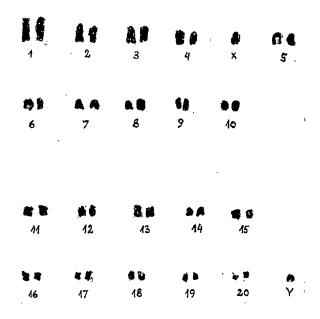


Fig. 2. Karyogram of a male cell found in a male rat irradiated during foetal life.

From the results shown in Table 1 the percentage of female cells in the bone marrow of male progeny irradiated during foetal life seems to be proportional to the dose of irradiation.

e 1. PROPORTION OF FEMALE CELLS TO THE TOTAL NUMBER OF DIVIDING BONE MARROW CELLS IN ADULT MALE RATS IRRADIATED in utero

Radiation dose (r.)	No. of tested animals	Female cells/ total No. of identified cells	Percentage of female cells
0	8	0/25	0.00
100	17	2/108	1.85
200	18	21/129	16.28
400	16	47/134	35.07

Although cytological analysis was not carried out in rats tolerant to their mother's skin grafts, the increase in the percentage of maternal cells in irradiated male progeny corresponds well with the increased tolerance after larger doses of irradiation, as described before1. Ramseier and Brent³ did not find increased tolerance after a larger dose of irradiation; however, they obtained a somewhat higher tolerance with a dose of 50 r. than with a dose of 150 r. The difference in the strength of antigens, and/or the time of exposure of foetuses may account for discrepancies between their and our results.

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Chemical Study of the Radiosensitization of Micrococcus sodonensis by Iodine Compounds

It has been shown that iodoacetamide¹ (CH2ICONH2) and potassium iodide² enhance the radiation inactivation of radiation resistant bacteria. Similar results have been obtained with iodoacetic acid3,4. Dean and Alexander2 found that sensitization with iodoacetamide was observed in Micrococcus sodonensis only when the bacteria and sensitizer were irradiated together, indicating that sensitization was not caused by blocking of intracellular sulphydryl groups before irradiation or by the production of a stable toxic product. It was later demonstrated that some short lived transients of iodoacetamide were responsible for the sensitizing effect. These transients would be formed by the reaction of iodoacetamide with the radiolytic products of water. The two principal species produced by irradiation of aqueous solutions are the solvated electron (eag) and the hydroxyl radical (OH). (For a comprehensive review of this subject see ref. 6.) The aim of the present investigation was to discover which of these species was involved in producing the toxic short lived transient and to determine the nature of this transient in iodoacetamide and potassium iodide. This could be done by studying the effect of scavengers for the solvated electron and the hydroxyl radical on the sensitizing ability of iodoacetamide and potassium iodide. Our results suggest that the iodine atom (I.), produced by the OH radical attack on the sensitizers, responsible for the observed sensitization.

Micrococcus sodonensis was grown for 9 h in TGY broth 0.5 per cent tryptone, 0.1 per cent glucose, 0.3 per cent yeast extract (Oxoid) at 30° C to give exponentially growing cells at a concentration of approximately 5×10^6 These cells were collected by centrifugation, washed in 0·1 molar phosphate buffer, pH 7·0 (made with triple distilled deionized water) and then diluted to a concentration of 5×10^5 cells/ml. in phosphate buffer containing the additive in question. Samples were irradiated at room temperature under either oxygen free nitrogen or nitrous oxide with cobalt-60 γ -rays. ensure anoxia the samples were gassed for 30 min prior to irradiation. The dose rate, determined by the method of Fricke, was 2 krad/min. Estimations of viable counts of samples were made, after suitable dilutions in 0·1 molar phosphate buffer, pH 7, by the pour plate method in TGY agar. An average colony count was 200/plate and all the points plotted in Figs. 1-3 are taken from an average total count of approximately 1,500 colonies. All the chemicals used were of research grade and were freshly prepared in sterile 0.1 molar buffer solution. To

Table 1. Summary of effects of iodoacetamide and potassium iodide, alone and with various additives, on the survival of Micrococcus sodonensis after anoxic gamma radiation at room temperature

Additive	Concentration (moles/l.)	D₀.₁ (krads)	DMF	Scavenger for
Control (N ₂) Control (N ₂ O) NaNO ₃ KCNS	2·5 × 10 ⁻³ 1·0 × 10 ⁻³	195 ± 20 195 ± 20 142 ± 15 185 ± 20	1 1 0·73 0·94	OH enq OH
IAA IAA + NANO ₃ IAA + KCNS IAA + N ₂ O	0.5 × 10-3	38 ± 5 21 ± 5 88 ± 10 5 ± 2	0·19 0·11 0·45 0·03	e ^{-aq} OH*
$\begin{array}{l} KI \\ KI + NaNO_3 \\ KI + KCNS \\ KI + N_2O \end{array}$	1·0×10 ⁻³	86.3 ± 20 4 ± 2 140 ± 15 4 ± 2	0·44 0·02 0·71 0·02	ОН

IAA, iodoacetamide; D_{0-1} , dose required to reduce the population to 0-1 per cent; DMF, dose modifying factor =

Radiation dose to produce 0.1 per cent survival in presence of test substance Radiation dose to produce 0.1 per cent survival in control

The smaller the DMF the greater the sensitization. * Unpublished work of Singh and Kabi.

ensure comparable scavenging the concentrations of radical scavengers used were selected on the basis of their relative activities towards eaq and the hydroxyl radical.

Figs. 1 and 2 show the sensitizing effect of iodoacetamide and potassium iodide in the presence of various scavengers for eaq and OH radicals. A summary of the data is presented in Table 1. It can be seen that the addition of sodium nitrate to the bacterial suspension enhanced the sensitization by iodoacetamide or potassium iodide. Sodium nitrate is a good scavenger for the solvated electron, and so it is likely that a competitive scavenging of eag enhances the sensitizing ability of these sensitizers. On the other hand, scavenging of the hydroxyl radical by potassium thiocyanate reduces this sensitizing ability (Figs. 1 and 2), demonstrating that the transient species involved are a result of the reaction of the hydroxyl radical with the sensitizers. This is further confirmed by the fact that maximum sensitization was obtained when the bacteria were irradiated in the presence of nitrous

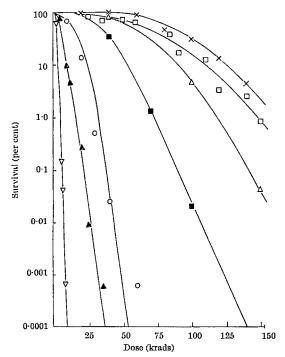
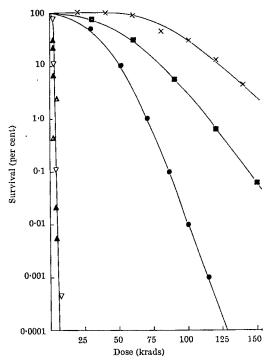


Fig. 1. Effect of various scavengers on the ability of iodoacetamide to sensitize M, sodonensis to gamma radiation. $\times \longrightarrow \times$, N_2 and N_2O control; $O \longrightarrow O$, IAA/N_2 ; $A \longrightarrow A$, $NaNO_3/N_2$; $A \longrightarrow B$, $IAA + NaNO_3/N_2$; $A \longrightarrow A$, $IAA + NaNO_3/N_2$; $A \longrightarrow A$, $IAA + NaNO_3/N_2$; $A \longrightarrow A$, $IAA + NaNO_3/N_2$;



oxide which is known to scavenge eaq radicals, converting them to hydroxyl radicals*.

It was difficult to obtain a reproducible curve for potassium iodide. This experiment was repeated many times and although we obtained good survival curves large variations were found in the D_{0} , from run to run. This ranged from 50 to 120 krads. The curve selected for Fig. 2 having a $D_{0\cdot 1}$ of 86·3 krads represents the mean of seven runs. This variation did not occur in the presence of other additives; it is likely that this particular system is extremely sensitive to trace impurities. Experiments with different concentrations of added sodium nitrate showed that at a concentration of 10^{-6} molar sodium nitrate and 10-3 molar potassium iodide a reproducible $D_{0\cdot 1}$ of 75 krads could be obtained.

During the radiolysis of aqueous solutions of I it is known that the following reactions occur^{9,10}

$$I^- + OH \rightarrow I^- + OH^-$$
 (1)

$$I^{\cdot} + e_{\overline{aq}} \rightarrow I^{-}$$
 (2)

$$I^{\cdot} + I^{-} \rightleftharpoons I_{2}^{-}$$
 (3)
 $2I_{2}^{-} \rightleftharpoons I^{-} + I_{3}^{-}$ (4)

$$2I_2^- \rightleftharpoons I^- + I_2^- \tag{4}$$

$$2I \cdot \rightarrow I_2$$
 (5)

In the presence of sodium nitrate the following reactions also occur¹¹

$$e_{\overline{aq}} + NO_3^- \rightarrow NO_3^{2-}$$
 (6)

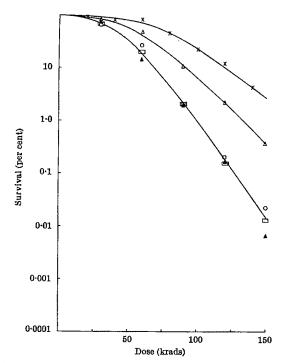
$$H + NO_3^- \rightarrow HNO_3^- \rightarrow OH^- + NO_2$$
 (7)

$$NO_2 + I^- \rightarrow NO_2^- + I^-$$
 (8)

From the survival data obtained it is clear that reaction (1) is the principal reaction leading to sensitization. I-, I2 and I are not the species involved, for they are stable and would sensitize equally well whether present during irradiation or added afterwards. Thus the transient species involved must be either I or I_a .

It has been shown that the reaction of eag with iodoacetamide is7

$$ICH_2CONH_2 + e_{aq}^{-} \rightarrow I^- + \dot{C}H_2CONH_2$$
 (9)



which is followed by reactions (1)-(5). Although the reaction of the hydroxyl radical with iodoacetamide has not been studied, it has been shown that the reaction of methyl iodide with OH radicals produces iodine atoms12, that is

$$CH^{3}I + OH \rightarrow CH^{3}OH + I. \tag{10}$$

By analogy it is expected that iodoacetamide would undergo a similar reaction, that is,

$$ICH_2CONH_2 + OH \rightarrow CH_2(OH)CONH_2 + I$$
 (11)

and I thus formed would further react according to reactions (2)-(5).

Scavenging the solvated electron in the presence of iodoacetamide will prevent reaction (9) and thus preclude the formation of the species I₂. Because N₂O greatly enhances sensitization the responsible species must be formed from the OH radical reaction, that is, the iodine atom. Thus it can be concluded that the short lived transient responsible for the radio sensitization in the presence of potassium iodide and iodoacetamide is the iodine atom formed by OH radical attack. Electron scavengers (such as NO_3) are able to enhance sensitization due to the prevention of reaction (2).

If this conclusion is correct, then methyl iodide should be a good sensitizer. Experiments using 10⁻³ molar methyl iodide are shown in Fig. 3. It can be seen that part of its sensitizing effect remains after washing the bacteria before irradiation and is probably caused by sulphydryl poisoning. The remaining effect produced by the presence of methyl iodide was small compared with iodoacetamide and potassium iodide. This is difficult to explain from a radiation chemical point of view, but other factors, such as the site at which these compounds are absorbed on to the bacteria, are also important. probably this which gives rise to the apparent discrepancy.

In conclusion, we have shown that the reaction of hydroxyl radicals with iodoacetamide and potassium iodide produces a transient which causes the observed radiosensitization. This transient is probably the iodine atom.

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PATHOLOGY

Inhibition of Mouse Sarcoma 180 by a Wheat Hemicellulose B Preparation

WE have found that the polysaccharide preparation, termed hemicellulose B, isolated from wheat straw is highly active in inhibiting the growth and inducing regression of sarcoma 180 subcutaneously implanted in The polysaccharide preparation has, however, only a slight effect on subcutaneous grafts of Ehrlich carcinoma and no recognizable effect on the growth of autologous grafts of spontaneous mammary adenocarcinomas. Certain polysaccharides from higher plants have been shown to inhibit growth of transplanted tumours, but the polymers also appeared toxic and none were well characterized $^{1-13}$. Statolon from P. stoloniferium induces the production of interferon in animal cells14.

Hemicellulose A and B were isolated from wheat straw holocellulose by the extraction procedure using sodium hydroxide solution¹⁵. Hemicellulose B was water soluble. The effect on transplanted tumours was assayed using sarcoma 180 and Ehrlich carcinoma, maintained in the ascites form. Both cultures were obtained from the Sloan-Kettering Institute. Seven day old ascites were injected subcutaneously in 0.05 ml. doses into the right groin of normal Swiss albino strain mice (designated by the National Institute for Genetics, Mishima, Japan) weighing about 20 g. This produced solid tumours at the site of injection. Spontaneous mammary adenocarcinomas were those in Swiss albino mice. Tumours of suitable sizes were surgically removed as completely as possible, and a small piece returned to the site of operation as an autologous graft in order to imitate incomplete removal and ensure local recurrence.

Hemicellulose B was dissolved in sterile distilled water and injected intraperitoneally daily for 10 days starting 24 h after tumour implantation. The rate of growth of the tumour was charted weekly in the conventional manner for 5 weeks, or as long as the mouse lived in the case of spontaneous tumours. In assays terminating at the end of 5 weeks, the mice were killed and the tumours were dissected out and weighed.

Table 1. REFECT OF CRUDE SAMPLES OF HEMICELLULOSE B ON SARCOMA 180

Source	No. of mice	Complete regression	Tumour weight average (minmax.)	Inhibition rate (per cent)
Soy bean Control	9 10	1/9 0/10	2·0 (0 - 6·7) 3·5 (0·6- 6·6)	42.9
Sunflower stalk	9	0/9	5.6 (2.0-11.3)	29.2
Control Slash pine	10 10	0/10 0/10	7·9 (5·1–10·9) 6·8 (2·1–14·1)	-9.6
Control Wheat straw	10 10	0/10 9/10	6.2 (2.9 - 9.7) 0.17 (0 - 1.7)	96-6
Control	îŏ	0/10	5.6 (0.2-11.2)	200

Table 2. EFFECT OF REPRECIPITATED WHEAT STRAW HEMICELLULOSE B (AND OF HEMICELLULOSE A FOR COMPARISON) ON SARCOMA 180

Dose/No. of days	No. of mice	Complete regression	Average tumour weight average (minmax.)	Inhibition rate (per cent)
200 mg/kg/8 Control	7 8	1/7 0/8	3·6 (0 -12·0) 10·5 (8·1-13·0)	65.7
100 mg/kg/10 Control	10 10	6/10 0/10	0.69 (0 - 2.3) 6.6 (3.9- 9.5)	89.7
100 mg/kg/10 Control	10 10	8/10 0/10	0.04 (0 - 0.2) 7.2 (2.7-11.5)	99.5
50 mg/kg/10 Control	10 9	4/10 0/9	$ \begin{array}{ccc} 1 \cdot 1 & (0 & -3 \cdot 4) \\ 9 \cdot 4 & (7 \cdot 2 - 13 \cdot 3) \end{array} $	88-3
Hemicellulose A 200 mg/kg/10	8	0/8	9-9 (6-6-14-1)	5-8
Control	š	0/8	10.5 (8.1-13.0)	- 0

Table 3. EFFECT OF REPRECIPITATED WHEAT STRAW HEMICELLULOSE B ON EHRLICH CARCINOMA (SOLID FORM)

Dose/No. of days	No. of mice	Complete regression	Average tumour weight (minmax.)	ratc (per cent)
200 mg/kg/2	10	0/10	4.0 (1.4-8.4)	-21.1
Control	10	0/10	3.3 (1.1-5.8)	
200 mg/kg/5	9	0/9	2.0 (0.9-4.0)	48.8
Control	10	0/10	3.9 (1.5-6.3)	
200 mg/kg/10	10	0/10	1.9 (0.8-2.7)	66-1
Control	10	0/10	5-6 (0-6-9-5)	
200 mg/kg/20	9	1/9	2.1 (0 -5.4)	30-0
Control	10	0/10	3.0 (0.7-5.6)	

In preliminary experiments, crude hemicellulose B from several sources was injected in 200 mg/kg doses daily for 10 days, starting 24 h after subcutaneous implantation of sarcoma 180. The tumour inhibiting effect of wheat straw hemicellulose B was striking because the tumours underwent complete regression in as many as nine out of ten mice (Table 1). Among the few remaining tumours, most were necrotized. The mice were in excellent physical condition throughout the period of hemicellulose injections with no evidence of toxicity developing. Results of further tests with reprecipitated wheat straw hemicellulose B are shown in Table 2, which also indicates the ineffectiveness of reprecipitated hemicellulose A.

Hemicellulose B of wheat straw has less effect on subcutaneously implanted (solid) Ehrlich tumour, as shown in Table 3. Complete regresssion did not occur. Similarly, wheat straw hemicellulose B was without effect on autologous grafts of spontaneous mammary adenocarcinoma. The various times necessary for the grafts to become definitely palpable and the various times of post-operative survival in twenty test mice were well within the limits of those of the controls, which were treated equally but without hemicellulose B.

The marked effectiveness of the hemicellulose B preparation against sarcoma 180 in contrast to its slight effect on Ehrlich carcinoma might imply that there is a common antigenic property between the polysaccharide preparation and a particular tumour but not with other tumours. These host-mediated effects, if existent, will be subjected to further examination.

The hemicellulose B preparation used is a mixture of polysaccharides with small amounts of other plant components present. This work does not show which of the components is active, and we intend to isolate and characterize the active component.

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Carcinogenicity of Hydrazine and I,I-Dimethylhydrazine for Mouse Lung

Вотн hydrazine itself and 1,1-dimethylhydrazine are known to be hepatotoxic1-5, but neither compound was investigated for carcinogenicity until Biancifiori and Ribacchi⁶ tested hydrazine sulphate as part of a study of the mechanism of carcinogenesis by the anti-tuber-culous drug, isonicotinic acid hydrazide^{7,8}. These workers reported the induction of pulmonary tumours in 100 per cent of twenty-two female Balb/C mice which survived daily oral treatment with 1.13 mg of hydrazine sulphate for 46 weeks (total dose of hydrazine sulphate, 283 mg).

It was later reported from the same laboratory, that the incidence of hepatomas as well as of pulmonary tumours was increased in CBA mice after similar oral treatment with hydrazine sulphate. More recently, the induction of reticulum-cell sarcomas by repeated intraperitoneal injections of hydrazine dissolved in physiological saline has been described¹⁰. In this case albino mice of unspecified strain were given sixteen intraperitoneal injections at intervals of 2-3 days until total doses of 400 mg/kg (about 10 mg/mouse) had been given. Thirteen out of thirty-four mice developed tumours in between 100 and 313 days, while only one tumour of the same type was seen among sixty untreated mice observed for a year.

The aim of the experiments described here was to test a number of other hydrazines for carcinogenicity in mice. The compounds selected for test are shown in Virgin female Swiss mice were used. From the results of preliminary toxicity tests (see Table 1) in which the compounds were administered in distilled water or polyethylene glycol 400 by stomach tube, 5 days each week, doses close to the maximum tolerated dose for daily administration were decided. Groups of twenty-five mice were then treated with the six compounds by gavage at the selected dose level on 5 days each week for 40 weeks. Eighty-five untreated animals served as controls.

(1) Hydrazine NH2.NH2 (2) Methylhydrazine sulphate NH2NHCH3.H2SO4 (3) 1,1-Dimethylhydrazine NH2.N(CH3)2 (4) Phenylhydrazine NH.NH. (5) Benzhydrazide CONH.NH2 (6) p-Hydrazinobenzoic acid NH.NH.

Fig. 1. Compounds tested.

The results are given in Table 2. A higher proportion of the mice treated with various hydrazines developed histologically verified lung tumours than do the control mice, but the difference was not significant. Of the treated groups, however, two developed far more lung tumours than the rest. Both hydrazine itself and 1,1-dimethylhydrazine increased the incidence of pulmonary tumours as compared with the control groups. These differences were significant (P < 0.001 and 0.5 > P > 0.2, respectively), though strictly speaking it is not admissible to make separate comparisons with the control group. The fact that in both cases some mice bore multiple lung tumours, however, supports the view that the apparent carcinogenic effect was real. Most of the effect was seen in animals examined at post-mortem between 50 and 60 Methylhydrazine sulphate, phenylhydrazine, benzhydrazide and hydrazinobenzoic acid were without apparent carcinogenic effect. Toxicity prevented the administration of the substances which gave negative or equivocal results at dose levels comparable, on a molar basis, with those of hydrazine and 1,1-dimethylhydrazine and this may account for their failure to induce tumours.

Table 1. TOXICITY TESTS IN 15 g FEMALE SWISS MICE

Substance	Dose (five times weekly by gavage)	Solvent (0·2 ml.)	Effect
Hydrazine	32 mg 8 mg 2 mg 0.5 mg	Water	5/5 died day 0-7 5/5 died day 0-7 5/5 died day 0-7 5/5 alive day 280
Methylhydrazine sulphate	32 mg 8 mg 2 mg 0.5 mg	Water	5/5 died day 0-7 5/5 died day 0-7 5/5 died day 0-7 5/5 alive day 280
1,1-Dimethyl- hydrazine	32 mg 8 mg 2 mg 0.5 mg	Water	5/5 died day 0-7 5/5 died day 0-7 2/5 died before day 30 2/5 died before day 150
Phenylhydrazine	32 mg 8 mg 2 mg 0.5 mg	Water	5/5 died day 0-1 5/5 died day 0-1 5/5 died day 0-1 2/5 died before day 280
Benzhydrazide	32 mg 8 mg 2 mg	Water	5/5 died day 0-7 5/5 died day 0-7 3/5 died before day 30 (2 before day 7)
p-Hydrazino- benzoic acid	0.5 mg) 32 mg 8 mg 2 mg (Water suspension)	4/5 died day 30-280 5/5 died day 0-7 5/5 died day 0-7 2/5 died day 0-7 280) 5/5 alive day 280

The pulmonary tumours which arose in mice treated with hydrazine or 1,1-dimethylhydrazine were alveologenic or bronchiologenic adenomas or adenocarcinomas of the types which commonly arise spontaneously in mice. No other tumours attributable to treatment were observed. Marked anaemia developed, as expected, in the group treated with phenylhydrazine; this necessitated a reduction in the dose during the sixth week of treatment. No other toxic effects were observed.

It is conceivable that the activity of 1,1-dimethylhydrazine is secondary to its demethylation to hydrazine, although this would need to be more or less complete in the light of the results. Alternatively, the dimethylhydrazine may be carcinogenic itself. Unfortunately, the result of the test with methylhydrazine sulphate throws no light on this problem, because it proved too toxic to administer at comparable molarity. Further studies are

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Successful Tissue Transplantation of Auxin-dependent Tumours and some of its Problems

AUTOTROPHY with respect to auxin has come to be regarded as a diagnostic characteristic of plant tumours¹⁻³. Experience shows, however, that spruce tumours in culture are heterotrophic with respect to auxin4. It was therefore decided to transplant living cells from in vitro cultures into healthy seedlings.

The methods employed for culturing primary explants of normal and tumour tissues of Picea glauca (white spruce) have been described previously. The two types of tissues were routinely grown on a medium described These explants have been maintained and transferred regularly for a number of years. In small fragments of undifferentiated, sterile tumour and of normal tissues, single cells were separated from cell clusters using an electric stirrer. Single cells were separated by filtration employing a slight modification of Bergmann's method⁶. The single cells were isolated from the suspension by a plating technique,; they grew into independent tumour and normal clones in 17 per cent of cases. The chromosome complements of each of these clones were examined as described before⁸. Almost 80 per cent of

Table 2. CARCINOGENICITY TESTS IN FEMALE SWISS MICE

Compound	Daily dose (mg)	Daily dose (µmole)	Solvent	No. of mice	at 40–50 we Tumour-bearing	eks Total	at 50-60 we Tumour-bearing animals/survivors	eks Total tumours
Hydrazine Methylhydrazine sulphate 1,1-Dimethylhydrazine Phenylhydrazine Benzhydrazide p-Hydrazinobenzolc acid Untreated control	0·25 0·5 0·5 0·5-0·25 0·5 1·0	8 3·4 8 4·6–2·3 3·7 6·6	Water Water Water Water Water Water	25 25 25 25 25 25 25 85	2/9 0/10 1/8 0/9 0/9 0/7 2/37	3 0 2 0 0 0 0	4/4 1/9 4/9 0/8 1/5 1/5	20 6 24 0 1 1

the cells of the initial normal explant were clearly diploid, twenty-two chromosomes, and only about 12 per cent were tetraploid, while the remaining 10 per cent appeared to be an euploid and polyploid; 5 n was the highest polyploidy ever observed in these cells. Normal clones with both diploid and aborrated chromosome numbers have been established to learn the consequences, if any, of chromosomal aberrations in these cells. Chromosomal irregularities here did not seem to be associated with any other irregularities and tumorization certainly did not result from it.

Variation in chromosome number up to more than 14 n with a high percentage of an uploidy characterized the tumour cells. In spite of the initial cytological instability, each established clone remained constant indefinitely; apparently the cell population of each clone is made up of cells the chromosome complements of which remain constant when kept at 20°-21° C on the nutrient substratum described before4. Changes of the kinds and concentrations of auxins and exposure to low temperatures resulted in chromosomal changes in both normal and tumour clones and will be described later. When stable clones were established and their chromosome complements had been determined, small pieces of tumour and normal tissues were grafted on to healthy seedlings of Picea glauca. The apparent lack of uniformity in the behaviour of these transplants can be seen by analysing the data (Table 1). The fact that transplantation of different clones with the same chromosome complements often had different results, as did different transplantations employing the same clone, may emphasize the importance of the non-genetic factors, too, in determining the outcome of transplants. Of the tumour transplantations 60 per cent were completely negative and 24 per cent grew and then regressed. Only 16 per cent became permanent positive transplants. It seems significant that, however small in number, only those small fragments of tumour tissue the chromosome numbers of which had been altered developed into tumours comparable in every respect with those from which they were cultured when transplanted into healthy seedlings of Picea glauca. Thus when diploid clones isolated from tumour explants were implanted they were either not taken by the host or fused with the host without giving rise to tumours in the manner of normal tissue transplantation.

No normal tissue transplantation resulted in tumor-Although 54 per cent of these grafts were rejected, some of the normal clones with altered chromosomal complements took and these tissue implants, too, fused with the host and soon assumed the host normal growth pattern.

Table 1. CHROMOSOMAL DEPENDENCE ON TAKES OF PURE CLONE TISSUES OF $Picea\ glauca\ \mathtt{TUMOURS}$

No. of chromosomes in the cells of donor tissues	Total No. of trans- plants	No. of completely negative trans- plants*	No. of transplants taken without development of tumour	Regression transplants	Positive transplants
22	5	3	2		
22	5	5			
33	3	2	_	1	_
33	$\frac{2}{3}$	1	_	1	_
44		2	_	1	_
11	2 5	-	_	1	1
55	5	3	_	1	1
70	3	3		*****	_
70	2	******		1	1
88	3	1		2	
88	2 5	2	_	******	
110	5	3	-	1	1
130	5	3	_	2	_
154	3	1	_	1	1
154	2	1			1
Total	50	30	2	12	6 (+2)

Each horizontal line represents results of grafting obtained with the same

cione. The term "take" is used to indicate the success of a transplant.

Completely negative transplants which showed no growth. Regression transplants grew temporarily but later regressed. Positive transplants which progressively grew into tumours.

These findings suggest that there are two physiologically different cytological phenomena. Chromosomal irregularities among the predominantly diploid normal cells may reflect cases where the perfect coupling of chromosome reproduction and mitosis has been disturbed by nonneoplastic factors. On the other hand, during tumorization an unknown factor (or factors) seems to render the cells neoplastic, causing chromosomal irregularies. because tumorization in these cells always seems to be associated with quantitative changes in the balance of chromosomes9. The definite lack of specificity in the chromosome numbers, even between individual cells of the same type, seems to suggest a secondary phenomenon which is the result of previous neoplastic change. These apparently secondary changes still have important consequences and could even be necessary co-factors for, or at least definite signs of, complete tumorization; clones isolated from tumorous masses with unchanged diploid chromosome complements did not show the auxindependence characteristic of these tumour cells in culture. These findings also reveal that in a given mass of tumorous tissues in vitro there are always a number of completely normal cells. This may explain why different investigators working with different isolates of tissue masses of Picea glauca, rather than with populations of single isolates, had arrived at different results10,11. Thus the behaviour of different isolates seems to be largely determined by the number of normal cells actually present in a mass of so-called tumour tissue, and results may be more uniform and meaningful where single cell cultures are being studied. Why different clones behave differently. and why inocula of the same clone behave differently, is far from understood.

Another difficulty is that the existence of a particular cell with an irregular chromosome complement does not prove that it was induced by a neoplasm. quite clearly, true neoplastic cells of Picea glauca are no longer diploid.

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IMMUNOLOGY

Increase in Antibody-forming Cells of Neonatally Thymectomized Mice receiving Calf Thymus Extract

DEVELOPMENT of the mammalian lymphoid system and attainment of full immunocompetence have been shown to depend on the presence of the thymus during early The possibility has been considered that the

multiple functions of the neonatal thymus are effected not only by migration of viable thymocytes or cells preformed elsewhere which become competent within the environment of the thymus, but also by means of a diffusible substance secreted by the thymus and affecting differentiation of lymphocytic cells outside this organ. Evidence for such a humoral mechanism has accumulated from studies of the ability of thymic tissue contained within cell-tight diffusion chambers to prevent the deficiencies resulting from surgical removal of the thymus. Specifically, mice implanted intraperitoneally with chambers allowing passage of fluids but not of intact cells showed neither depletion of lymphocytes of peripheral blood and lymphoid organs nor characteristics of the wasting syndrome which follows thymeetomy of newborn animals4. Furthermore, the ability to form antibodies in a primary response against sheep erythrocytes was, to a large extent, maintained, as was the capacity of neonatally thymectomized mice to reject skin homografts. The non-cellular nature of this thymic substance has been confirmed by studies of the restorative effects of a cellfree component extracted from thymus of xenogeneic In neonatally thymectomized mice, wasting disease was prevented and the normal lymphocyte population of peripheral blood and spleen was maintained by injection of such extracts^{7,8}. Furthermore, the abilities of such mice to reject both skin grafts and tumours were partially restored and graft versus host reactivity was partly returned to the spleen cells of thymectomized mice by calf thymus extracts^{9,10}.

The first steps towards identification of this thymic hormone have indicated that the bovine thymic extract prepared in this laboratory is protein, the most active component in stimulating DNA synthesis in lymph nodes of thymectomized mice being in that fraction precipitated by ammonium sulphate at 20–40 per cent saturation¹¹. In addition, this preparation was also effective in restoring the graft versus host response of spleen cells from thymectomized animals¹². We have investigated the ability of this fraction of thymic extract to restore the primary immune response of neonatally thymectomized mice against sheep erythrocytes. The haemolysin response was evaluated by the Jerne plaque assay¹³ in order to determine the number of functioning antibody producing cells.

Extracts of calf thymus and calf kidney were prepared as described previously¹¹. The thymus extract tested in this experiment was that fraction precipitated by ammonium sulphate at 20–40 per cent saturation, and was adjusted to a protein concentration of 5 mg/ml., while total kidney extract was used as control at a concentration of 10 mg of protein/ml. The extracts were administered according to the schedule in Table 1.

Inbred C3H/eb mice received 0.05 ml. of extracts intraperitoneally on the first day of life and again on the seventh day, with the aim of overcoming the antigenic effects of calf extracts. Thymectomy was performed on the third day by an adaptation of Miller's technique¹⁴ and completeness of thymectomy was confirmed, when the mice were killed, by inspection and also histologically. When 2 weeks old, animals of one group were implanted subcutaneously with whole thymuses from 1–2 day old

syngeneic donors. From 2 weeks old, animals of two other groups received intraperitoneal injections of thymus or kidney extract three times a week. Injections were terminated a week before immunization. When the animals were 9-13 weeks old antibody-plaque forming cells of the spleen were estimated according to the procedure of Jerne¹³ with the following modifications. Four days after intraperitoneal injection of 4×10^8 sheep erythrocytes, the spleen of each mouse was dispersed by pressure, first through a wire mesh and then through a 27 gauge needle, into Hanks solution. Samples (0.1 ml.) containing the desired number of cells were added to 1 ml. of 0.7 per cent Difco' agar + 0.56 per cent DEAE dextran dissolved in Hanks solution at 47° C, and 0.1 ml. of 4×10^7 sheep red cells. Duplicate quantities of spleen cells and antigen were mixed and layered on a base of 1.4 per cent agar in Hanks solution in 2 in. Petri dishes. After 1 h at 37° C, the plates were re-incubated for 0.5 h with guinea-pig complement diluted 1:25 in Kolmers saline. Unstained plaques were counted under the dissecting microscope. Each spleen was weighed and the number of spleen cells was estimated from a dilution of 1:100. In addition, before killing for the plaque assay, serum was collected from blood of the retro-orbital sinus of some of the mice. The haemolysin titre of this serum was measured as follows. Doubling dilutions of serum (0.1 ml. inactivated at 56°C) were made in saline (0.1 ml.). After addition of 1 per cent sheep erythrocytes (0·1 ml.) and guinea-pig complement diluted 1:20 in Kolmers saline (0.1 ml.) the tubes were incubated at 37° C for 0.5 h. The reaction was stopped with saline citrate and 100 per cent haemolysis evaluated the next day

The impaired splenic haemolysin response of thymectomized animals is evident in the results shown in Fig. 1. It can be seen that extract of kidney, used as a control of bovine protein, had no restorative effect on the deficiency caused by thymectomy. In contrast, extract of thymus elicited an increase in the number of competent spleen cells in five of the ten animals tested to a response approximately five times that of thymectomized controls. Although the magnitude of this restoration is small in comparison with normal function, it represents a considerable amount of the repair achieved by subcutaneous grafts of whole thymus. These results indicate that the spleen cells responsible for production of haemolysing antibodies are under the influence of a thymic hormone, and confirm previous findings based on experiments with diffusion chambers5.

A depression in the primary haemolysin response of neonatally thymectomized mice could result from a decrease in the number of cells producing antibodies, or it could reflect diminished activity of a normal number of cells among other possibilities. By the use of Jerne's plaque assay it was possible to consider the first alternative specifically, and it is clear that these results support previous evidence of a decrease in the number of functional cells¹⁰. On the basis of comparison of plaque size, it has been suggested that the antibody output of each cell is not reduced after thymectomy¹⁵. A comparison of the haemolysin activity of serum with the number of antibody forming cells in the spleen (presumably the chief site of haemolysin formation) suggests, however, that the

Table 1. SCHEDULE OF EXPERIMENTAL PROCEDURES

			Experimental treatment		
Age of mice	Intact	Thymectomized	Thymectomized + thymus extract	Thymectomized + kidney extract	Thymectomized + thymus implant
<1 day			0.05 ml. extract	0.05 ml. extract	
3 days		Thymectomy	Thymectomy	Thymectomy	Thymectomy
7 days	*******		0.05 ml. extract	0.05 ml, extract	
14 days	******		-		Subcutaneous thymus implant
From 2 to 4 weeks	9044A		0.1 ml. extract $3 \times \text{weekly}$	0.1 ml. extract $3 \times \text{weekly}$	*****
From 4 to 8-12 weeks	Particular.		0.2 ml. extract 3 × weekly	0.2 ml. extract 3× weekly	garandes.
1 week later 4 days later	Sheep erythrocytes Assay	Sheep erythrocytes Assay	Sheep erythrocytes Assay	Sheep erythrocytes Assay	Sheep erythrocytes Assay

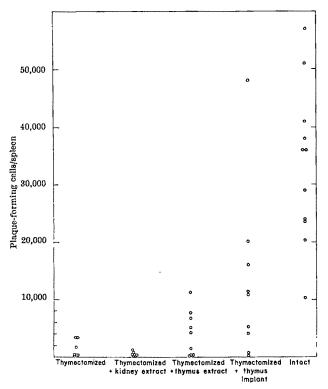


Fig. 1. Number of plaque-forming cells of spleen in primary haemolysin response four days after challenge with sheep erythrocytes.

quantity of antibody in a cell may also be reduced by thymectomy (Table 2). From a small sample this value appears to be increased under the influence of thymus extract.

Because the number of functional cells is reduced by thymectomy and partially restored by thymus extract, it is of interest to determine whether this action of thymic hormone was exerted on the total complement of nucleated spleen cells or more specifically on a particular type of cell. As can be seen in Table 3, the increment in functional antibody producing cells does not result from a general spleen cell proliferation; this suggests either a specific proliferation of target cells or the activation of already existing cells by thymus extract.

The limited extent of restoration of the spleen cell haemolysin response mediated by the thymus extract

Table 2. RELATION BETWEEN CIRCULATING ANTIBODIES AND NUMBER OF ANTIBODY-FORMING CELLS OF SPLEEN

Treatment	Reciprocal of titre of serum hacmolysins/10 ³ plaque-forming cells of spleen	Ratio of antibodies/cells
Intact	256 / (8)	7.8
Thymectomized	<3/33 (11) <3/1.3 (7)	< 2.3
Thymectomized + thymus extract	$ \begin{array}{ccc} & 1.3 & (7) \\ & 43 & (3) \\ & 6.9 & (5) \end{array} $	6.2

Figures in parentheses indicate number of animals contributing to average lues. Only those animals responding to challenge are included in the group receiving thymus extract.

Table 3. COMPARISON OF INCREASE IN NUMBER OF FUNCTIONAL ANTIBODY-PRODUCING CELLS TO INCREASE IN TOTAL NUMBER OF NUCLEATED SPLEEN CELLS

	Percentage change relative to thymectomized controls		
Treatment	In plaques/spleen	In nucleated cells/spleen	
Thymectomy + kidney extract	-71	+52	
Thymectomy + thymus extract	+174	+ 65	
Thymectomy + thymus implant	+856	+22	

Value for each group is average of between seven and ten animals.

requires an explanation in view of the greater degree of improvement that was found in diffusion chamber experiments, and the qualitatively stronger influence of the same thymic preparation on other immunological responses¹⁰. The chain of events leading to antibody production, as distinguished from homograft rejection and graft versus host reactivity, may involve more than one type of lymphoid population. One of these populations may be dependent on a thymic component which is not optimally active in this extract. Before this explanation can be considered, however, the possibility must be eliminated that xenogeneic extracts of bovine origin present a competitive antigenic challenge to the restored cells. In support of this notion are the results of preliminary experiments in which complete calf thymus extract (containing many additional proteins) administered from 2 weeks of age failed to enhance the spleen cell plaque response of thymectomized mice, and parallel treatment with complete extract of calf kidney even depressed the response. Investigation of these possibilities must await further purification of the active component of thymus extract or, alternatively, development of a system for the testing of syngeneic material. The significance of the present results, however, lies in the indication that a humoral factor extracted from thymic tissue restores the capacity to form haemolysing antibodies to a portion of the spleen cells of neonatally thymectomized mice.

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CYTOLOGY

Visualization of Human Somatic Chromosomes by Scanning **Electron Microscopy**

Some structural details of human somatic chromosomes cannot be examined by light microscopy because of their size and because of insufficient contrast after staining, nor can they be viewed by phase contrast. Barnicot and Huxley¹ attempted to get more information about fine structure from transmission electron microscopy. They stained unsectioned chromosomes with uranyl acetate and lead hydroxide, but the structure appeared somewhat diffuse and in spite of a slight gain in the clarity of the

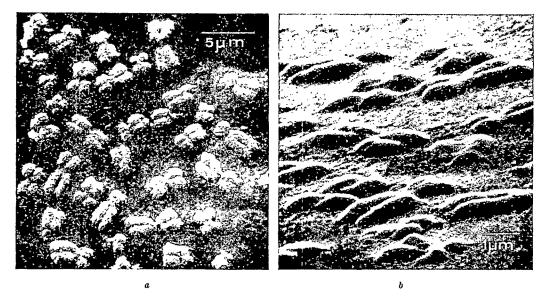


Fig. 1. a, Metaphase plate at a magnification of 2,800 and tilted to an angle of 30° from the normal plane to the direction of observation.

b, The same metaphase plate as a at a magnification of 9,600 and tilted to 80°.

image no additional features were revealed. Perhaps an ordered structure in the unfixed chromosomes was lost by the preparative procedures. Because of its tedious nature this technique is useful for special purposes rather than for routine examination.

A different aspect of human chromosomes was examined by electron microscopy², but no technical data were given. ("La structure fine des chromatides est pour l'instant à la limite des possibilités".)

In light microscopy as well as in transmission electron microscopy the image is made up of beams which have penetrated the chromosomes. The scanning electron microscope, however, which was developed recently, shows an image of the surface of the object which is being scanned. The highest resolution of about 200 Å exceeds by far that of light microscopy. A further advantage of microscopy in the study of human chromosomes is in the operation of the instrument: specimens of about 10 mm in diameter can be moved, in a plane normal to the electron optical axis, in two orthogonal directions. Because of the great depth of focus the microscope gives a three dimensional aspect of the object. By tilting and rotating, the specimen can be put in the desired position. A particular preparation is necessary only in the case of electrically non-conducting specimens in order to prevent an electric charge accumulating on the surface. This was done by overlaying them with a thin film of evaporated aluminium.

For our first attempt to visualize human chromosomes by scanning microscopy we used metaphase chromosomes from cultured lymphocytes, with a 1 per cent solution of sodium citrate to produce hypotonic shock and acetic ethyl alcohol (1:3) as fixative. The supporting slides were examined under a low power light microscope and areas of about 10×10 mm² with adequate numbers of metaphases were cut with a diamond. Preliminary results of this simple technique are shown in the micrographs.

The metaphase plate seen in Fig. $1a~(\times 2,800;$ tilting angle 30°) shows a few details of the surface structure of the chromatids. The centromere region as well as the satellites are definitely lower than the arms of the chromosomes. Some of the chromatids exhibit a fine cleft which separates the half chromatids, as occasionally observed by light microscopy in stained specimens or by phase contrast³. We do not know yet to what extent this surface is coated by a layer of fixed residual cytoplasma.

By tilting the same metaphase plate to an angle of 80° (Fig. 1b; $\times 9,600$) it can be estimated that the thickness

of the chromosomes varies from about 1100 Å at the centromere to a maximum value of about 2600 Å in the mid portion of the long arms, as long as the thickness of a layer of residual cytoplasma is uniform on the slide and on the chromosomes. A real diminution of the chromosomal substance can be observed at the telomere, centromere and satellite regions.

In further work it will be necessary to examine chromosomes prepared in different ways⁴. New preparative procedures must be developed to give "naked" chromosomes which are not grossly modified and no essential alterations must be produced by fixation.

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Reproductive Death of Irradiated Cultured Mammalian Cells and its relation to Mitosis

A close relationship between mitosis and cell death has been demonstrated in rat retina¹, and similar observations have been made on various other cell systems. This type of cell death has often been called "mitotic death" or "division death", emphasizing its relationship to mitosis or division. Lately, this type of death has been called "reproductive death", stressing loss of reproductive integrity². It should be mentioned that all terms, including "metabolic death"³, express only part of the characteristics of cell death, but none to the satisfaction of everyone. Knowing the shortcomings of these terms,

"reproductive death" will be used, for the sake of convenience, for this type of cell death.

Evidence which suggests a close relationship between mitosis and cell death can be divided into three classes. (a) The radiation dose needed to kill cells at and after the first post-irradiation mitosis (reproductive death) was, in most rapidly dividing cells, substantially less than that needed for interphase death of these cells4,5. (b) The appearance of dead cells was associated with the appearance of mitotic cells3,5,8. In many situations, the fraction of dead or dying cells increased at the time of the first post-irradiation mitotic peak^{1,6-11}. (c) The conditions which modified the mitotic index of the cell population affected the appearance of dead cells in the tadpole^{8,10}. For example, low temperature delayed the appearance of the first post-irradiation mitotic peak as well as the appearance of dead cells¹⁰. The second dose of radiation decreased the mitotic index as well as the fraction of dead cells9.

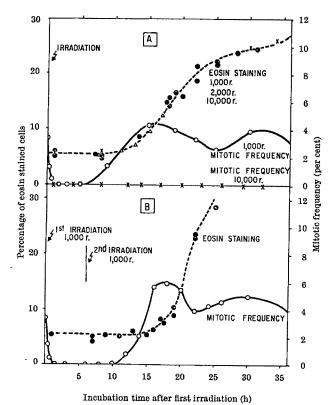


Fig. 1. Changes of the mitotic frequency and the percentage of cells stained by eosin with time in the irradiated cell population. A. The cell cultures were irradiated with a single dose at 0 h. − − − , Eosinstaining curve (♠, cells irradiated with 1,000 r., ∆, 2,000 r. and ×, with 10,000 r.). —, Mitotic frequency (○, irradiation with 1,000 r. and ×, t10,000 r.). B. The cell cultures were irradiated with doses of 1,000 r. at 0 and 6 h. − − − , Eosinstaining curve; —, mitotic frequency. The percentage of cells stained with eosin in the non-irradiated control is 5.0 per cent ± 0.8 per cont and the mitotic frequency of the non-irradiated control is 3.4 ± 0.1 per cent.

In suspension culture of mouse leukaemic cells, L5178Y, our previous work showed that, after a lethal dose of gamma radiation, the sudden increase of cells which stained with eosin (dead or dying cells) occurred shortly after the first as well as the second post-irradiation mitotic peak¹¹. This suggested that many of the lethally irradiated cells die at G₁ after completion of one or more mitoses¹¹.

The present communication provides further evidence to show that the post-irradiation mitotic peak is positively related to reproductive death of mouse leukaemic cells, L5178Y, in culture.

A suspension of L5178Y cells in the exponential growth phase was irradiated with 1,000 r. of gamma radiation from a cobalt source. After irradiation, the frequency of mitosis (percentage of cells in M) and of cosin staining (percentage of dead or dying cells^{11,12}) was determined as a function of time. Immediately after irradiation, there was a complete G_2 block (which prevented cells passing from G_2 to M). After 6 h, the block disappeared and the cells began to enter M with a resulting rise in mitotic frequency. The first mitotic peak occurred 15 h after irradiation. The increase in the percentage of cells stained with eosin began 10 h after irradiation and reached a maximum after 20 h (Fig. 1A).

In order to delay the appearance of the first mitotic peak, a second dose of gamma rays (1,000 r.) was given 6 h after the first irradiation (just before the disappearance of the first G_2 block). The second dose at 6 h caused not only a further delay in the appearance of the first mitotic peak, but also a comparable delay in the appearance of cells stained with eosin (Fig. 1B).

To show that such delay of both mitotic peak and cells stained with eosin is not caused by the higher dosc of radiation, 2,000 r. was given initially instead of the first dose of 1,000 r. Fig. 1A showed that the 2,000 r. produced the eosin-staining curve superimposable on that of the first 1,000 r. alone.

It should be mentioned that the cells irradiated with 10,000 r. died similarly to those that received 1.000 r. but that they did not show any clearly distinguishable mitotic peak (Fig. 1). We observed, however, the appearance of meshed nuclear structures in the post-irradiation period of 15–20 h. This could be a feeble attempt of the severely damaged cells to undergo mitosis. The other reason why cells irradiated with 10,000 r. are said to die of reproductive death is that it was observed that more than 35,000 r. was necessary to kill L5178Y cells by interphase death (unpublished work of Goldstein and Okada).

These observations constitute another piece of evidence that the post-irradiation mitotic peak is closely related to death of L5178Y cells and suggest that the passage of lethally irradiated L5178Y cells through M is an essential step for the cells to die at G_1 . The present observations agree with the idea of chromosome aberrations being the principal cause of reproductive death in cultured mammalian cells (see, for example, refs. 13 and 14).

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GENETICS

Abnormal Distribution of Haemoglobin Genotypes in Negro Children with Severe Bacterial Infections

SEVERE pneumococcal infections (meningitis and/or septicaemia) in children in this department have been unexpectedly frequent in cases of sickle cell anaemia (SCA). We have investigated this situation by examining the distribution of haemoglobin genotypes in a group of children with acute, generalized bacterial infections.

All records of patients hospitalized in our department from 1959 until December 31, 1966, with bacteriologically proved acute meningitis and/or septicaemia were reviewed. Salmonella infections were excluded because of their particular tendency to cause chronic osteitis in patients with sickle cell anaemia.

Altogether 265 cases were examined, of which sixtynine had to be rejected because the haemoglobin genotype had not been determined. Haemoglobin electrophoresis was not carried out either because the young age of the patient made paper electrophoresis unreliable, or because the patient died quickly after admission, before an appropriate blood specimen had been taken. remaining 196 cases the haemoglobin genotype was known and the diagnosis of septicaemia and/or meningitis was substantiated by a positive bacteriological culture from the blood, from the cerebrospinal fluid or from both.

Haemoglobin genotype was determined routinely by paper electrophoresis with a tris-barbiturate buffer. After November 1964 agar gel electrophoresis with citrate-citric acid buffer was used in the case of children less than 6 months old. The last method gives clear-cut results, even in the newborn. Bacteriological diagnosis was made according to standard cultural and immuno-logical techniques. The distribution of haemoglobin logical techniques. genotypes in the patients was compared with the expected distribution by standard statistical methods.

The distribution of haemoglobin genotypes was similar in patients with septicaemia and in patients with meningitis, so the two types of infections were connected. The causative organisms fell into five groups: pneumococci (forty-five cases), coliforms (fifty-seven cases), staphylococci (thirty-three cases), Haemophilus influenzae (twentyfive cases) and a miscellaneous group (thirty-six cases) including Neisseria meningitis, streptococci, proteus group, pseudomonas group, clostridia, and Flavobacterium meningosepticum. The distribution of the haemoglobin genotypes for the five bacterial groups is shown in Table 1.

Table 1.	DISTRIBUTION	OF HAEMOGLO	BIN GENOTY	PES
Bacterial groups	AA	AS	ss	Total
Pneumococci	18 (40·0%)	(8·9%)	23 (51·1%)	45 (100%)
Coliforms	40 (70·2%)	8 (14·0%)	9 (15·8%)	57 (100%)
Staphylococci	20 (60-6%)	7 (21·2%)	6 (18·2%)	33 (100%)
H. influenzae	19 (76·0%)	(12·0%)	(12·0%)	25 (100%)
Miscellaneous	28 (77·8%)	(11·1%)	(11·1%)	36 (100%)
Total	125 (63·7%)	26 (13·3%)	45 (23·0%)	196 (100%)

Haemoglobin S is the only abnormal haemoglobin known to be present in a significant frequency in the Bantu population of Kinshasa (formerly Leopoldville). The incidence of the sickle cell trait (SCT) in 488 adult women living in Kinshasa has been found to be 26.6 per cent (unpublished work of Vandepitte, Van Baelen, Cornu and Eeckels); the local incidence of sickle cell anaemia in a group of 1,000 newborn babies was 20 per thousand. The figures agree well with each other: assuming an equal frequency of sickle cell trait of 26.6 per cent in adult males and females, it can easily be calculated that the

frequency of the genotypes AA, AS and SS in a newborn population has to be 75·1 per cent, 23·2 per cent and 1·7 per cent, respectively. This distribution can be considered to modify itself during childhood to reach in adults the figures 73.4 per cent, 26.6 per cent and

The average age of our 196 cases of severe bacterial infection was 26 months. At that age the exact distribution of the three haemoglobin genotypes is unknown. For the purpose of this communication, it will be assumed that it is still the same as at birth, namely, 75.1 per cent AA, 23.2 per cent AS and 1.7 per cent SS. Compared with this expectation, the observed distribution is markedly abnormal for each bacterial group taken separately as well as for the whole 196 cases. The observed frequencies for the genotype SS are constantly greater than expected, whereas the observed frequencies for AS are constantly lower, except in the case of the staphylo-This abnormal distribution is most prococci group.

nounced in the pneumococci group.

Using the Kolmogorov-Smirnov test¹, the distribution observed in the pneumococci group can be shown to differ significantly from the expected distribution (P < 0.01). The same is true for the total of all groups (P < 0.01). There is, however, some doubt as to the homogeneity of this total group; the distribution observed in the case of pneumococci differs significantly from the distribution observed in the total of other bacterial groups (P < 0.01). After eliminating the pneumococci group from the total, the distribution of haemoglobin genotype in the 151 remaining cases can be shown to differ from the expected distribution in a way which is probably significant (0.01 < P < 0.05). Finally, it must be noted that when only the AA and AS cases are considered, the differences between the observed and expected frequencies of both genotypes are not statistically significant, neither in the pneumococci group nor in the total of the remaining cases.

In forty-five cases of pneumococcal septicaemia and meningitis, the proportion of patients with sickle cell anaemia was found to be significantly greater than expected. The only explanation which presents itself is an abnormally high sensitivity of children with sickle cell anaemia to pneumococcal infections. A markedly increased incidence of meningitis, mostly caused by pneumococci, has been described2 in patients with sickle cell anaemia. In 151 other cases of acute bacterial meningitis or septicaemia caused by various germs (excluding pneumococci), the proportion of cases of sickle cell anaemia is also greater than expected; the difference is probably significant. Although apparently to a lesser degree, as with pneumococci, cases of sickle cell anaemia might also present an increased sensitivity to other bacteria.

In all bacterial groups observed, except staphylococci, the proportion of sickle cell trait is unexpectedly small. Although the difference between observed and expected frequencies is not statistically significant, the possibility that sickle cell trait is linked with a lowered sensitivity to bacterial infections must be considered.

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Eradication of Culex pipiens fatigans through Cytoplasmic Incompatibility

Culex pipiens fatigans is the chief vector of filariasis in south-east Asia. Urbanization has often caused the numbers of this mosquito—and with it the danger of filariasis infection—to increase alarmingly¹. The natural vigour, tolerance and fast development of resistance to insecticides of this mosquito necessitate the development of other control methods, and cytoplasmic incompatibility² seems to be an ideal means.

Crossing between members of allopatric populations of the Culex pipiens complex can produce four different results. Most populations will produce normal offspring in reciprocal crosses, while some give offspring in one direction and embryos which will not hatch in the opposite direction. Other crosses are infertile in both directions. This lack of offspring is due to cytoplasmic incompatibility³, which is inherited cytoplasmically. It remains constant for indefinite numbers of generations in the female line. In an incompatible cross the sperm is blocked before it can fuse with the haploid egg nucleus, and if the embryos develop they do so from the haploid egg nucleus and die before hatching (unpublished work of E. Jost).

Twenty different crossing types are known in the Culex pipiens complex, and it is possible to specify at least one, sometimes several, strains of the complex which are incompatible with a certain population of Culex pipiens anywhere in the world. Desirable genic traits can be introduced into an incompatible strain without changing the cytoplasmic incompatibility, and so strains from temperate regions can be adapted to tropical environments.

Release of incompatible males into a natural population should be suitable for the control of *Culex pipiens*. Cage experiments show that females cannot discriminate between normal and incompatible males, and the competitiveness of males depends largely on nutritional conditions during larval development. Cage populations with an initial ratio of incompatible and normal males of 1:1 have been eradicated after three or four generations.

The World Health Organization sponsored a pilot experiment at the Filariasis Research Unit at Rangoon. A strain of Culex pipiens fatigans with the cytoplasm from a strain from Paris and the genome from a strain from Fresno, California—considered to be better adapted to ecological conditions in Burma than the pure Paris strain—was used. During August to October 1966, males were tested for incompatibility with females from twenty-five natural populations in Rangoon and its surroundings.

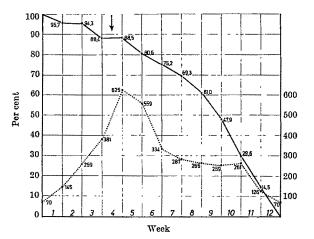
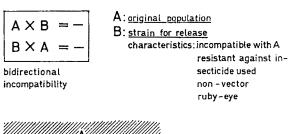


Fig. 1. Eradication of *C. fatigans* in the village of Okpo, Burma, February-May 1967. ——, Daily average percentage of hatching rafts per week 1-12; , daily average number of egg rafts per week 1-12.



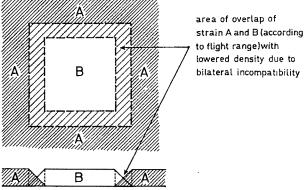


Fig. 2. Self-operating system. Replacement of strain A by strain B with subsequent equilibrium between A and B.

These females laid a total of 1,472 rafts with 130,445 eggs. Only 180 larvae hatched (0·14 per cent).

The field work was carried out at Okpo, a village about 10 miles north of Rangoon, surrounded by completely dry rice fields during winter. The size of the mosquito population was estimated to vary from a minimum of 2,000 males and 2,000 females to as many as 10,000 males and 10,000 females on a given day, depending mostly on fluctuations in the number and extent of breeding places.

Incompatible males were released between February and May 1967 (Fig. 1). From March 16 until May 6, 5,000 incompatible males were released daily. Because of the initially small number released the percentage of non-hatching rafts from incompatible crosses remained low (week 1-4, 4·3-11·6 per cent). Soon after the release of the optimum number of 5,000 incompatible males/day (arrow in Fig. 1) the percentage of inviable rafts increased to an average of 19.4 per cent (fifth week, March 21-27) in spite of an almost ten-fold increase in the size of the population during the first 4 weeks (seventy rafts/day during the first week, 625 rafts/day during the fourth week). During the next seven days (March 28 to April 3) a slight increase to 24.8 per cent occurred; during the following week (April 4-10) 30.7 per cent was observed. From then on the percentage of inviable rafts increased more rapidly. It reached 39.0 per cent during April 11-17 (week 8) and 70.4 per cent during April 25 to May 1 (week 10). A new wave of higher production in the breeding areas, beginning in week 10, was obviously cut off by the high percentage of non-hatching rafts. During May 2-8 (week 11) the percentage increased to an average of 85.5 per cent and finally, on May 9 and 10, 100 per cent inviable rafts were obtained. The production of new larvae was therefore stopped and no more adults were expected to emerge after about 10 days. Unfortunately, the monsoons started on May 11 and the experiment had to stop.

The results of this experiment show that the incompatibility principle can operate in nature and that, after the release of an adequate number of incompatible males, a C. p. fatigans population was eradicated in about 3 months or five or six generations. If in the future a population in an extended area is completely suppressed, the problem of filling the vacuum created could be serious. With the removal of C. p. fatigans with its high

density in most breeding places an opening will be available for aquatic insects with similar ecological requirements. Another species of mosquito could come to occupy the niche. It might be harmless or a vector of the same importance. A strain of C. p. fatigans unable to transmit filariasis, or even a strain with no desire to bite man, could be developed and liberated to fill the empty niche. This strain should be incompatible with the surrounding population. Fig. 2 shows a model for the eradication and replacement of a natural population (A) based on the possibility of creating an equilibrium between a new population (B) and the surrounding original population (A). The model requires the replacement strain B to be incompatible with the original population A in two reciprocal crosses. Strain B must be a non-vector, and it would be useful if it were also resistant to an insecticide if such is used initially to depress strain A. A visible, nondeleterious genetic marker, for example, ruby-eye, included in strain B could be very helpful in further observations of whether equilibrium between A and B is maintained.

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BIOLOGY

Isolation of Rabies Virus from Fruit Bats in Thailand

THE transmission of rabies by bats was first reported in 1921 (ref. 1). Vampire bats (Desmodus rotundus murinus) have been found to be an important carrier of sylvatic rabies in South America2, and they have been known to shed the virus in their saliva for long periods of time without apparent illness3. Since 1953, when the first rabies isolate from bats in the United States was made from a yellow bat (Dasypterus floridanus) in Florida, there have been more than five hundred isolates from bats in at least thirty-six States4. A survey of the literature has failed to reveal reports of rabies in bats in south-east Asia, and publications of the World Health Organization^{5,6} state that surveys of bat rabies in Asia have been consistently negative. The same statement about bat rabies in Europe, however, was refuted by Pitzschke⁷, who isolated rabies virus from a wide-winged bat (Epitescus serotinus) in Thuringia, Germany. The high prevalence of rabies in Thailand and the dense bat population prompted us to investigate the disease in bats in this area.

Seventy-nine dog-faced fruit bats, Cynopterus brachyotis, were trapped in Kanchanaburi, Thailand, and their brains were removed and examined by the fluorescent antibody technique. Two were found to be positive and weanling mice were inoculated intracranially with a 20 per cent suspension in phosphate buffered saline, pH 7.2. Eighteen days after inoculation the mice began to show a paresis of the posterior limbs. No other symptoms were noticeable. A creeping paralysis over the course of 4-5 days caused the death of all the mice, and they were shown to be positive for rabies by the fluorescent antibody technique. Hyperimmune antirabies equine serum effectively neutralized the virus in its ability to infect weanling mice. Dilutions of between $2 \times 10^{-1.2}$ and $2 \times 10^{-7.2}$ were made of a first passage mouse brain suspension and equal parts of these and either normal or hyperimmune horse serum were incubated for 1 h at 37° C. Intracranial injections of 0.03 ml. of each dilution were made into weanling All mice inoculated with the suspension plus normal serum died within 14 days of inoculation. Table 1 shows that only three out of ten mice died in the groups receiving either 10-1.2 or 10-2.2 dilutions plus hyperimmune serum. All mice receiving a more dilute brain suspension and hyperimmune serum survived more than 30 days.

Table 1. NEUTRALIZATION OF ISOLATE BY RABIES ANTISERUM Brain suspension + normal horse serum No. of Dilution mice No. injected dea Brain suspension + hyperimmune anti-rables horse serum
No. of
Dilution mice No. of Dilution No. of deaths injected deaths 10-1·2 10-2·2 10-3·2 10-4·2 10-5·2 10-6·2 5 5 5 5 5 5 5 10-11 2100000 10-5.5 10-3.5 10-4.5 10-6.5 65

These results imply that the dog-faced fruit bat, Cynopterus brachyotis, must be considered as a potential reservoir of sylvatic rabies in Thailand. The significance of bats as carriers of sylvatic rabies is enhanced by the report⁸ of transmission of rabies isolates from bats to a variety of animals by the intramuscular route and by the fact that rabies virus can survive for long periods of time in the brown fat tissue of bats.

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Photoelectric Technique for estimating Plasma Coagulation Time

THE usual way of determining time of coagulation, which is of diagnostic importance, is to observe clot formation. There has been no report of any way of measuring coagulation time with an accuracy of a few seconds. We report here our observations of the coagulation of human plasma, using a simple, sensitive and reproducible photoelectric device developed in this laboratory to study blood coagulation. The idea originated from a suggestion of Born1.

The details of the instrument are shown in Fig. 1. The position of the pen is set at maximum when a beam of chromatic light (2 mm aperture) is passed through a sample of clear fresh plasma. The clotting of plasma decreases the photoelectric output to the servo-amplifier, causing the pen to fall slowly.

Fig. 2 shows tracings of the time of coagulation using 2.5 ml. of plasma and 0.5 ml. of 0.025 molar calcium chloride. Volumes as small as 0.5 ml., however, can also The expanded recordings made with increasing sensitivities show that the time required for the voltage

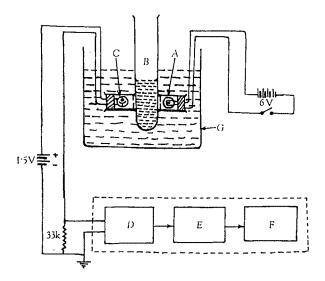


Fig. 1. Diagram of our apparatus. A, Incandescent lamp; B, plasma container; C, photoconductive cell; D, amplifier; E, servo mechanism (SE 21 Gilson Medical Electronics); F, chart drive; and G, thermostat water bath controlled at 37° C.

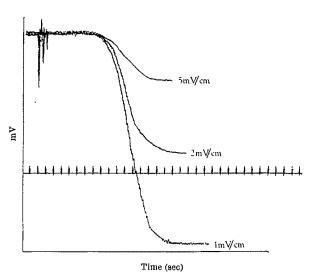


Fig. 2. Decrease in voltage drop as a function of time. Each marking corresponds to 12 sec.

to decrease to a steady minimum value is always 30 sec. Plasmas from different donors, however, show variations in this time. The disturbance of the pen serves as a reference for the time of calcium chloride addition.

The results show that accurate, consistent and reproducible values for the coagulation time can be obtained by using a photoelectric technique. It means that kinetic studies of coagulation can also be carried out.

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Reliability of Predictions of the Contamination of Milk with Fission Products in the United Kingdom

It has been shown¹ that in the United Kingdom a satisfactory guide to the contamination of the average mixed diet with strontium-90 and caesium-137 from nuclear weapons tests is provided by measurements of these nuclides in milk. This is so both for individual years when the rate of fallout is high, and also for the total dietary intake integrated over many years.

The following equation has been found² to give a satis-

The following equation has been found to give a satisfactory relationship between the annual average levels of contamination of milk with both nuclides and the deposition of fallout during the period 1958-64

$$C = p_r F_r + p_l F_l + p_d F_d$$

where C is the annual mean ratio of strontium-90 to calcium (pc./g) or concentration of caesium-137 (pc./l.) in milk; F_r is the annual deposit (mc./km²/yr); F_l is the deposit in the second half of the previous year (mc./km²); F_d is the cumulative deposit (mc./km²); and p_r , p_l , p_d are described as the "rate", "lag-rate" and "soil" proportionality factors, respectively.

The following values of the proportionality factors in this equation were derived

	Strontium-90	Caesium-137
Rate factor (pr)	0.70	3.00
Lag-rate factor (pi)	1.13	6-00
Soil factor (na)	0.11	0.03

Unlike the other factors, which were calculated from survey data by least square analyses, the "soil" factor for caesium-137 was deduced from experimental results because it was too small to be estimated adequately from the survey data.

Table 1. COMPARISON OF CALCULATED AND OBSERVED QUANTITIES OF STRONTIUM-90 IN UNITED KINGDOM MILK, 1968-66

Deposition of strontium-90* (mc./km²)		Strontium-90 in milk (pc. **Sr/g Ca)				
Year	January to June	July to December	Total	Calcu- lated		Calculated % Observed
1958	2.9	2·4	5·3	6.9	7·2	97
1959	6.0	1·7	7·7	10.5	9·8	107
1960	1.2	0·8	2·0	6.0	6·4	94
1961	0.8	1·4	2·2	5.3	5·9	90
1962	5.2	5·3	10·5	12.6	11·7	108
1963	10.0	9·0	19·0	24.6	25·6	96
1964	9.5	5·3	14·8	28.4	28·0	101
1965	3·5	2·4	5·9	17·7	19·0	93
1966	1·8	0·9	2·7	12·5	12·1	103

The proportionality factors used were based on data up to the end of 1964

* Some of the values shown differ slightly from previous estimates* because of a small change in the basis of calculation.

Table 2. COMPARISON OF CALCULATED AND OBSERVED QUANTITIES OF CAESIUM-137 IN UNITED KINGDOM MILK, 1900-66

Deposition of caesium-137 (mc./km²)			Caesium-137 in milk (pc./l.)			
Year	January to June	July to December	Total	Calcu- lated		Calculated %
1960	2.0	1.4	3.4	28	26	106
1961	1.3	2.4	3.7	20	21	96
1962	7.8	8.0	15.8	62	62	100
1963	15.0	13.5	28.5	134	135	100
1964	14.3	7.9	22.2	153	153	100
1965	5.3	3.6	8.9	78	98	80
1966	2.7	1.3	4.0	37	46	81
The nr	onortionali	w footore ne	od wore h	toh no hasa	o un to the	1301 to head

The proportionality factors used were based on data up to the end of 1964 only.

Tables 1 and 2 show that for both nuclides the calculated and observed levels agree closely for each of the years for which data were used in the calculation. It is now possible to test these equations more fully by applying them to the results for the two subsequent years, 1965 and 1966. As in the previous report, the country-wide mean deposition was calculated from the survey results of the Atomic Energy Authority (ref. 3 and personal communica-

¹ Born, G. V. R., Nature, 194, 927 (1962).

tion from R. S. Cambray) and information on rainfall provided by the Meteorological Office.

Table 1 shows that for strontium-90 the agreement between predicted and observed values continued to be close in 1965 and 1966. If new proportionality factors are calculated from the data for the whole period p_d remains unchanged, while p_r and p_l increase by 1 per cent and 7 per cent, respectively. The proportion of the total strontium-90 in milk attributable to uptake from the soil is 43 per cent in 1965 and 63 per cent in 1966, as compared with about 20–25 per cent in years of high deposition². These figures are comparable with those when the rate of deposition was previously declining in 1960 and 1961.

For 1965 and 1966 the equation underestimates levels of caesium-137 in milk by 20 per cent (Table 2). Although the agreement is adequate for practical purposes, it is of interest to recalculate proportionality factors on the basis of the fuller data. The revised factors are: p_r , 2·5; p_l , 6·0; p_d , 0·16. The chief change is in p_d , which is five times that estimated previously from the result of a limited number of experiments. Its value, however, remains so small that it fails to attain statistical significance. Uncertainties regarding this component are not surprising in view of the complex processes which affect the uptake of caesium from the soil⁴; results for a considerably longer period must be assembled before a soil factor for caesium-137 can be derived with confidence.

It is interesting that the more recent data confirm the conclusion previously advanced, on the joint basis of survey results and information on agricultural conditions, that in the United Kingdom the contamination of milk with both nuclides is influenced to an appreciable extent by the rate of fallout in the preceding year.

Although the present method seems to provide a reasonable basis for gauging the extent to which milk will be contaminated as a result of a postulated pattern of fallout, the procedure is approximate only. It has been previously explained. That the processes which control the transfer of strontium-90 to milk are much more complex than the equation implies; the periods to which the rate and lag-rate factors relate are arbitrary and slow changes in the soil factor are to be expected with time. During extended periods slow losses of both nuclides will occur from the root zone; the cumulative deposit in the soil will decrease for this reason as well as by radioactive decay. Present information provides no basis for eliminating these defects.

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Accumulation of Glycine in the Fat Body of the Silkworm, Bombyx mori L.

Investigations of intestinal transport of amino-acids in the locust^{1,2} and silkworm^{3,4} have shown no evidence for active accumulation in a transport from the insect gut of amino-acids. When glycine-2-¹⁴C was administered in vivo to fifth instar larvae of the silkworm, 96 per cent

of the radioactivity was incorporated into various tissues within 1 h whereas in vitro only 19 per cent of the activity was transported by the mid-gut of silkworm (unpublished work). These results suggested that continued absorption of glycine by the intestine could be aided by a facilitated diffusion mechanism in which amino-acids are rapidly removed from the site of absorption either by accumulation into other tissues or by degradation. Although the insect fat body has been assigned both accumulatory and dissimilatory roles, the mechanism of accumulation of amino-acids has not been investigated. Our present experiments show that the silkworm fat body possesses an efficient mechanism for accumulating glycine and that both the accumulation and the release of glycine are metabolically controlled.

Table 1. VARIATION OF UPTAKE OF GLYCINE WITH AGE IN THE SILKWORM FAT BODY

Age of silkworm	Percentage absorption for 50 mg fat body with S.E.	P	External concentration ratio
Middle fifth instar larvae	* (a) 24.31 ± 1.87		$\frac{2.36}{1.17}$
Just before spinning stage Mid-pupal stage	(b) 9.37 ± 0.12 5.29 ± 0.67 Nil	< 0.01	0.67

* Larvae from a different batch.

The uptake of glycine was studied by suspending 50 mg of fat body in 0.5 ml. of Ringer solution containing 5 μ l. of glycine-2-14C (specific activity, 0·1 mc./9·8 mg dissolved in 2 ml.) and 7·5 mg of carrier glycine (200 mmoles/l. Ringer solution). The mixture was shaken occasionally and, after the specified time, the fat body was removed from the medium by rapid filtration through glass wool and suitable samples of the medium and alcohol extracts of fat body were plated on planchets. Radioactivity was measured with a Geiger-Müller counter (end-window type). The fat body rapidly took up glycine. Within 6 min the total concentration for the total water of the fat body tissue was found to be between 2 and 3 (Table 1). This fast uptake was maintained up to a glycine concentration of 3 moles/l. (the maximum used in the experiment). Addition of ATP (1 µmole/ml. of incubation medium) had no effect on the absorption of glycine. The time course of the uptake is shown in Fig. 1.

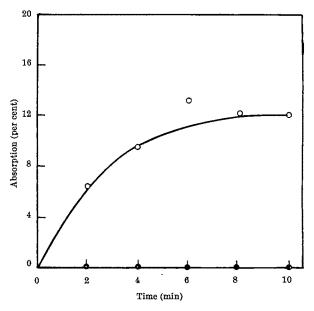


Fig. 1. Time course of uptake of glycine by the fat body of the silk-worm. O, Control; •, cyanide.

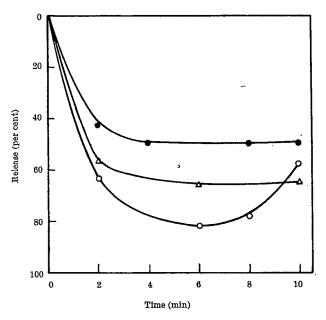


Fig. 2. Time course of the release of glycine from the fat body of the silkworm. O, Control; •, cyanide; A, DNP.

Experiments designed to study the effect of the inhibitors 2,4-dinitrophenol and cyanide have further emphasized the remarkably rapid uptake of glycine. considerable inhibition of uptake was observed with cyanide and to a lesser extent with DNP when the fat body was pre-incubated with the inhibitors (10^{-3} molar) in Ringer solution and used subsequently for absorbing glycine from a medium containing both the labelled substrate and the inhibitor. On the other hand, direct incubation of the fat body with the substrate and inhibitor showed no inhibition of uptake. The rate of uptake of glycine obviously far exceeds the rate of inhibition of the transfer mechanism.

The kinetics of the release of glycine from fat body are shown in Fig. 2. Equilibrium was reached within 10 min and the release was partially inhibited by cyanide (10-3 molar) and DNP (10⁻³ molar). On prolonged incubation there was a reincorporation of the released glycine into the fat body and both DNP and cyanide inhibited this reincorporation.

Fat body is also the most important site of synthesis of proteins and other reactions of amino-acids such as transamination, oxidation and decarboxylation, and so it was necessary to determine the metabolic fate of absorbed glycine. The fat body was fractionated after 30 min of incubation with labelled glycine. More than 90 per cent of the activity was recovered in the alcohol extract and this in turn could be accounted for as glycine. Paper chromatography of the extract showed mainly glycine and a trace of serine. During this period none of the labelled carbon was recovered in the respired carbon dioxide by manometric studies.

The well defined active uptake observed with the fat body tissues suggests that absorption is connected with an organized tissue structure. The dependence of the uptake on the discrete fat body structure is demonstrated by the disappearance of the ability to accumulate during the pupal stage (Table 1). Comparison between middle fifth instar larvae and spinning larvae shows a statistically significant reduction in uptake even at the early spinning stage. These observations are in agreement with electron microscopy studies of the fat body in Philosamia cynthia ricini (L.)^{7,8} during different stages of growth. These revealed the typical double-layered plasma membrane for the fat body cell during the fifth instar and its partial disintegration during the spinning stage. The membrane was not visible in the fat body cells of the pupae.

We thank Professor J. V. Bhat for his keen interest in the work.

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Role of Host Plant in Production of Winged Forms by a Green Strain of Pea Aphid Acyrthosiphon pisum Harris

THE importance of tactile contacts between aphids, or the effet de groupe as first described by Bonnemaison1, has been demonstrated in several species of aphids. Lees2 has reviewed work in this field, and has evaluated the claims of those who have invoked "nutrition" as the important factor in the production of these forms, rather than any crowding factor. He concluded: "Unequivocal evidence that starvation or malnutrition, or specific changes in the quality of the plant sap, can cause isolated aphids to become alatae or alata-producers is still lack-

The present work on Acyrthosiphon pisum has shown that, while there is certainly a response to crowding in adult apterae in that some of them produce some alate daughters immediately after the treatment, it is possible to induce aphids reared from first instar in isolation on seedlings to produce many more alate progeny simply by providing them, when adult, with a different food source. In the experiments described here, all aphids have been reared on broad bean (Vicia faba) seedlings (0.5-4 in. high). Only after producing a control "0" batch of larvae on the seedlings were they subjected to any of the treatments described here.

Crowding aphids off the host plant for 24 h in an empty glass tube, at a density of ten/tube, can provide sufficient stimulus for at least some species to produce winged offspring³. This treatment was given to adult apterae of Acyrthosiphon pisum, and a response developed when they were returned to seedlings to deposit young. In Table 1, batch 1 comprises the larvae produced by the mothers of a given experiment in the first 24 h after any particular treatment. Each succeeding batch covers a 2 day period of larviposition. The numbers of alatae produced by the mothers in any batch have been pooled and expressed as a percentage of the total number of larvae in that batch. When the mothers were feeding on seedlings an average of 31 per cent of the larvae born in the first batch after crowding became alatae (Table 1A). Starvation was not the factor inducing the mothers to become alata-producers because control insects which were starved in identical tubes for 24 h, in isolation, produced no alatae at all.

Three experiments were designed to show whether or not the condition of the host plant could influence the number of alatae produced by adult apterae. The results of these experiments are given in Table 1 (B-D). In the first experiment, the aphids were reared as described here and were directly transferred, without previous crowding, to small leaf cages on mature fully expanded green leaves

of 12 in. high bean plants. Their larvae were then removed in batches and were reared on seedlings until the fourth instar, when they were examined for the presence or absence of wing pads. Previous work had shown that larvae of this species are determined as alatae or apterae before birth, any environmental factor such as diet or crowding having no influence after birth. It had also been shown that in such cages contacts between the larvae and the mother do not induce the latter to produce alatae. Hence, any response by the mother, as reflected in the form of her offspring, could only have been caused by the different type of nutrition she was receiving during

Table 1B shows that within 72 h of feeding on the mature leaves, that is, by the end of the second batch, some mothers had started to produce alate daughters. The percentage of alatae was much greater in the third batch, and still greater in the fourth. Indeed, in the latter case the proportion of alate progeny exceeded the values obtained when aphids were crowded and returned to seedlings to reproduce (treatment A). Controls maintained in isolation on seedlings for their entire lives produced no alatae.

Table 1. RESPONSE OF APTEROUS VIRGINOPARAE OF Acyrthosiphon pisum when subjected to various treatments favouring the production of winged forms

		Percentage							
Treat- ment	No. of aphids	becoming alata	Pe	rcents	ige of al	atae in of larv	successi	ve bat	ches
		producers	0	1	2	3	4	5	6
A	60	77	0	31	5	1	0	0	. 0
Controls	80	0	0	0	0	0	0	0	0
\boldsymbol{B}	30	50	0	0	2	29	59	17	36
Controls	50	0	Ó	Ó	0	0	0	0	0
σ	30	100	Ô	70	57	70	69	56	47
D(i)	20	100	Ò	50	73.5	11*	Ö	0	0
D(ii)	20	100	Ō	27	68.5	75	1.5*	Ö	Ō
* First	batches aft	er withdray	val fr	om m	ature le	af.			
A	Crowded a	nd placed o	n see	dlings	s immed	liately a	ıfter.		
Controle	Starved in	ignlation a	nd be	mt on	rilhaga	ma .			

to Crowded and placed on seedlings.

Not crowded or starved but placed on mature leaves after producing "0" batch.

Not crowded or starved and kept on seedlings.

Crowded and placed on mature leaves immediately after.

Crowded and placed on mature leaves immediately after:

(i) Returned to seedlings after 4 days.

(ii) Returned to seedlings after 6 days. Controls

D

The second experiment (C) involved both crowding and host plant factors and can be compared with the initial crowding experiment (Table 1.4). Here the aphids were crowded ten/tube for 24 h, but, instead of being returned to seedlings to reproduce, they were isolated in cages on mature bean leaves, the larvae being removed and reared in the usual way. The effect of the new host plant on these aphids was most marked. The percentage of alatae in the first batch of larvae was more than twice as great as when the aphids were fed on seedlings after crowding, and 100 per cent of the mothers became alataproducers rather than 77 per cent. Furthermore, the aphids continued to produce large numbers of alatae throughout their reproductive lives.

The last experiment (D) was similar to the previous one (C) except that the aphids were returned to seedlings after either 4 or 6 days of depositing larvae on the mature leaves. The results show that most of the aphids produced 100 per cent apterae immediately after the transfer. A few produced from one to five alatae in the first batch on the new host, but then reverted to producing apterae only.

Clearly, as Johnson reported for Aphis craccivora, the type of host plant can play a most important direct part in the production of winged virginoparae in at least some species of aphids. Johnson reared the parent apterae of Aphis craccivora on mature leaves as well as allowing them to reproduce on them, but unless he added a crowding stimulus the response was comparatively weak. My experiments on Acyrthosiphon pisum, however, show that a strong response can be obtained when the aphids are provided with mature leaves after parturition has begun, and that this is true even when the aphids have been kept totally isolated.

I thank Dr A. D. Lees for supervising and encouraging this work.

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Delayed Damage to Phaseolus vulgaris L. Seeds by Water trapped during Soaking

Damage to Phaseolus vulgaris seeds through soaking is widely recognized. Successive workers1-6 have interpreted their results as indicating that this is more likely to be caused by the leaching of cell constituents than by oxygen shortage. Our evidence, however, which conflicts with none of theirs, indicates that the rate of oxygen supply to the embryo is the basic problem.

Seeds of the cultivar 'Masterpiece' were soaked in tap water and then placed on moist paper in closed boxes at 20° C in the dark. Our results (Fig. 1 and Table 1) show that short periods of soaking can be favourable to germination, but soaking for longer than 4 h has caused progressive damage. This damage is related to the entry into the intercotyledonary cavity of an excess of water which is then prevented from draining away because the cotyledons swell inordinately and are held pressed together at their edges by the testa. In these conditions the germination percentage declines, some of the seedlings which are tardily produced die soon after radicle emergence and the growth of the survivors is restricted. Yet all these manifestations of damage are completely prevented if the trapped water is released by stripping the testae off the The actual damage does not therefore soaked seeds.

Table 1. WATER CONTENT OF Phaseolus vulgaris L. SEEDS (LOT 2) AFTER 24 h SOAKING, AND EXTENSION GROWTH OF SURVIVORS PLACED IN GERMINATOR

			WITH - 2 OH WATER POINT	LLAL	
Soaking treatment (h) 1% $H_2O_2 \longrightarrow tap water \longrightarrow 1\% H_2O_2$			Water content (per weight) at time of In embryonic tissues	Mean length of tap root and hypocotyl after 7 days in germinator (mm)	
0	0	0 * 0 †	$ \begin{array}{c} 10.8 \pm 0.4 \\ 105.1 \pm 4.9 \\ 116.4 \pm 6.3 \end{array} $	0 0 14·7±3·5	8.4 ± 0.1 4.8 ± 1.2 ¶
0	24	0 { \$	117·3 ± 3·4 116·4 ± 6·3	16·4 ± 3·7 0	4·0 ± 0·2¶ 14·7 ± 1·6
6	18	0	117.8 ± 6.4	5.2 ± 3.9	15.5 ± 1.4
24	0	0	113.5 ± 3.6	2.0 ± 1.2	16.0 ± 0.9
-ō	23	ī	117.5 ± 4.8	1.6±0.3	9.9 ± 0.9

Experiments were carried out at 20° C.

* Dry seed.
† Unsoaked seed at radicle emergence.
† No other treatment.
\$ Continuous aeration by bubbling air through soaking water.
|| No other treatment but testa removed at end of soaking.
|| Based on survivors; approximately one-third of the seedlings did not survive until day 7.

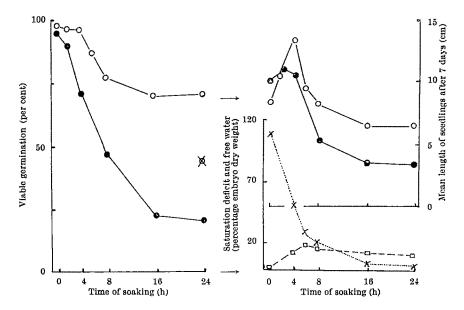


Fig. 1. Effect of soaking in tap water on germination (viable seedlings), seedling growth and water content of seeds of *Phaseolus vulgaris* L., 'Masterpiece': ●, seed lot 1; ○, seed lot 2; ⊕, seed lot 2, with air bubbled through the soaking water; ×, saturation deficit of seed (reaching zero after 24 h soaking); □, free water in the intercotyledonary cavity.

occur while the seeds are under water for 24 h (or indeed 48 h) but after they have been placed in an environment which is normally favourable to germination. This, and the worsened performance when air is bubbled through the soaking water, suggests a detrimental imbalance in the oxygen supply to the embryo (high outside, low inside). Treatment of the seeds with a I per cent solution of hydrogen peroxide before, during or after soaking has virtually the same results as removing the testa, because the solution penetrates into the seeds and gaseous oxygen is released by enzyme action and (Table 1) gradually expels the excess of water from the intact seeds. Consequently, these seeds are capable of germinating immediately on removal from the 1 per cent hydrogen peroxide soak. They will not germinate while in this solution because it is so strong as to be inhibitory but they will germinate while completely submerged in a solution of 0.06 per cent hydrogen peroxide.

Finally, even intact Phaseolus vulgaris seeds soaked in water for 24 h can on removal germinate and grow well if they have, before soaking, imbibed in aerobic conditions about one-third of the water needed for the radicle to emerge. This suggests that germination may be triggered off very early during imbibition, provided sufficient oxygen is available at this stage, and will then proceed unimpaired even though the oxygen supply may be severely restricted later.

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Germination Response to Leaching in Dormant Seed of Trifolium subterraneum L.

DORMANCY in Trifolium subterraneum L. (subterranean clover) has been reviewed by Morley¹. Dormancy in imbibed seed is usually high at harvest maturity but declines with time at rates which differ with strain. High temperatures are inhibitory to germination, and the younger the seed the lower is the inhibiting temperature. Ballard^{2,3} has shown that dormancy in new seed is readily overcome by cold treatment and also by subjecting the imbibed seed to an atmosphere enriched with carbon dioxide at temperatures of up to 25°C; above 25°C, carbon dioxide is unable to abolish completely the inhibition of germination at high temperatures. Dormancy may also be relieved by heat treatment of dry seed4, by removal of the seed coats and by other means. presence of an inhibitor in the seed which is soluble in water has in the past been questioned if not discounted.

Some apparent inconsistencies between germination in the laboratory and germination of clover seed in the field following summer rains are being investigated. In the course of these studies, we have observed a marked germination response, in the laboratory, to leaching seed of T. subterraneum with water. The response to leaching has been particularly marked at temperatures above 25°C, at which there has been little or no response to carbon dioxide treatment. Even at 10°C, however. responses to leaching have been obtained in addition to those obtained by treatment with carbon dioxide.

A recent study on new seed (conducted within a few weeks of harvest maturity) included thirteen strains of T. subterraneum representing a wide range of maturities. At 30°C, the following treatments were examined: (a) seed placed on filter paper in Petri dishes; (b) seed placed on filter paper in flat-sided glass bottles sealed with an atmosphere containing 2.5 per cent carbon dioxide; (c) seed placed in a Buchner funnel and leached continuously with de-ionized water at 10-15 ml./h; (d) testae removed from imbibed seed and the embryos placed on filter paper in Petri dishes; and (e) embryos leached as in (c). In treatments (a), (b) and (d), the filter paper was saturated with de-ionized water, and there was a slight excess of free water in each case. Treatments (a), (b) and

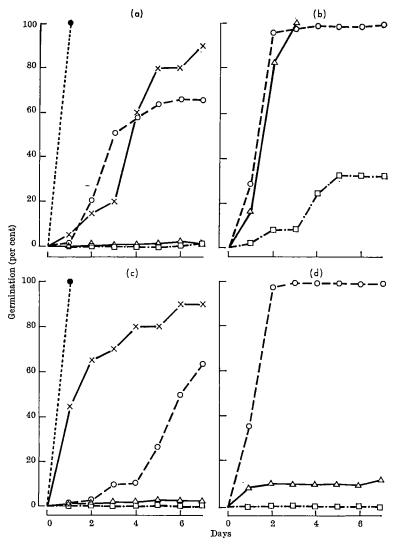


Fig. 1. Effect of seed treatments on germination in two strains of *T. sublerraneum*.

(a) and (b), 'Northam C' at 30° and 20° C, respectively; (c) and (d), 'Burnerang' at 30° and 20° C, respectively.

[], Petri dish; A, 2·5 per cent carbon dioxide; O, leached; x, embryos; , embryos leached.

(c) were also studied at 20° C. In most cases, duplicates of fifty seeds were used in treatments (a), (b) and (c) and twenty embryos in treatments (d) and (e). All treatments were conducted in darkness except for short periods each day when the seeds and embryos were examined in diffuse daylight. Seeds were classed as germinated when the radicles had emerged in the case of intact seed, and when the radicles had elongated in the case of embryos. The percentages of seeds which had germinated were recorded at intervals of 24 h for 7 days. These readings were then summed after the method of Timson?. The final results, recorded as summed germination, $\Sigma 7$, and averaged for the thirteen strains, are presented in Table 1. The time course of germination of two strains, 'Northam C' and 'Burnerang', is shown in Fig. 1.

At 20° C all strains showed some reluctance to germinate in Petri dishes. Twelve strains germinated readily when

Table 1. Effect of a range of seed treatments on the summed germination percentage (Σ 7) of new seed of thirteen strains of T. sublertaneum

Seed treatment	Cumulative germination percentage 20° C 30° C		
 (a) Petri dish (b) Carbon dioxide (c) Leached (d) Embryos (e) Embryos leached 	296 584 628 Not available Not available	18 24 354 384 698	

treated with carbon dioxide, but the remaining strain, 'Burnerang', showed little response. All strains, however, readily germinated when they were leached. At 30° C there was little or no response to treatment with carbon dioxide, but again there was a marked, though incomplete, response to leaching, and more than 50 per cent of the seed of twelve strains germinated within 7 days. The exception at this higher temperature was 'Dinninup', in which only 30 per cent of the seed germinated after leaching for 7 days. removal was partly successful in promoting germination at 30° C, and almost complete germination was obtained within 24 h by leaching the embryos (carbon dioxide was equally effective in promoting germination of the embryos of 'Daliak'—the only strain tested in this way).

In an earlier study, germination tests were conducted over a range of temperatures on seed of four early maturing strains of T. subterraneum which had been stored for 6 months in one case and 3 years in the other. All seeds from both age groups readily germinated in Petri dishes at 10°, 15° and 20° C. Germination rate was slightly enhanced by carbon dioxide treatment and was further slightly accelerated by leaching. At temperatures of 27° C and higher, germination of all seeds was poor in Petri dishes. Carbon dioxide did not promote germination in the younger seed, but gave a marked germination response in the older seed at 27° C and a slight response at 30° C. All seeds germinated rapidly when leached at 27° and 30° C, and even at 35° C appreciable germination was obtained (range, 15 per cent to 90 per cent after 5 days).

The evidence presented here supports the hypothesis that a water-soluble germination inhibitor is involved in the regulation of the germination of seed of T. subterraneum. Moreover, this inhibitor seems to be located partially, if not exclusively, in the embryo. We also have evid-

ence, based on Avena coleoptile section tests, for the presence of both inhibitory and promoting substances in the seed of T. subterraneum. Germination may therefore depend on promoting as well as inhibitory substances, as claimed in other plant species8,9. The possibility that the leaching response may be connected with moisture tension effects or alteration to gas exchange cannot be ignored. The available evidence suggests, however, that these alternatives are unlikely.

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Manganese Toxicity in Celery

NECROTIC lesions on the leaflets and petioles of celery, Apium graveolens L. (variety Latham's self-blanching), were observed in a crop grown on a reclaimed peat soil in West Cork. I have shown that these symptoms in celery, which have not previously been reported, were caused by toxic concentrations of manganese in the plant.

The celery was grown on a deep acid peat soil in the Raheen Series¹, which was formed in a low lying area where peat development was associated with poor drainage conditions. The peat was reclaimed in 1964 when it was drained and sea sand equivalent in neutralizing value to 2.5 tons of ground limestone/acre was applied. Oats were sown but failed. The field was fallowed in 1965. In February 1966 sea sand equivalent to 5 tons of ground limestone/acre was applied and subsequently a crop of celery (approximately 1 acre) was produced by the usual horticultural procedures.

In September, samples of (a) healthy, (b) slightly affected, (c) badly affected and (d) very badly affected plants were taken. Each sample was split into four parts, mature and young leaflets and mature and young petioles. Soil samples were taken from around the roots of the four plant samples. Plant and soil samples were analysed by the methods used in the Soil Laboratory, Johnstown Castle².

Table 1. CONCENTRATIONS OF MANGANESE AND ALUMINIUM IN MATURE AND YOUNG LEAFLETS AND PETIOLES OF CELERY (P.P.M. ON DRY MATTER BASIS)

	· Part of plant							
Extent to which	Mature				-	Young		
plants affected	Leafl	Leaflets Petioles		Leaf	Leaflets		Petioles	
-	Mn	Αl	Mn	Al	$\mathbf{M}\mathbf{n}$	Al	Mn	Al
Not affected (healthy)	245	102	70	58	138	66	60	30
Slightly affected	3,300	114	700	96	360	82	250	40
Badly affected	5,423	114	1,167	58	787	122	355	30
Very badly affected	10,270	170	2,635	102	1,795	152	633	ND
ND Not determine	A.		,					

The symptoms, which appeared mostly on the mature parts of the plant, occurred as brown necrotic lesions (approximately 0.25 in. in diameter) on the leaflets and as sunken shiny grey black lesions in the pith between the vascular bundles and as irregularly shaped lesions on the abaxial and adaxial surfaces of the petiole and petiolule, respectively (Figs. 1 and 2). The plants produced new heart leaves and retained an upright habit regardless of the degree to which they were affected.

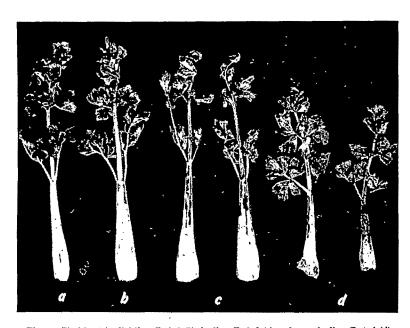


Fig. 1. Healthy (a), slightly affected (b), badly affected (c) and very badly affected (d) leaves showing lesions on leaflets and on abaxial and adaxial surfaces of the petiolules and petioles.



Fig. 2. Irregularly shaped lesions on adaxial surface of petiole and petiolule.

Healthy plants were very well developed and it was expected that a high yield would be obtained in the 70 to 80 per cent of the crop not affected.

Slightly affected plants were retarded in growth and brown necrotic lesions occurred apparently randomly on the leaflets. Lesions occurred in the pith between the vascular bundles on the adaxial surface of the petiolule and the upper part of the petiole (Fig. 1b). Small irregularly shaped lesions also occurred on the edge of the petiolule.

Badly affected plants were considerably retarded in growth. All the mature leaflets were affected; the brown necrotic lesions were more numerous and the leaflets tended to be chlorotic. The lesions occurred most fre-

quently along the edge and veins of the leaflets. In these plants, more intervenal lesions occurred on the abaxial surface of the petiole and petiolule. Irregularly shaped lesions also occurred on the adaxial surface of the petiole and petiolule.

Very badly affected plants were stunted. The lesions on the leaflets were very numerous, tended to coalesce and cover the whole leaflet except for a small area around the junction with the petiolule. The lesions on the petiole and petiolule were also very numerous and in extremely bad cases tended to coalesce to form large necrotic areas on the abaxial surfaces (Fig. 1d). Large irregularly shaped lesions also occurred on the adaxial surface of the petiole and petiolule (Figs. 1d and 2).

The concentrations of manganese in the affected leaflets and petioles were high and varied significantly with the extent to which the plants were affected (Table 1).

The manganese concentration in the leaflets of the very badly affected plants was a hundred times greater than expected. High concentrations of manganese had also been accumulated in the new leaflets and petioles in which, however, no symptoms had developed. Concentrations of

aluminium were very slightly higher in the affected plants, whereas boron and copper did not vary at

In October, variation of pH was determined in soil profiles taken from various parts of the field. In the areas in which the plants were very badly affected, field investigation showed that pH was only satisfactory in the top two to three inches of soil. Below this the peat was very acid (pH 3.9). In the part of the crop in which the plants were healthy and where a good yield was expected. sea sand had been well mixed with the peat. Here the pH in the surface 6-8 in. was satisfactory and the peat below 8 in. was very acid. It was evident that the extent to which plants were affected was correlated with the depth to which sea sand had been incorporated into the peat and therefore with the depth of peat with a suitable pH. Where this was 6-8 in. deep, the rooting medium was satisfactory for maximum plant growth. Where this was only 2-4 in. deep, roots tended to extend from the high pH surface zone into the very acid zone beneath, from which considerable quantities of manganese were apparently absorbed by the plant.

Hewitt has shown that celery is much more susceptible to aluminium than to manganese and that celery crop failure caused by acidic soil conditions usually shows the symptoms associated with aluminium toxicity4. The high concentrations of manganese in the plant and the development of the symptoms described were caused by the absorption of manganese from the acid soil beneath the plant where the sea sand had not been sufficiently incorporated into the peat.

The reason why so much manganese and so little aluminium were absorbed has not been determined. Conroy and Ryan have shown that, although the concentrations of manganese in the native rocks of the West Cork area are not high, sufficient quantities exist to provide sizable accumulations in times. They have shown that high concentrations of manganese occurred in the alluvial soils of the Manch and Ilen series1. It is probable therefore that the high concentrations of manganese have accumulated in the peat, and manganese toxicity in celery has occurred where inadequate mixing of lime with the peat soil has not decreased the availability of manganese in the root zone of the plant.

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Fixation of Nitrogen by Bitterbrush (Purshia tridentata (Pursh) D.C.)

THE frequency and importance of nodulated nonleguminous plants are becoming more widely recognized. Allen and Allen¹ have presented a summary of nonleguminous symbiosis in both the gymnosperms and angiosperms and emphasized analogies with the legume-Several workers2-4 have used rhizobia symbiosis. nitrogen-15 to demonstrate that fixation of atmospheric nitrogen does occur in the nodules of many non-leguminous plants.

In 1961, Wagle and Vlamis⁵ reported nodules on the roots of bitterbrush (Purshia tridentata), a member of the family Rosaceae, and presented evidence which suggested that nitrogen fixation occurs in the nodules. We have used nitrogen-15 to study nitrogen fixation by species of non-leguminous plants, including bitterbrush.

Bitterbrush seeds collected in central Oregon were soaked in 10 per cent thiourea for 5 min to enhance germination. The seeds were sown in a sandy soil with a high natural nodulation potential, in 1 pint polyethylene containers, on January 19, 1967. After germination and establishment each pot was thinned to six seedlings. Plants were grown in a greenhouse and the sunlight was augmented by banks of alternating warm-white and daylight fluorescent lamps suspended about 75 cm above the pots to give a day length of 16 h. Day temperature was 21° C and night temperature was 16° C. Plants were watered to maintain moisture slightly below field capacity. After 4 months the seedlings were gathered. Another group of seedlings was grown for a period of 2 months in a growth room with a day length of 16 h at approximately 2,000 ft.-candles at a day temperature of 27° C and night temperature of 21° C. After the seedlings had been removed from the pots, soil was washed from the roots. The nodules were then excised and approximately 0.3-0.5 g of nodule mass was placed in 20 ml. gas-tight serum bottles for exposure to nitrogen-15.

The nodules were exposed to nitrogen-15 within 10 min of excision for a period of 24 h at 20° C in the dark. The atmosphere in the serum bottle was enriched by reducing the pressure within the bottle by removing a known volume of air with a syringe. The pressure was restored to near atmospheric by injecting a calculated amount of nitrogen-15 at the appropriate pressure. The atmosphere in the serum bottle was calculated to contain 14 atm. per cent nitrogen-15. Gas analysis indicated, however, that the atmosphere ranged from 9 to 12 atm. per cent.

After exposure the nodules were removed from the serum bottles and prepared for analysis for nitrogen-15 by mass spectrometry which was carried out in the laboratory of Dr F. E. Broadbent, University of California, Davis.

Virtanen et al.2, who were among the first to use isotopic nitrogen in fixation studies, considered 0.015 atm. per cent of excess nitrogen-15 indicative of positive fixation. We used 0.02 per cent as positive evidence in our studies and the results are given in Table 1. Although there is considerable variation among the samples, they all have an excess of nitrogen above 0.02 atm. per cent. The variation may be due to differences in plant vigour. The nodules in sample 2 came from a vigorously growing 2-month-old seedling. Work in our laboratory with Ceanothus velutinus Dougl. indicates that there is considerable difference in the rate of fixation between nodules from actively growing and dormant plants.

Table 1. ATM. PER CENT EXCESS NITROGEN-15 CONTENTS OF BITTERBRUSH NODULES EXPOSED TO GASEOUS NITROGEN-15

Sample No.	Approximate fresh weight of nodule sample (mg)	Atm. % excess 15 N
1	330	0.075
$\bar{2}$	330	0.379
2 3	300	0.120
4	500	0.155
5	500	0.051

The nodules on bitterbrush have the same coralloid morphology and white colour as those of Ceanothus, and a Streptomyces species has been isolated from bitterbrush nodules which has characteristics similar to the Streptomyces species isolated from Ceanothus velutinus nodules. That this organism is the endophyte still remains to be confirmed by pure culture inoculation and nodulation.

Bitterbrush is widely distributed throughout the ponderosa pine (Pinus ponderosa Laws.) forests and open range lands in the western United States. Although much remains to be learned about the nodulation and nitrogen fixation process in bitterbrush, our results imply that it is making a significant contribution to the nitrogen economies of the ecosystems in which it occurs.

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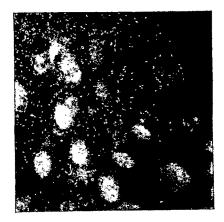
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MICROBIOLOGY

Intracellular Development of Infectious Bronchitis Virus

Cells infected with infectious bronchitis (IB) virus in tissue cultures of chick or chick embryo kidney have been described as showing shrinkage, vacuolation and detachment from the monolayer1-4. When the immunofluorescent technique was used, the growth of IB virus in chick embryo kidney cells, first in the nucleus and later in the cytoplasma, was described. A study was undertaken to obtain additional information about the location of IB virus, and to determine the type of viral nucleoprotein to assist in classification of the virus6.

Coverslip cultures of chick embryo lung and kidney from 15 and 19 day embryonated eggs, respectively, were stained by coriphosphine, before, and daily for 6 days after, infection with 1,000 EID_{50} of IB virus for each coverslip. The cultures were examined under a Reichert microscope with fluorescent illumination. Infected cell nuclei were seen to become smaller 24 h after infection and the intercellular junctions more distinct (Fig. 1a). Intracytoplasmic granules were clearly visible 48 h after infection with red fluorescence (Fig. 1b) characteristic of



Uninfected chick embryo lung cells stained with coriphosphine 72 h after infection of similar cells with virus (\times c. 500).

The number of normal nuclei and ribonucleoprotein. cells in any one field of the microscope at this time was greatly reduced. By 72 h, the granules had coalesced to become red-fluorescing intracytoplasmic inclusions (Fig. 1c), and the nuclei of infected cells were pyknotic. Few normal cells remained.

Many isolates from what appears to be clinical IB disease have been found in this laboratory not to be pure IB virus, but commonly mixtures of two viruses, most often IB virus and avian adenovirus. Neutralization of the IB component of such a mixture with specific IB antiserum, and the passage of such virus serum complexes in chick embryo kidney tissue culture, were found to be the best way of recovering the adenovirus component from mixtures of IB virus and adenovirus, and of making a preliminary identification of a contaminating desoxyribonucleoprotein virus, as cells infected with adenovirus show bright green fluorescence in their nuclei.

In four out of 150 separate chick embryo lung or kidney tissue culture preparations from embryonated eggs from the same IB-free flock extensive cytoplasmic vacuolation appeared to develop spontaneously in uninoculated monolayers. Repeated attempts to reproduce such vacuolation by passaging collections of affected monolayers in normal cultures proved unsuccessful. Inoculation of material into the allantoic cavity of 10 day embryonated eggs and onto the chorio-allantoic membranes of 8 day embryonated eggs failed to demonstrate the presence of an infectious agent.

It appears that vacuolation of the cytoplasm of monolayers infected with IB virus is not necessarily related to the presence of an infectious agent as previously suggested1,2,4. Furthermore, no indication was obtained that

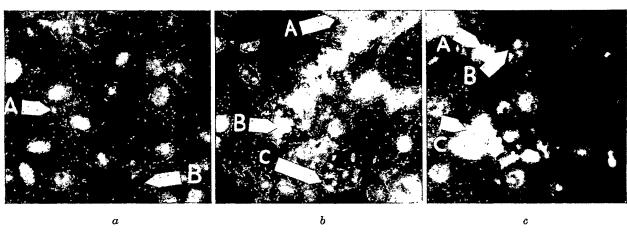


Fig. 1. Chick embryo lung cells, infected with 1,000 BID_{50} Massachusetts strain IB virus and stained with coriphosphine (\times c. 500). a, 24 h after infection; A and B show granular appearance of cytoplasm. b, 48 h after infection; A, B and C show the intracytoplasmic granules. c, 72 h after infection; A shows a pyknotic infected nucleus, B and C point out the intracytoplasmic granules coalesced to become inclusions.

virus may develop initially in the nucleus, but it was noticed that the shrunken size of the nuclei of infected cells was accompanied by brighter fluorescence when stained with coriphosphine, although this was taken to be non-specific.

On the basis of criteria previously proposed, it seems that IB virus is a ribonucleoprotein type virus, developing as intracytoplasmic granules that coalesce to form inclusions.

I thank Mrs Barbara Hay for technical assistance. D. M. Berry

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BIOCHEMISTRY

Effects of Antibiotics on Protein Synthesis in Muscle and Implications with regard to the Action of Insulin

Many compounds are specific inhibitors of protein synthesis and the mechanism of their action has been much investigated. Not all compounds which inhibit protein synthesis in micro-organisms do so in ribosomal systems of mammalian origin, for example, chloramphenicol¹⁻³. In yeasts an interesting dichotomy has been described between materials inhibiting mitochondrial but not ribosomal systems, for example, tetracycline and macrolides, and vice versa, for example, cycloheximide4.

The incorporation of amino-acids into protein by isolated rat diaphragm has been used extensively for studies of the mechanism of the action of insulin and has the advantage of representing an isolated intact cell preparation in which to study protein synthesis. The isolation of polysomal aggregates from muscle^{6,7} reveals the basis of the tissue's capacity to synthesize protein. Because the system is probably less active than that of liver^{8,9}, and isolated muscle mitochondria possess considerable incorporating activity^{10,11}, the extent to which the in-corporating capacity of the intact tissue is microsomal or mitochondrial is not clearly established.

Investigation of the inhibitory influence of various antibiotics and other materials on protein synthesis in isolated rat diaphragm muscle is of value both as a comparison of animal versus microbial systems and as an indication of the location in vivo of the synthetic activity. It is also useful to correlate the extent to which materials much used to regulate the metabolism of isolated organelles will affect intact cells and their response to hormones.

The procedure for the preparation and incubation of diaphragm and for the assessment of incorporation of amino-acids into protein and their accumulation in the tissue has been described before 12-14. The agents used were added directly to the incubation medium which contained no added glucose unless figures for glucose uptake are given.

Table 1. Effect of various antibiotics on the incorporation of ¹⁴C-GLYOINE INTO THE PROTEIN OF ISOLATED RAT DIAPHRAGM

Additions to the medium	Insulin present (0·1 U/m!.)	Radioactivity incorporated into protein (c.p.m./mg protein)
No addition	-	58.7 + 5.8
Cycloheximide $(1 \mu g/ml.)$	_	$12 \cdot 2 \pm 1 \cdot 2$
Cycloheximide (10 µg/ml.)	_	6.0 ± 0.70
Cycloheximide (100 μ g/ml.)		1.8 ± 0.11
No addition		42.1 ± 2.7
Tetracycline (100 µg/ml.)		37.1 ± 2.9
No addition	+	88.4 + 3.5
Tetracycline (5 µg/ml.)	÷	88.7 ± 7.3
No addition	-	42.9 ± 1.7
Erythromycin (100 μ g/ml.)		43.0 ± 2.3
No addition	_	52.5 ± 3.1
Chloramphenicol (100 μ g/ml.)	****	53·3 ± 2·9
No addition	+	91.0 ± 4.0
Chloramphenicol (100 μ g/ml.)	+	90.8 ± 5.4

Each figure is the mean \pm standard error of the mean of six observations. Incubation was for 2 h and the ¹⁴C-glycine (specific gravity about 5 mc./mmole) was added undiluted at about 0·1 μ c./ml.

Incorporation of amino-acids into protein by diaphragm is inhibited by puromycin¹⁶, actinomycin^{16,17}, proflavin¹³ and by numerous metabolic inhibitors¹⁸. Incorporation is also inhibited by cycloheximide (Table 1) which in yeasts is an inhibitor of cytoplasmic ribosomal as opposed to mitochondrial synthesis4. It is not affected by the presence of tetracycline, erythromycin or chloramphenicol the inhibitory influence of which on mitochondrial protein synthesis is established^{11,19}, either in the absence of insulin or when incorporation is subject to insulin stimulation (Table 1). These figures indicate that most incorporation observed with isolated diaphragm muscle results from the activity of extramitochondrial synthesis, and the figure of 20 per cent for mitochondrial activity suggested by Florinis must be an upper estimate. Muscle, as opposed to yeast in a growth phase, is obviously unlikely to form mitochondrial materials very fast. Although mammalian protein synthesizing systems are for the most part resistant to inhibition by chloramphenical, inhibitory influences that have been reported mostly represent situations in which there is introduction of new messenger-RNA²⁰⁻²³. Chloramphenical shows no toxic effect on incorporation even in the presence of insulin. This reinforces a point previously made with actinomycin²⁴ that the action of insulin in muscle on the numerous responsive parameters is not brought about through stimulation of nucleic acid syn-

Table 2. Effect of insulin on the uptake of glucose and of aminoisobutyrate by isolated rat diaphragm after incubation with puromyoin or cycloheximide

Antibiotic added	Insulin added	Uptake of glucose (mg/g wet weight/h)	$ \begin{pmatrix} \text{Aminoisobutyrate accumulated} \\ \text{(Ratio } \frac{\text{c.p.m./ml. tissue water}}{\text{c.p.m./ml. incubation medium}} \end{pmatrix} $
None Cycloheximide	-		$\begin{array}{c} 1.55 \pm 0.094 \\ 1.24 \pm 0.064 \end{array} \} P < 0.05$
Cycloheximide Cycloheximide	- +	$\begin{array}{l} 2.35 \pm 0.32 \\ 3.62 \pm 0.13 \end{array} \} P < 0.01$	
Puromycin Puromycin	 +	$2.39 \pm 0.10 \atop 4.00 \pm 0.33$ $P < 0.001$	$1.33 \pm 0.047 \\ 1.79 \pm 0.083$ $P < 0.001$

Each figure is the mean \pm standard error of mean of six observations. The concentration of puromycin or cycloheximide added was 100 μ g/ml. and in each case, the tissue was pre-incubated for 3 h with or without the inhibitor before the test incubation of 1 h,

Other materials used on occasion as inhibitors of protein synthesis include amino-acid and nucleotide analogues. Neither azauridine (1 mmolar), 5-fluorouracil (10 μmolar), DL-norleucine (20 mmolar) nor DL-ethionine (20 mmolar) have any appreciable inhibitory influence on incorporation by diaphragm even during incubation periods as long as 6 h. It could be argued that the materials do not readily penetrate the tissue—but the uptake of amino-acids is well established, the incorporation of nucleotides readily observable and the rapidity with which molecules as large as puromycin, actinomycin and cycloheximide exert their influence is impressive. Uptake of chloramphenicol by reticulocytes has recently been studied23.

Table 3. Effect of insulin in the presence of cycloheximide on the accumulation and incorporation into protein of ¹⁴G-glycine by isolated rat diaphragm

Insulin added	(Ratio c.p.m./ml. tissue water c.p.m./ml. medium)	Radioactivity incorporated into protein (c.p.m./mg protein)
-	$2.04 \pm 0.18 \\ 2.95 \pm 0.21$ $P = 0.01$	${16.5 \pm 0.70 \atop 22.3 \pm 1.10} P = 0.001$

Each figure is the mean \pm standard error of mean of six observations. The tissue was pre-incubated for 30 min in the presence of 2 μ g/ml. of cyclohexlmide before addition of the ¹⁴C-glycine (0·2 μ l./ml.).

Table 4. Effect of administration of cycloheximide *in vivo* on the capacity of the subsequently isolated diaphragm muscle to incorporate ¹⁴C-Glycine into protein and to respond to insulin

Animals injected with	Isolated diaphragm with insulin	Uptake of glucose (mg/g wet weight/h)	Radioactivity incorporated into protein (c.p.m./mg of protein)
Saline		$3.58 \pm 0.25 \\ 6.38 \pm 0.20$ $P < 0.001$	$\frac{145}{949}$ $\pm \frac{22}{140}$ $P < 0.01$
Saline	+	6.38 ± 0.20 } ~ ~ 0.001	
Cycloheximide		2.89 ± 0.31 6.55 ± 0.17 P < 0.001	21.7 ± 0.92
Cycloheximide	+	6.55 + 0.17	30.4 + 2.90 1 - 0.02

Each figure is the mean \pm standard error of mean of six observations. The cycloheximide (10 mg/150 g of rat²³) was injected intraperitoneally (in saline) 90 min before removal of the diaphragm.

Extensive incubation with puromycin lowers the capacity of muscle to take up amino-acids25, although the effect is not seen in shorter incubations26. suggested that the membrane carrier for amino-acids may be a protein with a rapid rate of turnover. Wool and Cavicchi²⁷ have also provided evidence that puromycin and cycloheximide interfere with the response of a muscle ribosomal system to insulin, and they suggest, by analogy with similar findings with adrenocorticotrophic hormone²⁸, that a primary action of insulin is to promote formation of a labile protein which is responsible for increasing the activity of the polysomes. I have confirmed the findings25 of the inhibitory effect of puromycin and cycloheximide on accumulation of aminoisobutyrate (Table 2). But an effect of insulin to promote aminoisobutyrate accumulation remains observable, albeit on a smaller absolute scale, and promotion of glucose uptake is also seen. A normal percentage increase of incorporation by insulin is observable (Table 3) although it is reduced by pre-incubation with cycloheximide. cycloheximide is administered in vivo before removal of the diaphragm, the tissue again responds to insulin both with respect to glucose uptake and amino-acid incorporation (Table 4). These observations do not support the concept that the primary actions of insulin on muscle, whatever they are, are dependent on proteins with a rapid rate of turnover and renewal. Because cycloheximide is thought to bind to ribosomes, a more mundane interpretation of the results of Wool and Cavicchi²⁷ and possibly those of Garren et al.28 would be that the ribosomal systems isolated after treatment with cycloheximide remain contaminated with the inhibitor and, despite some apparent incorporating capacity, are effectively dead. In these conditions hormonal response is unlikely to be observed. The question remains why cycloheximide in vivo was not found totally to inhibit incorporation by diaphragm. This is probably a technical question of dosage

as can be shown with puromycin¹⁷. A more important question is why ribosomal systems from animals treated with inhibitors exhibit incorporating capacity at all (for example, refs. 29, 30). Without a clearer understanding of the full enzymology of protein synthesis this is difficult to answer.

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Growth Hormone and Carbohydrate Metabolism in vitro

In the recent communication under this title, Bolodia and Young' say that "... no satisfactorily reproducible effect of growth hormone in vitro on carbohydrate metabolism has been described". No grounds are given for rejecting the comprehensive evidence of Ottaway and Bulbrook² for effects on the glucose uptake of rat diaphragm which are practically identical with those now described by Bolodia and Young, nor for the evidence of Bronk and Fisher³ that very small concentrations of bovine growth hormone produce anti-insulin effects on the perfused rat heart. Bronk and Fisher showed that growth hormone reduced glucose uptake and permeability to galactose in the absence of insulin as well as in its presence. It follows that growth hormone produces an effect which offsets the action of insulin but is not acting as an anti-insulin.

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Hydrocortisone Induction of Tyrosine Transaminase in Regenerating Rat Liver

THE "minimal deviation" hepatomas—well differentiated rat hepatocellular carcinomas—demonstrate many abnormal enzyme regulatory mechanisms. It has been suggested that these alterations are critical in neoplastic transformation. The corticosteroid induction of the hepatic enzymes tryptophan pyrrolase (TP) and tyrosine transaminase (TT) has been extensively studied in these tumours. Administration of corticosteroid causes a considerable increase in the activities of TP and TT in the normal adult rat liver within 4–5 h (refs. 3 and 4). In hepatomas, however, there is little or no increase in TP activity after administration of corticosteroid, and baseline enzyme activity is usually depressed.

It has been shown that the corticosteroid induction of TP is markedly depressed during the period of DNA synthesis after 70 per cent hepatectomy in the rat^{5,6}. This indicated that during the preparation for cell division this particular enzyme regulatory mechanism was also

altered in a non-neoplastic cell.

In contrast, the corticosteroid induction of TT in several tumour lines was found to be normal and baseline enzyme activity was either normal or increased. We have examined the pattern of steroid induction of TT in the regenerating rat liver after 70 per cent hepatectomy to determine whether the induction was altered during the preparation for mitosis in a non-neoplastic cell.

Male Sprague-Dawley rats weighing approximately 250 g underwent either a 70 per cent hepatectomy? or a sham operation on the morning of each experiment. The rats had been adrenalectomized 5-7 days previously

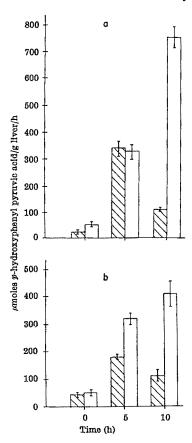


Fig. 1. Tyrosine transaminase activity after hydrocortisone administration a, 2 h, and b, 21 h, after operation. Values on abscissa are hours after injection of steroid. There were five rats in each group and brackets indicate \pm standard error of the mean. Hatched columns, sham operated; white columns, hepatectomized.

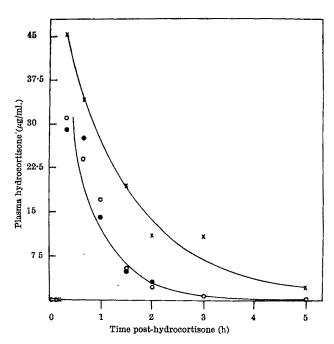


Fig. 2. Disappearance of plasma hydrocortisone after intraperitoneal administration. Each point is average for two rats. ●, Unoperated; O, sham operated; ×, 70 per cent hepatectomized.

and had been maintained on 0.9 per cent saline and lab chow given freely. Hydrocortisone phosphate (5 mg/100 g) was administered intraperitoneally 2 or 21 h after operation. No new DNA synthesis occurs in the early post-operative period after 70 per cent hepatectomy, but at 21 h the synthesis of DNA has achieved a significant rate⁸. The rats were killed 0, 5 and 10 h after administration of hydrocortisone, and TT was assayed by the method of Lin and Knox⁴. Previous experiments had shown that neither prior adrenalectomy nor administration of hydrocortisone appreciably altered the mitotic response after 70 per cent hepatectomy. It had also been shown that neither of these procedures nor sham operation raised hepatic mitotic activity above that of an intact unoperated rat^{5,8}.

Hydrocortisone induction of TT activity in both unoperated and sham operated adrenalectomized rats was similar to that described before, with respect to both the pattern of induction and the degree of peak activity.

At both 2 and 21 h after 70 per cent hepatectomy the kinetics of induction of TT were altered (Fig. 1). Enzyme activity 10 h after injection was significantly greater than at 5 h, contrasting with the return towards baseline values shown by the sham operated rats. After 5 h, the time of expected peak induction, activities were similar in both groups of rats. Basal enzyme values in the 70 per

cent hepatectomized rats were also elevated.

These findings initially suggested that control mechanisms for TT were altered during hepatic regeneration, but not temporally related to DNA synthesis as had been observed for TP. In contrast to TP, TT appeared to be hyperinducible. It has been shown, however, that repeated small doses of hydrocortisone, in contrast to a single large dose, cause a pattern of induction of TT in unoperated rats similar to that seen in the hepatectomized group in this investigation. It seemed possible therefore that removal of 70 per cent of the functional liver might result in an alteration of hydrocortisone metabolism, leading to sustained exposure of the residual liver to circulating steroid. To test this possibility, plasma concentrations of hydrocortisone were determined in 70 per cent hepatectomized and sham operated rats after a single intraperitoneal dose of steroid at the same dose level used in the previous experiment¹¹⁻¹³.

Fig. 2 indicates that plasma clearance of hydrocortisone is markedly slowed in the case of 70 per cent hepatectomy. The residual hepatic parenchyma was therefore exposed to a greater concentration of circulating steroid for a longer period of time than controls; values 3 h after injection were ten times greater than controls.

It should be noted that this phenomenon must be considered when experiments utilizing an agent metabolized by the hepatic parenchyma are performed in partially

hepatectomized rats.

It seems probable that TT is fully inducible in the regenerating rat liver and that the alteration of induction kinetics is caused by a prolonged exposure to steroid rather than a fundamental change in enzyme regulation.

The differences in the hydrocortisone induction of TP and TT during hepatic regeneration may reflect a relationship between their mechanisms of control and the metabolic events accompanying the preparation for cell division. These factors may also be operative in the "minimal deviation" hepatomas.

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Ergosterol Peroxide: a Fungal Artefact

ALTHOUGH the isolation of ergosterol peroxide from the extracts of several different fungi has been reported1-4, the question of its authenticity as a metabolite in these cases does not seem to have been raised. In the present case, the peroxide was found to be present in extracts of sporophores of Piptoporus betulinus or of Daedalea quercina. These extracts, however, had been exposed to daylight for several days and the peroxide could not be detected initially in fresh extracts. Ergosterol (R_F 0.40) and ergosterol peroxide $(R_F \ 0.23)$ were both easily detected by T.L.C. on silica using 1 per cent methanol in chloroform as eluent, characteristic intense blue-black and dark green colours, respectively, being developed after spraying with ceric ammonium nitrate and heating briefly at 100° C. A sample of the peroxide isolated after chromatography on silicic acid was identical in all respects with a synthetic sample.

It can be shown that, in the conversion of ergosterol to its peroxide under a slow stream of oxygen, both light and a photosensitizing substance (for example, eosin⁵) are essential. The crude brown gummy materials extracted with methanol from P. betulinus and D. quercina were each able to act as photosensitizer, and 100 mg or more of either extract allowed complete oxygenation of ergosterol (10 mg) in methanol (10 ml.) under an ordinary 150 watt pearl lamp in 18 h. This photosensitizing property, which was not impaired by boiling the crude extract with acid or base, is one which is exhibited by a wide range of coloured compounds to varying degrees. (The fungal anthraquinones emodin and physcion⁶, for example, were found to have up to 20 per cent of the sensitizing activity of eosin, a minimum of 50 µg allowing complete oxygenation of 10 mg of ergosterol in 18 h.) Hence, as most fungal extracts contain at least small quantities of pigments, isolated ergosterol peroxide may be, in general, an artefact rather than a natural product.

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PHYSIOLOGY

Apparent Peroxide in the **Blood of Pregnant Mice**

In previous communications^{1,2}, it was shown that the total apparent peroxide in mice was decreased by an oral contraceptive and increased by pregnancy. The uterus, ovarian fat plus ovaries, mammary tissue and adrenals of pregnant mice each showed a significant increase, but the total amount of increased peroxide in these organs accounted for only about 6 per cent of the whole mouse increase. In a further attempt to locate the peroxide induced by pregnancy, the blood of pregnant mice was examined.

The pregnant albino hairless mice and the peroxide estimations using an anoxic box were as previously described2.3. Ten control and ten pregnant mice, initially 13 weeks old, were used for this experiment, Blood was drawn from the right ventricle of the dead mouse and weighed in a thin walled glass bulb. The glass bulb was macerated with the blood.

For the calculations of peroxide in total blood it was assumed that blood is 10 per cent of the body weight.

Table 1. MICROMOLES OF PEROXIDE IN MOUSE BLOOD

	Mean peroxide	Peroxide/g.
Control	1.52	0.59
Pregnant	5-96 P < 0-01.	1.71
	L < 0.07"	

As shown in Table 1 there was a significant increase of peroxide in the blood of pregnant mice and this accounted for about 73 per cent of that in the whole mouse.

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Selective Antagonism of Arsenicals and Antimonials

WE have long been looking for an antagonist of arsenicals and antimonials to decrease selectively their toxic action, without interfering with their therapeutic effectiveness. The dimercaptans behave in the opposite way: for example, 2,3-dimercaptopropanol (BAL), in one-eighth of the dose of 'Arsenoxide' employed, abolishes antitrypanosomal activity, and only multiple doses of toxic arsenic can act as antidote1. Having observed BAL to abolish the activity of penicillin and chloromycetin in spirochaete infections without affecting antistreptococcal activity2, we realized that selective antagonism against particular cells, which could be host cells, is possible. We found that 'Penicillamine' (dimethylcysteine, DMC) which is known to detoxify bivalent heavy metals (mercury, lead) $^{3-5}$ is also a powerful antidote against arsenicals and antimonials, which does not interfere, within a wide range, with their antiparasitic effectiveness in vivo and in vitro.

During the past 6 yr we have investigated the influence of DMC and its analogues on arsenicals and antimonials in vivo and in vitro on schistosomes, leishmania and trypanosomes, as well as in different hosts.

As an antidote against 'Arsenoxide' (Mapharsen), DMC compares favourably with BAL and is less toxic. (Subcutaneous LD_{50} in mice for BAL is 150 mg/kg, for dl-DMC is 450 mg/kg and for d-DMC is 650 mg/kg.) The LD_{95} of 'Arsenoxide' is 50 mg/kg and the compound is detoxified by 20 mg/kg of dl-DMC given twice 30 min before and after the arsenical. As for bivalent metals, d-DMC is a more effective antidote to arsenicals than its optical isomer.

Table 1. Effect of dl-dimethyloysteine on the antitrypanosomal and toxic action of 'arsenoxide' in mice

Experiment No.	Mice infected with	'Arsen- oxide' (mg/kg s.c.)	dl- DMC (mg/kg)	No. of mice used	No. of cleare infec Tempor- arily	d of tion	No. mice died
2/94	T. equi- perdum "," Toxicity	2·0 2·0 2·0 0 50	0 40×2 80×2 80×2 0	3 3 3 5	0	2 3 3 —	1 0 0 3 5
2/96	Antagonism T. equi-	50	80×2	5	******		(24 h _i) 2
,	perdum ,,	1·0-1·5 2·0	0	10 9	6 2	4 7	6 4 (2 neg*)
	**	2.0	80 × 1	12†		12	(2 neg*)
	Toxicity	50	0	4	*****		(neg*) 4 (24 h)
	Antagonism	50	80×1	12†			0

Without trypanosomes in the blood stream.
 Subdivided into three groups of four mice receiving DMC 2, 1 and 0.5 h before injection of 'Arsenoxide'.

Doses of DMC from 1 to 80 mg/kg, given twice to sixtyseven mice infected with T. equiperdum and treated with 2 mg/kg of 'Arsenoxide', did not inhibit the therapeutic activity of the arsenical, although thirty-nine animals received antagonistic doses (two of 10-80 mg/kg) sufficient to give protection against lethal 'Arsenoxide' intoxication. In tryptose-phosphate broth, 25 µg/ml. of 'Arsenoxide' kills the trypanosomes within 40 to 60 min; addition of 100 µg/ml. of DMC does not modify this activity, which can be slightly delayed by 200 µg/ml. of DMC. To abolish effectiveness, both in vitro and in vivo, very large excesses of DMC are required, which also applies to the anti-bacterial action of mercurials. For example, *L. enriettii* in culture is killed within 24 h by 0·125-0·25 mg/ml. of tartar emetic, an action which is not modified by 5-10 mg/ml. of DMC, but is totally abolished by 25-50 mg/ml., that is, hundred-fold excess, which leaves a vigorous population of parasites even after 10 days.

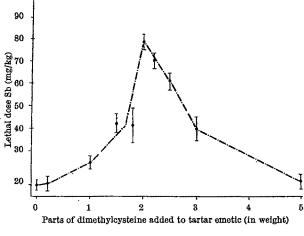


Fig. 1.

The curve of tolerance of antimonials to which increasing amounts (0.25-5.0 parts by weight) of DMC are added is parabolic: maximum detoxification, which in the case of tartar emetic reaches 300 per cent, is followed by additive toxicity (Fig. 1). In these experiments with antimonials, both d- and l-DMC were effective. On the other hand, the clearing effect of 15 mg/kg of tartar emetic on the parasitaemia of mice (T. equiperdum), completely abolished by 30 mg/kg dimercaptosuccinic acid, was not modified by 30 mg/kg of dl-DMC.

It is of theoretical and therapeutic interest that the selective antagonism is limited to specific structures. Cysteine is known, to deactivate as much as it detoxifies, and we found that dimercaptosuccinic acid in doses of 10-30 mg/kg abolishes the antitrypanosomal activity of 10-15 mg/kg of tartar emetic.

This selective antagonism with DMC led us to prepare drugs with increased therapeutic indices. For example, antimony-tris dl-ββ-dimethylcysteine (Sb (DMC₃)), containing 19.5 per cent antimony, has a toxicity: clearing ratio as high as 15 in T. equiperdum infection of mice. This compound can be further detoxified by the addition of 1 mole of DMC without loss of effectiveness. The arsenic substituted dl-DMC derivative of 'Arsenoxide' (3-amino 4-hydroxy-phenarsine di-ββ-dimethylcysteine trichlorhydrate), with a 12.08 per cent arsenic content, toxic to mice in 200 mg/kg doses clears T. equiperdum infection in doses of 2.5-3.0 mg/kg and induces a permanent cure in a dose of 12 mg/kg. The resulting safety margin is considerably greater than that of 'Arsenoxide'.

A preparation (NAP) to which we attribute the structure

complexed with an additional amount of DMC, seemed by its reduced toxicity and high antiparasitic action (S. mansoni, T. equiperdum, T. venezuelensis) to be particularly suitable for chemotherapeutic use, and we have been investigating it since 1964 in the local Anti-bilharzia Center of the Venezuelan Ministry of Health in patients infected with S. mansoni. Ron Pedrique et al. gave a very favourable clinical report on this drug; there were no significant side effects and a high curative rate (80 per cent) in patients treated intramuscularly, during 5

days, with a total dose corresponding to 300 mg of antimonv.

Two fundamentally different processes seem to be involved in this selective antagonism. (a) The antagonist inhibits some, but not all, biochemical effects of the agonist. This is undoubtedly the case for the inactivation of BAL in the antispirochaete action of penicillin, which leaves the antibacterial activity unaffected2. (b) The antagonist enters into a reaction with the agonist forming conjugates with different biological properties, for example, with lower toxicity and high antiparasitic activity, which may apply to DMC.

The overall inhibition of activity obtained with very large excesses of DMC may depend on a different mechanism: donation of sulphydryl groups which reactivate the receptor site of the parasite and/or block directly the effect of the drug on it. The latter mechanism, attributed to the excessive doses of DMC, may be analogous to the mechanism of inhibition of the antiparasitic activity of arsenicals and antimonials by the dimercaptans, BAL and dimercaptosuccinic acid2,9.

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Significance of Respiratory Quotients in Toad Bladder and Kidney

THE respiratory quotient is an established guide for deducing the main class of substrate which is completely oxidized by the intact aerobic organism¹. Incomplete oxidation of substrates has, however, been shown to occur in vivo in individual organs, such as kidney2,3 and heart4, and so an interpretation of the respiratory quotient cannot be made for observations of gaseous metabolism in an isolated tissue or a single organ without simultaneous evidence of complete substrate oxidation.

The observations of incomplete substrate oxidation by organs are consistent with the concept that the complete aerobic oxidation of substrates in the whole organism results from sequential or partial substrate oxidations in a series of organs. Thus the respiratory quotient of the organ is determined not only by the substrates used, but also by the degrees to which substrates are oxidized, and used for syntheses, and the intermediary metabolic

pathways involved.

Some recent reports of tissue metabolism in vitro and in vivo have not considered these limitations in the interpretation of the respiratory quotient. For example, Maffly and Coggins have reported that the rate of Δ carbon dioxide production parallels Δ short circuit current in the toad bladder preparation and, from published data on oxygen consumption by the toad bladder, they deduce that the respiratory quotient of this tissue is in the range

of 1.2-1.4. They consider the latter figure to be close to unity and suggest that the energy for the transport of sodium ions is derived from the complete oxidation of endogenous carbohydrate, particularly because the toad bladder has been shown to utilize exogenous glucose avidly.

Maffly and Coggins also suggest that the high respiratory quotient of dog kidney² may be accounted for by conversion of carbohydrate to fat⁶. It is not clear whether they also imply that in dog kidney the energy for sodium transport may be derived from the complete oxidation of glucose. If glucose is taken up by the kidney and converted to fat, the respiratory quotient might indeed be raised; however, the known smallness or absence of glucose uptake⁷⁻⁹ by the dog kidney makes it highly improbable that glucose extracted from renal arterial blood is being used both for sodium transport and for fat production. If glucose is being converted to fat, the energy requirements for sodium transport must be largely derived from the oxidation of other substrates, for sodium transport and fat synthesis are both endergonic processes. Thus while these speculations about the relationship between gaseous and intermediary metabolism and sodium transport in the toad bladder and kidney may have some basis, specific interpretations of the respiratory quotient are questionable in the absence of complete quantitative studies of the metabolism of the endogenous substrates of, and the metabolic pathways in, organs.

Similarly, Nieth and Schollmeyer¹⁰ have reported that the large uptake of non-esterified fatty acid by human kidney is consistent with an observed renal respiratory quotient of 0.7. (It should be noted that if the respiratory quotient is calculated using the data they present the figure is closer to 0.8.) They also calculated that the complete oxidation of the total substrates taken up by the human kidney could be accounted for by the simultaneously measured renal oxygen consumption¹¹. tunately, Nieth and Schollmeyer made no estimate of the renal uptake of glutamine which must have occurred during their observations. Calculations from the uptake of glutamine by human kidney12 indicate that between 15 per cent (normal man) and about 35 per cent (during acidosis) of the estimated renal consumption of oxygen may be accounted for by glutamine oxidation. Calculations made from observations in the dogs indicate that the mean renal uptake of glutamine may account for about 17 per cent (alkalosis) to 35 per cent (acidosis) of the renal consumption of oxygen. Thus if one assumes that uptake and complete oxidation of glutamine were also occurring in the human subjects11, the calculated respiratory quotient would be even higher than 0.8. The addition of glutamine to the substrates taken up by human kidney then makes the observed oxygen consumption grossly insufficient for complete oxidation of the total substrates. Thus it seems that the substrate oxidation in the studies of in vivo human renal metabolism was incomplete, and that the respiratory quotient cannot be used to determine the substrates which are used.

The basis for the respiratory quotient of kidney or toad bladder is not yet clear. In the case of the dog or human kidney, it seems that an important endogenous substrate is non-esterified fatty acid²,¹¹,¹³. Complete oxidation of non-esterified fatty acid should yield a respiratory Complete oxidation quotient of ~0.7, but incomplete oxidation could result in a respiratory quotient of more than unity2, the degree of elevation depending on the relative amounts of fatty acids completely and incompletely oxidized. Furthermore, because the sum of the substrates taken up by the dog3 or human kidney cannot be completely oxidized by the simultaneous oxygen consumption, there must be partial dissimilation of some substrates, for example, nonesterified fatty acid or glutamine, which would be consistent with a high respiratory quotient related perhaps to renal work.

I should also like to emphasize that the bases for the respiratory quotient in mammalian kidney and toad 'bladder or other tissues transporting sodium transcellularly, such as frog skin, may not be alike because the substrates which can be taken up by each of these organs are different (Table 1). The dog kidney in vivo shows a definite substrate specificity in that lactate. pyruvate^{8,14,15}, glutamine, α-ketoglutarate^{15,17} and palmitate13 are avidly taken up from the arterial blood, whereas glucose, glutamate and oleate12 either are not taken up or are utilized at relatively low rates. Maffly and Edel-man¹⁸ have reported that the toad bladder similarly displays a varied but differing hierarchy of affinities for substrates, in that α-ketoglutarate is utilized at a very low rate, while glucose and pyruvate are utilized briskly (Table 1). Such differences in substrate specificities among organs transporting sodium ions may indicate that their patterns of intermediary metabolism are dissimilar. If there are both specific substrates17 and specific exergonic reactions18 coupled to ion transport in each organ, they may not be the same for kidney, toad bladder, frog skin or other sodium-transporting structures.

Table 1. Substrate selectivity and affinity of three tissues which transport sodium transcellularly

	Toad bladder	Dog kidney (in vivo)	Frog skin
Glucose	++++18	± 10	
Pyruvate	+ + 18 + + 22	++++*	+++28*
Lactate		++++14	
Acetate	+++ ¹⁸ † + ²¹ †	?	+ + 24 †
Acetoacetate	++28†	_ 15	
a-Ketoglutarate	+18†	++++16	++23*
Succinate	++18+	0‡	+++ ^{28#}
Palmitate		$++++^{13}$	
Oleate		± 13	
Glutamate		± 8,9	+++***
Citrate		+ + 20	++++28*
Glutamine		++++18	
Oxaloacetate		?	++++***
Fumarate			++28*
Malate	+ 18		+ 23*

A qualitative scale of substrate affinities is used because the data are derived from observations made in widely varied circumstances. Moreover, the methods used for quantification of substrate uptake were different in each study. \pm , Small net utilization or production; 0, no net uptake; +, small net uptake; +, + + +, in the case of the kidney, a large enough net uptake, in some instances, to account for observed or estimated renal consumption of oxygen.

- * Data from Huf based on effect of substrate at 5 mmolar on $\dot{Q}o_2$, rather than on net utilization studies.
 - \dagger Data based on appearance of $\rm ^{14}CO_{2}$ from $\rm ^{14}C\text{--}labelled$ substrate.
 - # My unpublished results.

In this context then, the overall relationship between Δ oxygen consumption (or Δ carbon dioxide production) and a net sodium transport need not show the energy requirements for sodium transport. Indeed, Maffly and Edelman¹⁸ have shown that in the toad bladder increments in net sodium transport are related to $\Delta Q o_2$ when pyruvate is the added substrate. In contrast, addition of acetate increases Qo2 without any increment in net sodium transport. Obviously, Δ sodium transport/ ΔQ_{O_2} (or Δ sodium transport/ ΔQ_{CO_2}) is different for acetate and for pyruvate. In the absence of information about the intracellular organization of the specific exergonic reactions which are linked to sodium transport, little further understanding can be obtained about mechanism or actual energy requirements for sodium transport by relating either $\Delta \dot{Q} co_2$ or $\Delta \dot{Q} o_2$ to Δ sodium transport.

The results of experiments on the quantities of both the specific substrates taken up by and the individual products leaving a tissue simultaneously would aid an understanding of the significance of the respiratory quotient of an organ. Most important, this might perhaps permit the identification of specific reactions which have sufficient free energy changes to drive sodium transport.

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Arrangement of Cholinoreceptors on the Neuronal Membrane of Two Pulmonate Gastropods

CHOLINERGIC transmission has been demonstrated in ganglia of some gastropod molluscs1,2. The large neurones of gastropods are very suitable for investigation by the microelectrode technique, and use of this method to compare the activity of cholinergic drugs of different chemical structure can reveal new facts about the occurrence and arrangement of active groups on the cholinoreceptive membrane.

We used the freshwater gastropod molluses Planorbis corneus and Limnaea stagnalis. Most of the neurones in the ganglia of these molluses are spontaneously active. The membrane potential is 40-60 mV and the action potential is about 100 mV. Addition of acetylcholine either to the perfusion fluid or by electrophoretic microapplication causes depolarization and an increase in spike frequency in most of the cells. Some cells respond to acetylcholine by hyperpolarization and a decrease in spike frequency. Some cells are unaffected by acetylcholine3. The action of different cholinomimetics and cholinolytics suggests that the cholinoreceptors are nicotinic ones resembling somewhat the receptors of mammalian skeletal muscle4. The cells which respond to acetylcholine by depolarization are very sensitive to stimulation by nicotine but do not react to arecoline at all; they are more sensitive to butyrylcholine than to methacholine bromide; d-tubocurarine and gallamine exert a strong cholinolytic action, but atropine and ganglion blocking agents are almost inactive.

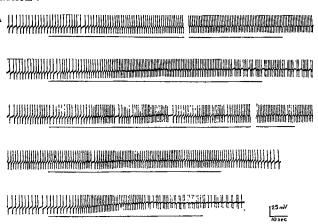
Mammalian skeletal muscles are known to be very sensitive to bisquaternary compounds which have an internitrogen chain containing nine or ten atoms (about 14 Å); these are decamethonium, suxamethonium,

d-tubocurarine and gallamine. High sensitivity has also been shown for bisquaternary compounds which have a chain containing sixteen atoms (about 22 Å) between nitrogen atoms; these are hexacarbacholinobromide, prodecomium bromide, hexadecamethonium, the compound KB-72, the dicholinic esters of higher dicarbonic acids (suberic and sebacinic) and others (see ref. 6). This suggests that there is a definite arrangement of single receptors on the postsynaptic membrane. In some cases, the distance between the anionic points of neighbouring receptors is about 14 Å ("C-10 structure"), in other cases it is about 22 Å ("C-16 structure"). The C-16 structure seems to appear earlier in the development of the locomotor muscles than does the C-10 structure. The C-16 structure is probably more universal, for the signs of its presence are found not only in the muscles but also in the mammalian autonomic ganglia5.

Is there some definite arrangement of receptors on the neuronal membrane of molluses? To answer this question we have investigated the action of some bisquaternary compounds with an internitrogen distance of 14 Å and 20-22 Å.

The isolated peri-oesophageal ganglionic ring was placed in a bath (about I ml.) and perfused with saline, at a rate of 15 ml./min3. The visceral and the large parietal ganglia were opened and a large cell (100-200µ) was impaled by a microelectrode filled with 2.5 molar potassium chloride (diameter of the tip about 0.5μ , R=15-60 megohms). The electrode was connected through a cathode follower to a d.c. amplifier and then to a cathode oscilloscope. The compounds to be studied were added to the perfusion fluid; in some experiments, acetylcholine was microapplied electrophoretically.

Decamethonium and suxamethonium (internitrogen distance 14 Å) caused only slight depolarization and a small increase in spike frequency even in a concentration of $5 \times 10^{-5} - 2 \times 10^{-4}$ molar. The cholinomimetic effect of these drugs was 20-50 times weaker than that of acetylcholine and approximately equal to that of tetramethylammonium (Fig. 1A). The antiacetylcholine action of decamethonium and succinyldicholine was also very weak. curarine and gallamine possess a strong antiacetylcholine action4.



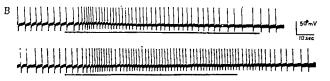


Fig. 1. A, The action of decamethonium (C-10), succinyldicholine (D-2), tetramethylammonium (TMA), suberyldicholine (D-6) and acetylcholine (Ach) on the same neurone *Planothis*. B, The action of sebacinyldicholine (D-8) and acetylcholine. In A and B the time during which the drug was applied is shown by the lower trace. Intervals are indicated by interruptions: in the case of C-10, 90 sec; in the cases of TMA and D-8, 30 sec. Here and in Fig. 2 the amplification was large. Only the lower part of spikes can be seen.

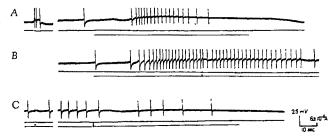


Fig. 2. The action of acetylcholine and of the compound KB-72 on neurone *Planorbis*. Acetylcholine was added to the perfusion fluid or applied by 600 msec pulses (see middle trace). A, The action of acetylcholine. B, The cholinomimetic action of KB-72. During the perfusion with KB-72 acetylcholine was applied twice. C, The action of acetylcholine (microapplication and adding to the perfusion fluid) after perfusion with KB-72 for 5 min.

Thus the results obtained with tubocurarine and gallamine suggest that there is a C-10 structure on the neuronal membrane, but the results with decamethonium and succinyldicholine show no evidence of this structure. We think that the results obtained with depolarizing drugs are much more important. Cholinomimetic action requires more precise complementarity to the cholinoreceptive surface than cholinolytic action. The high potency of tubocurarine and gallamine can be explained not by the presence of two nitrogen atoms but by other molecular features which can favour the firm binding to the receptive membrane. Such features would be the size of their molecules, the presence of flat rings and so For example, tubocurarine has a strong ganglion blocking action although there is no C-10 structure in the autonomic ganglia.

Dicholinic esters of suberic and sebacinic acids (internitrogen distance 20 Å-22 Å) exerted a very strong cholinomimetic effect on the neurones of Planorbis (Fig. 1A and B); their effect was usually stronger than that of acetylcholine. Depolarization and the increase in spike frequency induced by suberyldicholine and sebacinyldicholine lasted longer than when induced by acetylcholine, and desensitization was less pronounced.

Another compound with about 20 Å between nitrogen atoms, KB-72, a depolarizing myorelaxant⁸, induced depolarization and an increase in spike frequency in neurones of Planorbis. KB-72 was nearly as potent as acetylcholine but its effect developed later and lasted longer (Fig. 2). KB-72 also had a strong antiacetylcholine action: it prevented stimulation by the latter (Fig. 2C).

These data suggest the existence of C-16 structure on the neuronal membrane of Planorbis: a mutual disposition of receptors with about 20 Å between the anionic points of two neighbouring receptors In the neurones of Limnaea stagnalis suberyldicholine was about five times less active than acetylcholine There is still no clear evidence of the existence of a C-16 structure in this species; perhaps only a small part of cholinoreceptors is arranged in C-16 structure.

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Rapid Reflex Interference with Peripheral Vascular Tone

THE prevalent conception that blood vessels are only capable of contracting and dilating slowly is based on the behaviour of excised segments^{1,2}. Under natural conditions, however, far more rapid vascular activity has been observed. The veins of the bat's wings³, the arterio-venous anastomosis in the rabbit's ears⁴ and the arteries in the conjunctiva in man⁵ are such examples.

The so-called atrial waves, which have been observed in the peripheral circulation in connexion with the atrial systole⁶, are another example of rapid vascular behaviour. It has been taken for granted that these waves are transmitted mechanically. A study of the atrial waves some years ago⁶ indicated, however, that they were transmitted not mechanically but nervously, because they seemed to be uninfluenced by friction and damping and did not follow hydrodynamic laws for wave transmission; moreover, they could be wiped out by nerve block.

These studies were based principally on records of the arterial pulse in the arm. Finger plethysmography of

standard type, has since been used, because it was expected that the atrial waves could be traced in the digital vasculature, which is controlled to a great extent by the nerves, besides being a playground for all kinds of vasomotor reactions. The results confirm that the atrial waves are transmitted by reflex, not mechanical, means; thus as a rule they are more distinct in the fingers than in the brachial or radial arteries and can be recorded in the finger even when the arm circulation is occluded (results to be published) (Figs. 1 and 2). This vascular reflex mechanism involves the baroreceptors in the atrias and roots of the large veins 10 and their afferent fibres, the efferents being the sympathetic fibres to the smooth vascular muscles.

Pressure was applied to the carotid sinus in order to see whether reflex effects of a similar type could be experimentally produced by the stimulation of proprioceptors in other cardio-vascular areas. A series of experiments on healthy persons (results to be published) showed that there is a rapid and transient volume increase of the finger, corresponding to the atrial waves in connexion with the carotid sinus pressure. The arterial pressure is not affected by this brief pressure and the reaction is sometimes seen even when the circulation in the arm is obstructed by a cuff on the upper arm.

Experiments were then performed during the catheterization of patients for diagnostic purposes (results to be published) to see if brief distension of the central blood

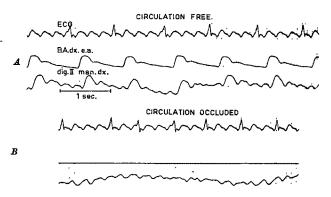


Fig. 1. Atrial flutter in a man aged 46. A, Upper tracing ECG with flutter waves, lower tracing finger plethysmogram with distinct atrial waves of the same frequency and duration as flutter waves in ECG, middle curve less distinct atrial waves in extra-arterially recorded pulse curve from brachial artery. B, Circulation occluded by cuff on upper arm. Atrial waves still distinct in finger plethysmogram while they are wiped out in peripheral pulse.

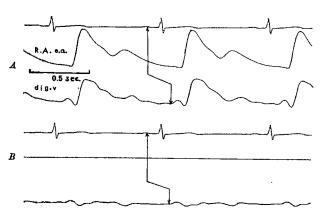


Fig. 2. Healthy man with sinus rhythm. A, Distinct atrial waves in plethysmogram (bottom curve), less distinct waves in extra-arterially recorded pulse curve from radial artery (middle curve). Transmission time of atrial wave as measured from beginning of atrial mechanic systole (approximately 0-05 sec after beginning of P-wave in ECG) to up-stroke of atrial wave about 0-20 sec (arrow). B, Circulation occluded by cuff on upper arm. Atrial waves still distinct in finger plethysmogram. Transmission time unchanged.

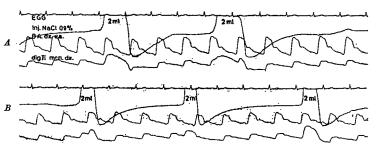


Fig. 3. A, Effect of flushing catheter with 2 ml. of saline, tip of catheter in vena cava inferior near atrial entrance. Volume increase of fluger shortly after beginning of each flushing (lower curve), no response in extra-arterially recorded pulse curve from brachial artery. B, Same effect with tip of catheter in external lliac veln.

vessels produced the same volume variation of the finger as the atrial systole and pressure on the carotid sinus. This was found to be the case, as seen in Fig. 3.4, which shows a rapid and transient swelling of the digit in connexion with the flushing of a catheter placed with its tip at the atrial entrance of the inferior vena cava. This is thus an imitation of the effect of the atrial systole.

The reaction was also seen during obstructed circulation. Sometimes a mere poking of the vessel wall with the tip of a catheter elicited a distinct volume reaction in the finger. The reflex time, which is the same for the atrial waves, the reaction to carotid sinus pressure and the intravascular flushing, is remarkably short—only a few tenths of a second (Fig. 2).

The reaction was elicited when the catheter was flushed with its tip placed in "classie" baroreceptor areas¹⁰, for example, the root of the inferior vena cava and the root and arcus of the aorta—but it was also elicited when the tip was placed in abdominal arteries and veins, as in Fig. 3B. Stretch receptors of the Vater–Pacini type have been shown to be embedded in and around the adventitia of such vessels¹¹. They are very sensitive to deformation¹² and bursts of spikes have been recorded from their afferent fibres in connexion with the rise in systolic pressure¹³. It is possible that their reflex effects, hitherto unknown, are the ones demonstrated here.

In conclusion, it can be said that there are signs of a rapid reflex interference with the peripheral vascular tone, produced by the stimulation of well known as well as less acknowledged reflexogenic areas of the cardio-vascular system. These effects can be traced in peripheral arteries. but they are more distinct in small, nervously controlled blood vessels such as the finger vasculature. The effects on the digital volume are small but may be theoretically important, for they signify that a co-

ordination is possible between the heart beat and the peripheral vascular tone.

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Influence of Hypoxia on Glucose Transport across the Human Placenta

EXPERIMENTAL models for foetal hypoxia have predominantly employed acute asphyxiation1,2, although in the human it is likely that when impairment of foetal oxygenation does occur it is often chronic and incomplete3. It has been shown4 that by giving mothers 10 per cent oxygen to breathe (for short periods of time) it is possible to halve foetal oxygen tension without harming the foetus. This experimental technique was used to study glucose and acid-base status in mother and foetus during hypoxia.

Five conscious patients at term in the first stage of labour were given a mixture of 10 per cent oxygen and 90 per cent nitrogen to breathe through the anaesthetic face mask for periods of 15-30 min. Maternal and foetal blood samples were collected simultaneously before and towards the end of the 10 per cent oxygen administration. Prompted by some unexpected results we also sampled maternal and foetal blood in five other patients before and 20 min after an intravenous injection of 10 g of glucose in 20 ml. of water. The maternal samples were taken from the fingertip after warming at 40° C for several minutes; the foetal capillary samples were collected from the scalp by the method of Saling⁵. All blood was collected in fine nylon tubing containing a heparinized thread. Oxygen and carbon dioxide pressures and pH were measured within 5 min of collection using an I.L.-113 ultramicroelectrode assembly at 38° C. Base excess was calculated from the Severinghaus nomogram after correcting for percentage oxygen saturation of haemoglobin⁶. Foetal haemoglobin was assumed to be 15 g per cent. Blood for glucose estimation was transferred to small tubes and mixed with sodium fluoride. After microcentrifugation, the glucose concentration in the plasma was estimated by a microadaptation of the technique described by Keston and Teller. The confidence limits of the methods for measuring glucose and oxygen have been described^{4,10}.

The effects of breathing 10 per cent oxygen are summarized in Table 1. Maternal blood glucose increased in every patient, the mean change being +34.6 mg per cent (t, 8.28; P < 0.001). Foetal blood glucose either remained constant or decreased (t, 1.59; P > 0.25). Oxygen tension was halved in the mothers, the mean change being -43 mm of mercury (t, 7.80; P < 0.002). It was

significantly lowered in the foetal samples, the mean change being -8.3 mm of mercury (t, 4.60; P < 0.02). When further measurements were made in one patient, E, 15 min after the end of the experiment, the oxygen tensions had reverted to baseline values. A slight decrease in carbon dioxide pressure, not statistically significant, occurred in all patients and could be explained by the overbreathing which we observed. There were no consistent changes in pH or base excess.

Table 1. RESULTS OF ADMINISTRATION OF 10 PER CENT OXYGEN

		Glue mg per		P(mm	O ₂ Hg	PC		р	н	B. mE	/1.
		M	F	M	\mathbf{F}_{-}	M	F	M	F	M	\mathbf{F}
A	Baseline 18' of 10 per cent O.	61 88	48 48	81 53	$\begin{array}{c} 26 \\ 24 \end{array}$	$\begin{array}{c} 25 \\ 20 \end{array}$	37 33	7·43 7·50		-6.0 -6.0	
В	Baseline 18' of 10 per cent 0,	77 106	61 50	87 24	$\begin{array}{c} 22 \\ 10 \end{array}$	26 26	42 34	7·45 7·42		5·0 6·0	
C	Baseline 15' of 10 per cent O ₂	93 130	55 53	99 31	$\begin{array}{c} 22 \\ 13 \end{array}$	29 26	42 39	7·39 7·35	7·27 7·23	-6·5 -11·0	-5·2 -7·8
D		110 160	75 63	86 46	27 16	28 27	41 40	7·40 7·45	7·32 7·32	- 4.5	$-3.3 \\ -2.0$
E	Baseline 24' of 10 per cent O ₂	65 95	58 60	88 52	20 12	28 26	45 42	7.47	7·28 7·29	-4.0	$-3.1 \\ -2.4$
	15' later	85	58	95	21	23	39	7-47	7.83	-7·0	-2.7
п	= 5 \$\vec{x}\$ baseline \$\vec{x}\$ after 10 per cent 0.	81 116	59 55	84 41	23 15	27 25	41 38	7·42 7·44	7·32 7·31	-6.3	-2·7 -4·2

Table 2. RESULTS OF A MATERNAL GLUCOSE LOAD, 10 g INTRAVENOUSLY

	Glu	cose		
	mg pe	mg per cent		m Hg
	M	F	M	F
F Baseline	95	77	82	20
20' after 10 g of glucose*	125	118		
G Baseline	45	20		14
20' after 10 g of glucose*	85	43		
H Baseline	70	45	109	10
20' after 10 g of glucose*	102	77		
J Baseline	89	60	94	21
20' after 10 g of glucose*	130	93		
K Baseline	83	33	78	13
18' after 10 g of glucose*	110	70		
$ar{x}$ Baseline	76	47	91	16
$ar{x}$ Baseline $ar{x}$ After 10 g of glucose	110	82		

^{*} Injected intravenously.

In order to discover the expected change in foetal blood glucose for a rapid maternal rise of 30-40 mg per cent, a further five patients were given 10 g of glucose intravenously. The results summarized in Table 2 show that similar changes occur in both maternal and foetal blood. The mean maternal increase was 34 mg per cent (t, 13.41; P < 0.001) and the mean foetal rise was 33.2 mg per cent (t, 11.01; P < 0.001). The changes in blood glucose in the two series of experiments are compared in Table 3: Δ M1, Δ M2 and Δ F2 are very similar, but a t test applied to $\Delta F1$ and $\Delta F2$ shows that these values are significantly different (t, 9.05; D.F., 8; P < 0.001).

Table 3. COMPARISON OF FOETAL AND MATERNAL GLUCOSE CHANGES DURING 10 PER CENT OXYGEN AND FOLLOWING 10 g OF GLUCOSE

	10 per cer	nt oxygen		10 g of	glucose
	\mathbf{M}_{1}	$\mathbf{F_1}$		M.	F.
Æ	+27	0	\boldsymbol{F}	+30	+41
\boldsymbol{B}	+29	11	\boldsymbol{G}	+40	+ 23
\boldsymbol{c}	+37	- 2	H	+32	+32
D	+ 50	-12	J	+41	+ 33
$oldsymbol{E}$	+30	+ 2	K	+27	+37
ž	+ 35	5	$oldsymbol{ec{x}}$	+34	+ 33

M, Difference between maternal blood glucose concentrations before and after either 10 per cent oxygen or 10 g of glucose. F, Difference between feetal blood glucose concentrations before and after either 10 per cent oxygen or 10 g of glucose. F_1 compared with F_2 : t, 9-05; D.F., 8; P<0-001.

The dissociation of foetal and maternal glucose concentrations during the period of hypoxia was unexpected. Normally foetal and maternal glucose concentrations are closely correlated, with maternal greater than foetal. This has been confirmed for foetal and cord blood^{9,10}. Changes in maternal blood glucose are transmitted to foetal blood: large rapid increases in glucose concentration (+160 mg per cent) caused by the intravenous injection of 50 g of glucose to the mother caused a comparable change in the foetus (+155 mg per cent)10. Ely11, in her study of the placental transfer of hexoses in the guineapig, showed that the spontaneous fluctuations in maternal glucose concentration which occur under light anaesthesia are quickly reflected in the solution perfusing the foetal aspect of the placenta. In our experiment, maternal glucose concentration increased without producing the expected foetal increase. There are three possible explanations: (1) glucose does not cross the placenta during the period of hypoxia; (2) glucose does cross the placenta but its effect on foetal glucose concentration is eliminated by an increased use of glucose somewhere in the foetus or placenta; (3) an increase in the concentration of foetal blood glucose is cancelled out by haemodilution.

The third explanation is the least likely. Although no direct measurements of haematocrit were made, hypoxia is known to cause haemoconcentration rather than haemodilution in adult and foetal animals of several species12. The possibility that there was increased glucose utilization during the hypoxic period cannot be dismissed, but there is no direct evidence of such an increase. If there had been a shift to anaerobic metabolism by the foetus or placenta with increased glucose utilization (because of the inefficiency of glycolysis as an energy source), nonbuffer tissue acid production would have caused some degree of metabolic acidosis. No consistent changes in pH and base excess occurred in the foetus. The changes were similar to the maternal changes in every case and gave no indication of a qualitative difference between maternal and foetal responses to hypoxia. Huckabee¹³ found that in human volunteers breathing low oxygen mixtures there was a critical arterial oxygen pressure (26-32 mm of mercury) below which "excess lactate" (which he regarded as the most sensitive index of the shift to anaerobic metabolism) accumulated. It is of interest that the two patients (B and C) in whom the base excess and pH did fall were those in whom the maternal oxygen tensions, 24 and 31 mm of mercury, fell below Huckabee's critical threshold. The phenomenon of dissociated maternal and foetal glucose changes, however, occurred in all patients, not only those with an associated metabolic acidosis.

Increased glucose utilization would occur with increased oxygen consumption, but the effect of hypoxia in several species including the human neonate is to decrease oxygen consumption¹⁴. Changes in temperature which alter oxygen consumption in the neonate cannot¹⁴ be invoked when the foetus is in utero.

Hypoxia has been thought to have no effect on transplacental passage of sugars. Karvonen $et\ al.^{15}$ measured

the rate of disappearance of non-metabolized sugars from the foetal circulation of the guinea-pig when the mother was given 10 per cent and 80 per cent oxygen. Varying the concentration of inspired oxygen did not alter the rate of transfer. Transport of glucose across the guinea-pig placenta shows a tendency to saturation at high concentrations, competitive inhibition of fructose and galactose and movement against a concentration gradient in experimental glucose/galactose competition, which suggest that a selective carrier mechanism is involved11. The energy requirements of such a system with transfer usually down a concentration gradient are so far unknown. It would be unremarkable if the system had some minimum requirement for oxygen. If the transport of glucose were an oxygen-dependent process, the failure of foetal glucose to increase concurrently with maternal glucose during

moderate hypoxia would be explained.

Comparison of Tables 1 and 2 shows that the baseline foetal oxygen tension (10 mm of mercury) in patient H given 10 g of glucose is as low as the lowest value produced

by maternal 10 per cent oxygen inhalation in patient B. When the maternal oxygen tensions are compared they are seen to be 109 and 24 mm of mercury, respectively. Maternal rather than foetal hypoxia seems to be the relevant factor in reducing glucose transfer.

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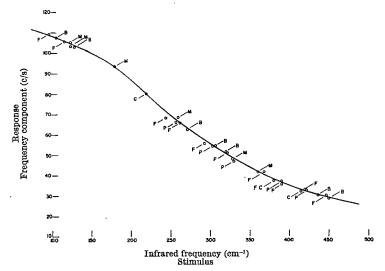
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Olfactory Coding

OLFACTORY signals must be coded both externally and internally. Externally, the odorous molecules present distinctive patterns based on their ability to excite several more or less widely separated intervals in a linear continuum of osmic stimuli^{1,2}. In other words, each odorous molecule carries a characteristic combination of primary stimuli. Internally, the characteristic pattern of the stimulus molecules must be transformed into a patterned neural discharge which somehow reflects the specificity of the input signal from the odorous molecules. To solve the olfactory code, we must find a correlation



Correlation curve for the response of the rabbit. B, Benzaldehyde; C, camphor; F, fenchone; M, methyl salicylate; P, pinene.

between physically observable properties of the external stimulus and the internal response,

In the odorous molecules there is evidence that the different primary stimuli are related to the various low vibrational frequencies of the molecule^{1,3-5}. At the level of the sensory end organs, the coding appears to be effected by the more or less selective stimulation of certain types of receptors, so that the kinds of receptor that "fire" depend on the quality of the stimulus and the number that "fire" depends on its intensity^{1,2,6}. The signals then pass by parallel connecting fibres to the olfactory bulb^{7,8}. There, the signals are transformed into a system of frequency components the numerical values of which represent the quality and the amplitudes of which represent the intensity of the stimulus^{8,10}.

This is shown by experiments with stainless steel macroelectrodes implanted at several places (usually four) in the olfactory bulbs of rabbits or, more recently, some human subjects, so that the discharges resulting from various kinds of smell stimuli can be recorded on magnetic tape. With conscious subjects, the bursts of activity continue for several seconds and can be electrically analysed to identify the frequency components present in the discharges evoked by different smells. The results show that the frequency components are distinctive according to the stimulus category, that is, substances with a certain type of odour (such as peppermint or musk) have many of the same frequency components in the discharge.

If the external stimuli are distributed along a linear continuum of possible molecular vibration frequencies, and if their internal counterparts are frequency components

in a continuum of possible frequencies in the discharge passing from the bulb to the higher centres of the brain, it is reasonable to look for a systematic relation between particular features of the input from the environment and corresponding features in the signals passed on into the brain. There should be a correlation between particular vibration frequencies in the stimulus molecules and certain frequency components in the response, and this correlation should be the same for chemically distinct stimuli. There appears to be a correlation.

For benzaldehyde and anisole, detailed information is available about the "normal modes" of the molecules, so that we seem to have full details of their vibration frequencies including the "difference frequencies" which are generated when two normal modes are excited in the same molecule at the same time. Table 1 shows the low molecular vibration frequencies of these two compounds together with all the high amplitude frequency components and certain of the weaker ones (of which there were many) in the responses of a rabbit to benzaldehyde and of a man to both benzaldehyde and anisole.

Fig. 1 shows a correlation curve for the responses of the rabbit which was constructed by plotting all the vibration frequencies for benzaldehyde against the frequency components in its response and drawing the smooth curve which could be made to pass near the largest number of points with the least curvature. For four additional test compounds, detailed normal mode assignments were not available, but far infrared spectra showing distinct peaks were secured using a Perkin-Elmer Model 301 far infrared spectrophotometer. These provide a partial inventory of the molecular vibration frequencies of these substances. With fenchone, camphor and methyl salicylate, all the far infrared frequencies below about

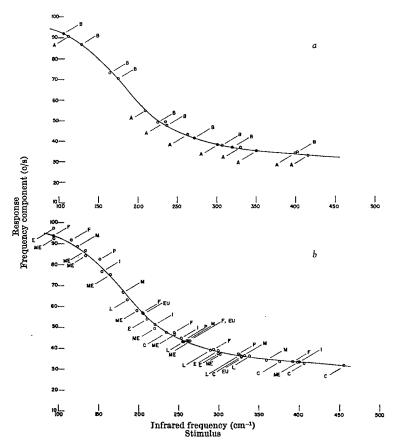


Fig. 2. a, Correlation curve for the response of L. H. A, Anisole; B, benzaldehyde. b, The same curve with data for nine additional compounds superimposed. C, Citral; E, eugenol; EU, eucalyptol; F, fenchone; I, indole; L, limonene; M, methyl salicylate; ME, menthol; P, phenylacetic acid.

450 cm⁻¹ can be correlated with frequency components in the discharge by the same curve (after a small adjustment in the middle range) to give the result shown in Fig. 1. The spectrum of pinene has eight distinct peaks between 450 and 100 cm⁻¹ of which five are correlated in Fig. 1.

Fig. 1.

We used the same method for the human subject, L. H., except that the preliminary curve was based on vibrational data for both benzaldehyde and anisole to give the result shown in Fig. 2a. It embodies all the molecular vibration frequencies of benzaldehyde between 425 and 100 cm⁻¹ and nine of the eleven anisole frequencies in the same range. In Fig. 2b far infrared and frequency component data for nine additional compounds are superimposed on the same curve without any adjustment.

Most of the molecular vibration frequencies can be correlated systematically with frequency components in the discharges from the olfactory bulb and usually with the strongest of these components, and the correlation is independent of the chemical identity of the stimulus molecules. The fact that both the rabbit and the man showed frequency components with no corresponding molecular vibration frequency is probably the result of contamination because the odorous stimuli were presented at a high concentration and without special purification. Also, as pointed out elsewhere, not all molecular vibrations are necessarily osmically active11,12.

Thus there seems to be a one to one relationship between properties of the stimulus and properties of the response to olfactory signals, both of which are objectively observable, and the way is open to a detailed exploration of the olfactory code.

These correlation curves are not the only smooth curves which can be drawn when all the frequency components are plotted against all the molecular vibration frequencies, but the number of possible alternatives is not large, and none of the others includes as many molecular frequencies

or as many of the strong components of the responses.

It is perhaps unfortunate that the word "frequency" should apply to both the stimulus and the response, and it must not be supposed that the vibrations of the odorous molecules excite nerve discharges of any physically related frequency by some process of direct mechanical coupling. All that can be said is that a particular vibration frequency in the molecule enables it to "fire" one kind of receptor but not another, and the receptors use a frequency coding of a very different sort to distinguish their signals from those of receptors of other types.

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PSYCHOLOGY

Degree of Learning, Proactive Interference and Retention

It is well known that retention of verbal material declines with the number of lists learnt1 if all lists are learnt to the same criterion. Underwood¹ concluded from this that the decline in retention is caused by proactive interference from the material previously learnt. This conclusion, however, has been questioned by Warr^{2,3}, who reports that the number of trials to reach a standard criterion decreases with successive lists (a finding also due to Deutsch and Mamakos4), and suggests therefore

that the decline in retention is caused by the decline in degree of learning. In support of this argument he presents data showing that, when successive lists are practised, not to a criterion but for a constant number of trials, the usual decline in retention over successive lists fails to If, however, degree of learning is the most important factor determining retention over successive lists, there should have been an actual increase in retention in Warr's experiment, for the degree of learning increases with successive lists when number of trials is held con-In the present experiment, we investigated retention over successive lists with degree of learning experimentally held constant.

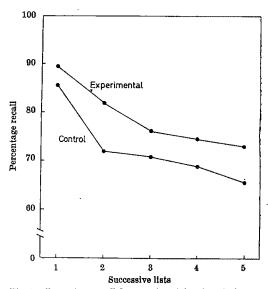


Fig. 1. Percentage recall for experimental and control groups.

The experimental procedure was modelled on that of Warr^{2,3}. The material to be learnt consisted of five lists of twelve pairs of two syllable adjectives taken from the lists compiled by Melton and Safier. The subjects were two groups of eight university students, who learned the lists by the method of anticipation. Exposure time for the stimuli was 2 sec and the intertrial interval 30 sec. Successive lists were learnt at 24 h intervals and testing for retention conducted 24 h later, immediately before the next list was learnt. Retention testing was conducted in the same way as original learning, except that response items were not presented and only one trial was given. The control group learned each list to a criterion of a single trial with all items correct. The experimental group learned the first list to this criterion, but on subsequent lists learning trials were continued until the subject had made the same total number of correct anticipations (Warr's^{2,2} operational definition of "degree of learning") as on the first list.

14	Table I		List		
	1	2	3	4	5
Mean trials to criterion	11-13	9.63	8.25	7.25	6.75
SD	5.25	4.38	4.97	3.60	8.25
Mean correct anticipations per item	6.39	5.74	4.74	4.51	4.51
SD	2.43	2.08	2.29	1.71	1.74

mobio 1

As in Warr's experiments, the control group showed an increase in speed of learning successive lists, and a consequent decrease in degree of learning: Table 1 presents mean trials to criterion and mean anticipations per list item for each list. Fig. 1 shows percentage recall for each list in both groups. The decline in performance shown by the control group is very similar to the result obtained by Warr3: from 85.5 per cent correct on list 1 to 65.7 per

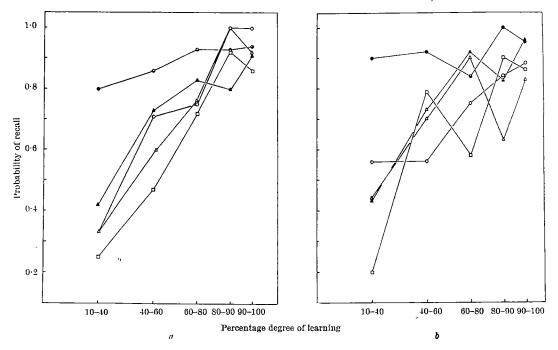


Fig. 2. Relationship between probability of recall and percentage degree of learning for a, control group and b, experimental group. List 1; ▲, list 2; ○, list 3; △, list 4; □, list 5.

cent on list 5 compared with his 82.5 per cent (list 1) to 60.8 per cent (list 6). More important, a decline of a comparable order is shown by the experimental group: 89.6 per cent (list 1) to 73.0 per cent (list 5). Analysis of variance of the data presented in Fig. 1 shows the decline in performance over successive lists to be significant (F, 6.12; df, 4 and 56; P < 0.01), but there is no significant variation caused by either experimental treatment or the interaction between treatment and lists. It seems reasonable to conclude therefore that previous learning of similar material can depress retention even when degree of learning is controlled. A factor rather like the proactive interference stressed by Underwood¹ is thus seen to be of some importance.

Nevertheless, it is clear from our data that the factor of degree of learning emphasized by Warr is also important in determining retention. Figs. 2a and b show the relationship between probability of recall and percentage degree of learning. (To obtain the latter, the number of correct anticipations made by a subject to each item in the list is expressed as a percentage of the maximum number of correct anticipations made by that subject to any item in that list (see ref. 3).) In order to have roughly equal numbers of readings at each point plotted in Figs. 2a and b, values on the abscissae are grouped in the ranges: 10-40 per cent, 40-60 per cent, 60-80 per cent, 80-90 per cent and 90-100 per cent. It can be seen that, in both groups, there is little relation between degree of learning and probability of recall on the first list. In the control group, the effect of degree of learning becomes greater on successive lists. The effect of proactive interference is seen in the fact that, for a given degree of learning, probability of recall is lower the later the list—a finding reported befores. In the experimental group, however, the relationship between degree of learning and probability of recall is more obscure, only the lowest level of degree of learning having a very obvious effect. Productmoment correlations were calculated for the two groups between percentage degree of learning (ranked 1 to 5 as in Figs. 2a and b) and probability of recall, irrespective of list, yielding the following results: for the control group r, 0.81; for the experimental group r, 0.62; P < 0.001in both cases.

These data suggest that proactive interference is a very important determinant of retention of verbal material, able to exert its effects even when degree of learning is experimentally controlled. If, however, degree of learning is not very high, the effect of proactive interference is chiefly exerted on those items with the lowest degree of learning.

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GENERAL

Amazing Antiquity of Mining in Southern Africa

THE only ancient manganese mine yet recorded is in southern Africa, at Chowa near Broken Hill, Zambia1. Its rubble infilling contained crudely flaked mining tools chiefly of manganese, used as choppers, wedges and chisels, together with hammerstones, perforated stones, upper and lower grindstones and a single polished stone axe, but no metal objects or potsherds. The Kafulamadzi Hills 3 miles away revealed Later Stone Age assemblages in quartz, together with manganese tools identical to those found in the working. On this basis, Dart postulated in 1934 the existence of stone age mining in southern Africa.

In 1964 Boshier conducted trial trenching in workings at the Ngwenya Iron Mine in western Swaziland. Sites there yielded flaked stone mining tools similar to those from Chowa found in 1934 (ref. 2).

So far forty excavations have been completed, seventy collections made and 300,000 artefacts recovered. It has unexpectedly emerged at Ngwenya, particularly from the site called Lion Cavern, that the mining is of

great antiquity.

Lion Cavern is at the southern end of a steep scarp face of the haematite hill, Lion Peak, where ancient miners cut into the face of a cliff, more than 500 ft. high, a shelter-shaped working about 25 ft. wide, 30 ft. deep and The floor of the shelter was covered by 20 ft. high. haematite soil and rubble. The first excavation, just inside the cavern's drip-line, bordered on the inner aspect of a large 5 ton haematite boulder fallen from above and almost blocking the entrance to the cavern.

The deposit, 9 ft. deep in this area, had the following characteristics: (a) 0-7 ft. contained stone-mining tools with a few sherds, which were restricted to the upper 4 ft.; (b) 7 ft. of worked bedrock 9 ft. down contained an atypical assemblage of stone artefacts chiefly quartzite and quartz. Irregular flakes predominated, and there were a few chisels. The industry was ascribed provision-

ally to the Later Stone Age.

Dr Minze Stuiver gave as the date³ for the basal level Y-1713, 9640 ± 80 B.P./ 7690 ± 80 B.C. We therefore decided to remove the obstructing haematite boulder by undercutting it on its talus-slope side and excavated the underlying deposit. Here the deposit had a maximum depth of more than 11 ft., with details as follows: (a) 0-6 ft. contained a few mining tools; (b) 6-8 ft. contained undoubted Middle Stone Age artefacts and possible Later Stone Age tools; (c) 8 ft. of worked bedrock deeper than 11 ft. yielded 23,000 artefacts belonging unquestionably to a middle stage of the Middle Stone Occasional stone mining tools were also found. Well defined ash levels showed that the assemblage was in situ.

Quartz, white quartzites, grey and white dappled quartzite, black indurated shales and greenish cherts were the principal materials used by the miners. These rock types occur mostly on a ridge overlooked by, and about 0.25 miles from, the cavern. The exposures there are patently flaked. Dappled grey and white quartzite exposures occur about a mile and more northwest of the site.

Samples of charcoal nodules from the middle to lower levels of the Middle Stone Age level were sent to the Yale and Groningen Laboratories, and the following dates were given:

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Y - 1827
                 22,280 \pm 400 B.P./20,330 \pm 400 B.C.
GRN - 5020, 28,130 \pm 260 \, \text{B.p.} / 26,180 \pm 260 \, \text{B.c.}
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The dates harmonize with the archaeological evidence and suggest that there was mining here for a very long time, terminated perhaps by the fall of the haematite block.

Mr John Strathern, chief geologist at the mine, confirmed the artificial nature of the rock surface underlying the Middle Stone Age stratum. He classified the bedrock as a specularite-rich, crumbly greasy red haematite, and stated that plain crumbly greasy red haematite occurs in large areas and in far more easily accessible locations at Castle Peak, only half a mile to the south. He therefore considered specularite to have been the incentive for the concentration of mining at Lion Cavern.

The worked floor must extend for at least another 10 ft. beyond the outside edge of this second excavation. Its outer part, as yet unexcavated, may date back another few thousand years. Evidence of an earlier culture may also be located there; there is an enormous Later Acheulian site, estimated to contain ten million artefacts barely a mile away.

At least 50 tons of haematite rich in specularite must have been removed from Lion Cavern; two-thirds of it during the Middle Stone Age. Boshier found it was held traditionally in high regard by Swaziland counsellors.

Haematite and specularite occur at many sites ranging back to Earlier Middle Stone Age times within 20 miles of Ngwenya.

These datings demonstrate that haematite has been mined at Ngwenya, on and off, for at least 28,000 vr. They afford the first dated presumptive evidence that all foreign ores and pigments found in prehistoric deposits all over the world were the result of deliberate mining. Dates of cavern strata containing haematite in Rhodesia and South Africa have ages ranging from 37,000 to 42,000 B.P. Incidentally, the claim made almost 35 vr ago, that 'manganese was being deliberately mined in Zambia by a foreign people familiar with its potentialities in Late Stone Age times", and Boshier's expectation2 that "In this field (of ancient mining) Bomvu Ridge (Ngwenya) is of supreme significance; its thorough investigation might well furnish us with knowledge of the genesis of South African mining", have been fully justified. We thank A. McKerron, N. A. D. Campbell and A.

Boshier for showing us the potential of Ngwenya; the staff of the Swaziland Iron Ore Development Company for their help; Dr Minze Stuiver, director of the Yale Radiocarbon Laboratory, and Dr J. C. Vogel, director of the Groningen Laboratory, for dating; and the Anglo-American Corporation of South Africa and the Swaziland Iron Ore Development Company for financial support.

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¹ Dart, R. A., Trans. Roy. Soc. S. Africa, 22, 55 (1934). Boshier, A. K., Scientific South Africa, 2 (7), 317 (1965).
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Amino-acid and Peptide Synthesis from Hydrogen Cyanide

THE recent interesting findings of Matthews and Moser¹ and the earlier work of Lowe et al.2 involving the abiogenesis of amino-acids and their precursors from simple molecules (HCN, H₂O, NH₃) raise two important issues which stem from my observations3 of the amino-acid content of atmospheric precipitation.

First, abiogenic processes leading to amino-acid systems are usually considered in connexion with remote times, yet amino-acids and their precursors can be detected in rainwater and snow, and the experiments carried out so far indicate at least the possibility that their genesis

may be abiogenic.

Second, both groups of investigators subject their systems to acid hydrolysis by refluxing with azeotropic hydrochloric acid. Unless this acid is freshly distilled before use, however, it will be found to contain small amounts of amino-acids after reflux—a phenomenon which itself is worthy of further study. I do not mean to suggest the invalidation of the hydrogen cyanide synthesis of amino-acid systems, but simply that a reappraisal of the data may be useful.

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Matthews, C. N., and Moser, B. E., Nature, 215, 1230 (1967).
 Lowe, C. U., Rees, M. W., and Markham, R., Nature, 199, 219 (1963).
 Sidle, A. B., Tellus, 19 (1), 128 (1967).

BOOK REVIEWS

ALGEBRAICAL GENIUS The Mathematical Papers of Sir William Rowan Hamilton

Edited by H. Halberstam and R. E. Ingram. Vol. 3: Algebra. (Cunningham Memoir, No. 15.) Pp. xxiv + 672. (London: Cambridge University Press, 1967.) 210s. net. Hamilton's decisive contribution to algebra is his discovery of the quaternions. It is the source from which modern algebra has derived some of its most fertile ideas and methods: the freedom to discard the commutative law of multiplication and the rigorous construction of new algebraical systems from those already known, thus putting algebra on a sound logical basis. Hamilton was deeply concerned about the foundations of algebra which, in his day, were considered to be far less secure than those of geometry. The introduction of negative numbers and, to a greater degree, of complex numbers appeared to be obscure and even illogical to many of his contemporaries (and perhaps still to some of our own). In a long essay, written in 1833, he propounds the seemingly bizarre doctrine that "algebra is the science of pure time"; in more prosaic language this means that he proposed to take the positive real numbers as a starting point whence the continuum of all real numbers, positive or negative, can be derived as equivalence classes of pairs of positive numbers, equivalent pairs having the same "difference". Granting now the real numbers, he proceeds to give his celebrated construction of the complex numbers as pairs of reals, obeying the multiplication law

$$(a_1,b_1)(a_2,b_2) = (a_1a_2 - b_1b_2, a_1b_2 + a_2b_1)$$

From there it was a natural step to pass to an algebra of triplets endowed with a product that preserves the usual rules of algebra. But for thirteen years Hamilton was held up by the fact (as we now know) that no "division algebra" of triplets can possibly exist. Time and again, after a night of strenuous efforts, his children asked him in the morning, "Papa, can you multiply triplets?", to which he replied, with a sad shake of his head, "No, I can only add and subtract them". It was on October 16, 1843, that "a spark flashed forth" and Hamilton discovered that a satisfactory multiplication could be found for quadruples, or quaternions, as he called them, rather than for triplets. He believed that this discovery was his greatest scientific achievement and he hoped that quaternions would turn out to be a powerful tool in dealing with geometrical and physical problems. He devoted numerous articles to the applications of quaternions, some of which contain results of remarkable elegance. Looking back, more than a hundred years after Hamilton's death, one would admit that the usefulness of quaternions fell short of his expectations despite isolated successes, which persist to our own days, in various branches of mathematics. On the other hand, the theoretical ideas which led him to his discovery had a more far-reaching influence on the development of algebra than might have been anticipated at the time.

The papers on quaternions and related topics occupy about three-quarters of this volume, the remainder being taken up by Hamilton's researches on the quintic, now mainly of historical interest, and his ingenious invention of the "icosian calculus", a precursor of modern graph theory exemplified by the network of edges on an icosahedron.

Hamilton's memoirs were composed in an age in which a scholar's time and a printer's wage were considered less precious than at present, and an author was at liberty to expound his work in full detail and to adorn it with personal digressions, usually cast in impeccable prose, which gave mathematical writings a much more personal flavour than is possible today. It is interesting to learn how deeply Hamilton was influenced by John T. Graves, whose name is not widely known to mathematicians of our time.

It need scarcely be stressed that the volume has been beautifully produced by the Cambridge University Press. The editors, Professor H. Halberstam and Dr R. E. Ingram, have provided a lucid introduction and valuable addenda, which round off the monumental work of one of the great creative minds in the history of the mathematical sciences.

W. Ledermann

PULSE COMPRESSION AND RADAR

Radar Signals

An Introduction to Theory and Application. By Charles E. Cook and Marvin Bernfeld. (Electrical Science; A Series of Monographs and Texts.) Pp. xvi+531. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 156s.

Modern radar systems, although they function in accordance with the same basic principles as the primitive radars of some twenty years ago, are a million times more sensitive. The use of larger aerial systems, higher operating frequencies, more powerful transmitters and receivers of lower noise levels have all contributed to the improvement, but the most important contribution has come from the realization, during the 1950s, that in pulsed radar the range resolution and accuracy are functions of the signal bandwidth and not of the transmitted pulse width. The maximum detection range can be obtained by using the maximum energy per pulse and thus the widest pulse in accordance with the energy requirements of the system or with the available transmitter power. Nevertheless, even after this pulse width has been chosen, it is still possible to satisfy the range resolution conditions by coding the transmitted signal with wideband modulation information. The extraction at the receiving end of the information does require, however, a more complex receiving system than that normally required for a simple pulse radar. Such a complex receiving system is called a matched filter processing system, and the signal processing generally implies operations performed on the received signal in the r.f. or i.f. portions of the radar receiver.

Both the concepts and techniques involved in the pulse compression, matched filtering radar signal processing system are fully described in Radar Signals. The first five chapters are devoted to the broad theoretical aspects related to matched-filter techniques, with detailed discussions of the principle of stationary phase, the radar ambiguity function, and parameter estimation. The next four chapters deal with specific radar waveforms, including the linear FM waveforms and discrete coded waveforms. In the final four chapters various practical problems associated with matched filter systems are considered. These include effects of distortion on matched filter signals, the design of lumped constant and ultrasonic dispersive delay lines, and the application of microwave and optical techniques to matched filter designs.

The text is addressed primarily to the systems engineer, and although it is extremely clearly and well written and amply supplied with more than 300 illustrations, it demands of the reader a fairly high standard of mathematical knowledge and acquaintance with waveforms and wave analysis. Each chapter is adequately supplied with a list of references, varying in number from eight to thirty-eight, and totalling 268. These, together with a bibliography of sixty-nine references and detailed author and subject indexes, indicate the care which the authors

and publishers have taken in the preparation of an up to date and comprehensive treatise on the subject.

The authors state in the preface that the volume is the outgrowth of a series of notes prepared for a graduate level course covering the theory, application and design of large time-bandwidth radar signals, given to co-workers at the Sperry Gyroscope Company, and they express the hope that it might be used as an introductory textbook for other students following such a course. Certainly each chapter is more or less complete in itself, as would correspond to a lecture course, but it is doubtful whether it would be suitable as a textbook for use in Great Britain. It is undoubtedly, however, an important contribution to the literature on radar engineering and an extremely valuable exposition of, and reference to, the present knowledge of complex radar waveforms.

S. Weintroub

ALL ABOUT ELECTRODEPOSITION

Fundamentals of Metal Deposition

By E. Raub and K. Müller. Translated from the German. Pp. viii+268. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 125s.

This book is a welcome addition to the literature of electrodeposition in the English language. It should be of great value to students in this field and to many of those already in industry who wish to understand the important fundamental processes on which their technology depends.

The first two chapters deal with the physical chemistry of electrolyte solutions and fundamentals of electrode processes. These will provide useful revision for those with a good background of physical chemistry and sufficient coverage of the subject for those who have not. The third and fourth chapters are concerned with cathodic discharge processes and various aspects of the structure of electrodeposits. In the first of these the treatment of electrocrystallization does not seem to be fully up to date and the section on hydrogen deposition is disappointing. There are few references here to recent researches concerned with ad-ions, mechanisms of incorporation of deposited ions into the crystal lattice, surface diffusion and other growth processes. Details of information concerning the mechanistic steps in hydrogen deposition are also inadequate, as is the literature cited. The final chapters are concerned with the physical and chemical properties and the distribution of deposited metals on the cathode. These chapters are up to date and the section on microthrowing power and levelling is excellently presented and illustrated.

The literature cited is biased towards German publications and the research interests of the authors. This is not unreasonable in view of the authors' long experience and expertise in the field. The text is easy to read, excellently illustrated throughout, and the standard of translation is high. Despite the few criticisms made, this book should be found on the bookshelves of all interested in electrodeposition.

I. A. MENZIES

INTRODUCING CATALYSTS

Introduction to the Principles of Heterogeneous Catalysis

By J. M. Thomas and W. J. Thomas. Pp. x+544. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 120s.; \$21.50.

In the scope of a textbook of medium length, the authors have presented a comprehensive introduction to a subject which has many aspects. It differs from some previous texts, among them those to which the authors have referred in their preface, by the inclusion of a brief chapter on the selectivity of catalysts and a discussion of

stepwise heterogeneous reactions, and a substantial one which introduces the design of catalytic reactors. This unusual breadth of the discussion of the principles of catalysis has resulted from the collaboration of a chemist with a chemical engineer.

Other chapters present the theory of adsorption; experimental methods in the study of adsorption; porosity and surface area; the role of lattice imperfections; geometric and electronic factors; and the mechanism of typical heterogeneous reactions. The review of experimental aspects of adsorption includes accounts of several of the techniques which have been developed more recently, such as flash desorption, infrared absorption, slow electron diffraction and the application of nuclear magnetic resonance and electron spin resonance methods to heterogeneous systems.

In this book consideration has been given to metals, oxides and non-metals, whereas many previous studies have been biased to a particular catalyst type. Consideration of the diversity of catalysts has led the authors to lay particular stress on the environment of the atomic site of catalytic activity. This aspect of the theory of catalysis is increasingly attracting attention (for example, in the Faraday Society discussion in 1966) because of the contribution which ligand field theory has made to understanding of the transition complex in the surface of a solid.

The book is excellently presented, with copious references, especially in the experimental section, a good author index, and a subject index which, if used in conjunction with the list of contents, is comprehensive. It can be recommended to research workers who want an up to date manual of this topic, and will be found to be stimulating by many who are already well informed about heterogeneous catalysis.

A. COUPER

PHOTOCHEMISTRY TRIBUTE

Photochemistry and Reaction Kinetics

Edited by P. G. Ashmore, F. S. Dainton and T. M. Sugden. Pp. xvi+378. (London: Cambridge University Press, 1967.) 75s. net; \$13.50.

A NUMBER of authors have contributed to this volume, the common link being that most of them were former research students of Professor R. G. W. Norrish and three have made distinguished contributions in their own countries to fields of study closely related to the interests of Norrish. The book is therefore a spontaneous token of affection to a scholar and personality of world wide reputation.

The series of review type articles covers the principal interests of Norrish and is divided into two parts, one dealing with photochemistry, the other with combustion kinetics. Separating these parts is a review of polymer chemistry.

There are general contributions by W. A. Noyes and Bernard Lewis, who review the contributions to science which have been made by Norrish. These are followed by a discussion of photochemistry in the liquid phase in which the importance of "cage effects" is stressed, both in inorganic and organic systems. In the gas phase, photochlorination is fully dealt with and the now well established technique of flash photolysis is the subject of two contributions. This part concludes with a detailed chapter on energy transfer in molecular collisions. Norrish's contributions to polymer chemistry are examined in the light of current knowledge in a review which deals almost entirely with free radical processes.

The final part of the book, dealing with combustion, is introduced by Semenov and in four reviews gives a clear account of the broad outlines of a difficult field of research. The individual topics dealt with include the mechanism of hydrocarbon oxidation in the gas phase, the pheno-

menon of the cool flame, sensitization and inhibition of

ignition and pyrolysis of paraffins.

This book can certainly be recommended to all chemists; it provides an easily read account of so many important fields of study. It is well provided with references and illustrated by clear diagrams. The editors and authors have provided their former professor with a tribute which, I am sure, must give him great pleasure and which justly matches his own reputation. JAMES C. ROBB

CHEMISTRY OF COAGULATION

Blood Clotting Enzymology Edited by Walter H. Seegers. Pp. xii+628. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 220s.

In a sense this book is disappointing in that it does not, as its title suggests, devote much space to the enzymology of blood coagulation; instead this is a comprehensive review of the mechanism of blood coagulation from the particular point of view associated with Dr Seegers. It is unfortunate that the author has not given more space to comparing and contrasting his own view with that proposed by other workers, which he dismisses with the phrase ... it is speculative in nature and proposes a sequence of events that is not consistent with the known facts of prothrombin chemistry". It is, in fact, the chemistry of prothrombin which is at the very centre of the discord between Seegers and those other "speculators". believes that prothrombin isolated by procedures laid down by him is a multifunctional complex which is dissociated into separate activities by thrombin. Other authors believe that his prothrombin preparations are a mixture of proteins, and have supported this belief by resolving the complex, by various procedures, into different components of which the precursor of thrombin is only one. Seegers claims that in these experiments the prothrombin has been modified and no longer represents the native substance.

The individual view of Seegers and his associates is apparent in the chapters devoted to the clinical aspects of coagulation and to immunochemistry, but the book also includes contributions from other authors on topics about which Seegers holds no particular view. There is an excellent review on the fibrinogen fibrin transformation incorporating the latest ideas on the mechanism of fibrin polymerization, and an interesting chapter on the ultrastructure of the fibrin clot. There is also a stimulating discussion on the effect of different surfaces on the proteins involved in blood coagulation.

Other aspects of coagulation which are included are the various inhibitors of blood coagulation, the role of platelets in haemostasis and, finally, a short account of

the chemistry and function of vitamin K.

In spite of these reservations, the student of blood coagulation should find this book useful because it presents an alternative interpretation of the phenomena of clotting, the merits of which can be decided by the reader. M. P. ESNOUF

ANTIBODIES FOR CLOTTING

The Use of Antibodies in the Study of Blood Coagulation By K. W. E. Denson. Pp. ix + 244. (Oxford and Edinburgh: Blackwell Scientific Publications, 1967.) 52s. 6d.

THE mysteries which surround blood coagulation are such as to make the development of a new method for its study an important event. In recent years the addition of techniques of protein chemistry, particularly electrophoresis and ultracentrifugation, to the long-established

coagulation methods has provided new information about the physical characteristics of certain clotting factors. The present volume describes a new approach to the study of the coagulation mechanism, through the provision and elaboration of a technique, which may prove a useful addition to those already mentioned.

The work described in this book was originally submitted

as a thesis for a DPhil degree in the University of Oxford and is essentially the application of immunological methods, in the form of the use of antisera to clotting

factors, to the study of blood coagulation.

The book is in two parts. The first part begins with a description of the two modern concepts of blood coagulation, the "cascade" hypothesis and that which envisages prothrombin as the basic factor, and continues with a detailed description of methods for preparing purified coagulation factors and thus their antisera. There follow two chapters on the characterization of the functional activity of the antisera of six clotting factors and one chapter dealing with the immunochemistry of clotting factors and their antisera.

The second part of the book describes, in great detail. the application of the specific antisera to clotting factors to various aspects of the coagulation mechanism and ends with a general discussion, including the author's con-

clusions and suggestions for further studies.

The detail in which the methods and experiments are described and the results presented is impressive and the treatise is obviously the result of a vast amount of detailed and painstaking labour, on which the author is to be congratulated.

This volume will be of greater value to those wishing to apply these techniques and procedures to their own investigations than to those with a more general interest in coagulation, although the summaries and conclusions at the end of most chapters will assist those not immediately concerned with the full experimental details, while the review of the literature is adequate for the needs of the treatise.

The approach described in this treatise has undoubtedly added, and will continue to add, to our knowledge of the coagulation mechanism. While it may help to resolve some of the differences between the "cascade" hypothesis, founded as it is on investigation of defects in the haemostatic mechanism, and that based on prothrombin, it remains to be seen whether its application will enable new light to be thrown on the all-important and pressing problem of hypercoagulability and thromboembolic disease.

G. B. D. Scott

IMMUNOLOGICAL EXPLOSION

Advances in Immunology

Edited by F. J. Dixon, jun., and J. H. Humphrey. Vol. 6. Pp. xvii+571. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967) 148s.

This volume contains a varied selection of topics. Many of the articles, however, have the common feature that they report results which appear to be just opening the way to the most productive phases of study. For example, P. G. H. Gelland A. Kelus in their article on anti-antibodies devote two pages to idiotypic antibodies. This is a most remarkable phenomenon first observed independently several years ago by Oudin and by the authors, and the far-reaching theoretical implications have aroused such interest that a fuller discussion will be needed very soon. Again the in vitro stimulation of a primary response has been sought for many years and much of Dutton's review is taken up with discussion of unsuccessful or ancillary studies related to this basic problem. The most convincing demonstration that a primary stimulation in vitro was possible, by Mishell and Dutton, must have been made while this review was in preparation and of necessity gets

only brief mention. As a further example the capacity of animals to synthesize antibodies which could distinguish between different types of nucleic acid was in doubt until several years ago. Among other reasons, the close similarity of structure of nucleic acids of different species made it uncertain that an effective antibody response could be obtained. In 1964, Plescia and colleagues (authors of the present article) found that complexing with methylated bovine serum albumin was sufficient to make many nucleic acids effective antigens. Very recently, work at Cambridge has led to the development of methods for solving the chemical structure of RNA. This should make possible detailed correlation of specificity and structure and it opens up many possibilities for the use of specific antiserum in both biochemical and chemical studies in this field. Yet again, the long article by A. E. Gabrielson and R. A. Good on the chemical suppression of adoptive immunity is concluded by a few pages on anti-lymphocyte serum. Although the mechanisms are not understood, this technique may well be the most effective method of suppressing undesirable immune responses and the volume of recently published work on this topic will clearly necessitate a full review very soon.

It is inevitable that with a subject progressing at the present explosive pace of immunology, many reviews will be outdated rapidly, but this does not reduce their value. The exhaustive discussion by Dutton, for example, of in vitro studies of the immunological response includes several long tabulated summaries of much often partially successful work and is not easy to read, but it will remain a valuable guide to the literature for many years to come.

In a less popular area, P. Lachman has reviewed the biochemical (or should it be molecular?) aspects of conglutinin and immunoconglutinin. The former is a high molecular weight globulin found in the serum of cows and several closely related species and structurally has nothing in common with immunoconglutinin which is an immunoglobulin, mainly IgM but also present in the IgG fraction. Both the conglutinins combine with a component or components of complement after the latter's reaction with the antibody—antigen complex, and thus give rise to the characteristic reaction named conglutination. Rapid progress in defining the complement system has made it possible to investigate in more detail the secondary conglutination reaction and the progress achieved is discussed in this article.

A transatlantic collaboration between Sterzl and Silverstein has led to an interesting review of developmental aspects of immunity in which emphasis has been placed on the cellular changes occurring at the onset of immunity in immature animals and when an immune response is initiated in the adult. Immunopathology finds a place in the discussion of experimental glomerulonephritis by Unanue and Dixon, thus completing the wide range of topics reviewed in this volume. Although no doubt it would be profitable, few immunologists will wish to read this issue from cover to cover, but all will find that more than half the articles will be of interest and value to them.

R. R. PORTER

ONE PHYLOGENETIC SYSTEM

Phylo-genetic Systematics.

By Willi Hennig. Translated by D. Dwight Davis and Rainer Zangerl. Pp. 263. (Urbana, Ill., and London: University of Illinois Press, 1966.) 93s.

Although this book has a certain importance as an attempt to think out how far one can derive a phylogenetic arrangement of species from imperfect material—especially from groups with no or only a fragmentary fossil record—it cannot be described as successful. The publishers' blurb describes it as "far in advance of the thinking of most systematic zoologists". Considering

that it is published in the United States, whence some of the best thinking on evolution, phylogeny, taxonomy and metataxonomy has come in recent years, this is a truly remarkable statement, unsupported by the book. It is a strange fact that, with the exception of Timoféeff-Ressovsky until recently, there have been few workers of wide repute on population genetics and evolutionary genetics in Germany, so that the basic processes of evolution have been neglected there for years. outstanding exception of Rensch, there have been very few workers on higher evolutionary processes, while the best-known palaeontologist is probably Schindewolf, whose ideas of typogenesis are rightly rejected by Hennig. Germany, in the past century almost too avant-garde in evolutionary studies, has for many years been undistinguished in this field; yet phylogenetic systematics cannot flourish without a sound knowledge of evolution.

The book is a translation of a complete revision, not available in print, of a previous book, so that the accuracy of translation cannot be checked. Much is hard reading, with obscurity of logical connexion between sentences, a flat and poor style, and frequent repetition of stylistic clichés. One perfect example of how not to put German into English is Ziehen's definition of order, which is given as "the totality of progressively graduated vicinal similarities of more or less determined positional relationships of several or many, even an infinite number, maximally all, 'somethings' within a finite or infinite whole". Yes! And the reader is left to struggle without any help towards elucidating the meanings of the terms. The reader is treated pretty cavalierly throughout; casual statements are made on important matters with no references or only an author's name without further data, although the reader needs to be able to check the author's assertions; legends for text-figures often fail to explain major features of the diagrams; proof-reading and checking are careless (for example, Fig. 66 refers to the Lepidoptera, not merely the butterflies; in Fig. 39, 3 and 5 should be transposed; Vertigo genesii is not a "parasitic snail larva", as the title of the reference quoted makes clear); and important discussions and statements of fact may appear not where they should logically be but in summaries—for example, that on sterility and crossability as means of defining biospecies. The author has the irritating habit of giving very short quotations from a large number of authors with no indication of context, a few words here or a phrase there which have struck him. Where so much divergence of terminology or emphasis exists, this is extremely dangerous, and the critical reader is left with the feeling that he ought to check every short quotation to make sure that the quoted author really meant it in the way that Hennig takes it. One at least certainly did not.

Hennig appears to define the species from an evolutionary point of view as a segment of a phyletic line bounded by two acts of speciation—the piece between two branchings. He firmly rejects the view held by nearly every other worker that if considerable transformation has taken place along this piece, it should not be all included in the same species, and justifies this remarkable procedure by the equally remarkable reason that because profoundly different stages in the life-history are included in the same species, morphology is not a criterion of the species. He dismisses the agamospecies as exceptional, and roundly asserts that no definite case of a species arising by hybridization is known in zoology. Neither statement seems judicious to me, and the title of the book and the treatment of the subject throughout scarcely justify a complete neglect of plants. He appears to believe that closeness of morphological similarity within a group means that the forms in it have diverged only recently-"We placed the fourteen species of the Acrania in one family because relatively little time seems to have passed (as may be inferred, in the absence of other indications, from the minor degree of their morphological divergence) since the existence of their last common stem

form". On present evolutionary and genetical theory this is at best very unsafe, and should be supported by numerous examples from actual fossil records before being accepted even tentatively. He discusses convergences and parallelisms briefly and unsatisfactorily, and assumes as an "auxiliary principle" that the origin of convergence of a character in two forms should never be assumed but always proved. This is done because of his conviction that "phylogenetic systematics would lose all the ground on which it stands" if such characters were always considered as convergences or parallelisms with proof to the contrary required in each case. It is difficult to understand how such a confusion of thought can go unrecognized. In the first place, there is no simple opposition between counting all such characters as showing parallelisms unless there is proof for any to the contrary, and on the contrary counting them all as indicative of common ancestry unless there is evidence otherwise. There is also the very usual position in which we simply do not know what their significance is and would be wholly unjustified in coming to any conclusion. And in the second place, the aim of phylogenetic systematics is surely to produce an arrangement of known forms in as close accord with their sequence of evolution as possible; this so-called "auxiliary" or "heuristic" principle is, in fact, a prejudgment of the very results we are trying to discover. This is no doubt why Tuxen disagreed with Hennig on this point, but he is merely dismissed by Hennig as having "probably misunderstood my heuristic principles". When the results of a science are produced by the methodological assumptions rather than by the evidence available, it is not a science; and in this particular example, it is clear that phylogenetic systematics of this sort should lose the ground on which it stands if it can do no better than this. It is particularly unfortunate that this book should appear at a time of so many attacks on evolutionary studies, systematics and taxonomy by biologists who should know a little more before communicating their ideas so freely; this sort of work gives A. J. CAIN them an easy target.

VERTEBRATES REVISITED

Structure and Habit in Vertebrate Evolution
By G. S. Carter. (Biology Series.) Pp. xiv+520.
(London: Sidgwick and Jackson, 1967.) 63s.

THE aim of Structure and Habit in Vertebrate Evolution is to "relate the structural and biological changes during evolution to changes in the animal's habits of life". It treats in turn the structure and palaeontology of each of the main vertebrate groups and follows this with a general discussion of the significance, in terms of phylogeny, of the changes that have come about at each major advance in vertebrate reorganization.

Some of the points that emerge from this treatment are interesting enough; for example, the adaptation of the respiratory methods, and the structure and physiology of the blood, of amphibian ancestors to a freshwater and then a terrestrial habitat. Throughout, the circulatory system receives fuller and more functional treatment than in most comparable books.

In correlating structure with habit the author is more interested in stressing the possible habits open to vertebrates as a result of some pre-adaptation of structure than in inquiring into the habits that might have selected structures. Thus he is more interesting on the structure and function of accessory respiratory organs and the evolutionary possibilities they provided than on the selective reasons for homothermy or the mammal jaw articulation.

The palaeontology of the vertebrate groups is given a good summary treatment with up to date fossil evidence, but the tentativeness of the approach to important phylogenetic connexions becomes exasperating when it is

applied to every group. Admittedly, it is by no means clear whether arthrodires are ancestral or closely related to the chondrichthyes or the docodonts to the monotremes, but the non-committal attitude to the possible relationships is carried to an extreme that blurs the main evolutionary events and spoils the stated aim of the book which is to study the major changes in vertebrate evolution.

The book should appeal to the student because points of difference between groups are numbered and listed, but this can also confuse where, for instance, the characters of fossil mammals and mammal-like reptiles are dogmatically contrasted in a numbered table and on the next page modified. There are, finally, a number of curious misprints for which there is no excuse—what are microtuberculates?

WILMA GEORGE

INSECTS BY THE MILLION

Insect Colonization and Mass Production Edited by Carroll N. Smith. Pp. xxi+618. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 216s.

THE interested layman as well as scientists in other disciplines must often wonder at the paradox of some entomologists trying to eradicate insect pests and others trying to grow them in the laboratory. This book may provide an answer, for, while it is important to control or eradicate harmful insects, the work is facilitated by knowledge about the life cycle, physiology and behaviour of the insects in controlled laboratory conditions, as well as in the field. Scientists are beginning to realize that successful colonization of insects and other arthropods is essential for efficient and productive research into virtually every aspect of entomology, including control and eradication. For example, a method developed by American entomologists during the Second World War, for rearing human body lice in large numbers on rabbits, led to the development of excellent and rapid techniques for insecticide testing and louse control.

The problem of resistance to insecticides which has assumed serious proportions recently, and its genetical basis, are best studied in laboratory colonies of insects in controlled conditions. Resistance has also highlighted other methods of control, for example biological control using insect parasites, predators and pathogens. All these require the mass production of the host insects on which the parasite, predator or pathogen could be reared in large numbers for release in the field. Recent work on insect attractants has provided information which suggests that insects or products derived from them can be used for the control of a variety of species. In some cases, these attractants have been synthesized, but in others, as in the case of the pink boll worm moth—a serious pest of cotton plants—the only source of the sex attractant at present is reared pink boll worm moths.

Considerable success has been achieved recently in the control and eradication of the screw worm, a serious pest of domestic stock, by releasing in the field mass produced sterilized males. This method of control is now being investigated for the control of various agricultural insect pests and disease carrying arthropods.

Many vital questions of vector host relationships in insects which transmit animal and plant diseases can best be answered through carefully controlled laboratory studies with laboratory grown insects. All these points provide justification for the laboratory colonization and mass production of economically important arthropods.

The five sections in this book deal with colonization of animal parasites and bloodfeeding arthropods (186 pages); insects of domestic and stored products (68 pages); plant feeding insects and mites (214 pages); insect parasites, predators and pathogens (42 pages); and the mass production of insects for the sterile release technique (67 pages).

The various groups of arthropods in each section are dealt with by authorities competent in their particular fields.

It is obviously impossible in a book of this size to include methods of colonization of all the economically important arthropods. The establishment of small laboratory colonies of insects such as rat fleas, Culex pipiens mosquitoes, bed bugs and cone nosed bugs, adequate for normal experimental procedures, is relatively simple and is included in the section on blood sucking insects. I feel, however, that some of this space could have been devoted to phlebotomine sandflies, which are difficult to colonize and which transmit human disease in many parts of the world. The colonization of body lice, biting midges, black flies, stable flies and tsetse flies is justifiably included, because of new successful techniques or because of the inherent difficulties involved. Papers in the Bulletin of the World Health Organization (31, 1964), on culture methods of arthropod vectors and their biological control agents, deal with colonization of some of these groups and of others not covered in the present

The second section, on insects of domestic and stored products, describes methods of rearing and handling house flies, cockroaches, and beetle and moth pests of stored products. The third section is of more interest to the agricultural entomologist and deals with colonization of diverse plant feeding insects and mites such as pine beetles, pink boll worms, cabbage loopers and corn root worms, many of which cause extensive damage to crops.

Perhaps the most interesting sections are the last two, which deal with biological control in one form or another and the colonization and mass production of insect parasites, predators and pathogens such as nuclear polyhedrose viruses of lepidopterous pests. This and the mass production of screw worms, fruit flies and mosquitoes for actual or potential control by release of artificially sterilized males into natural populations have shown the feasibility of using non-chemical methods for controlling insect pests. And this, when so much is heard about actual or potential, real or fancied, hazards of insecticides, is reassuring.

M. G. R. VARMA

TO SPLIT OR NOT TO SPLIT

The Lichen Symbiosis

By Vernon Ahmadjian. (A Blaisdell Book in the Pure and Applied Sciences.) Pp. viii + 152. (Waltham, Mass., and London: Blaisdell Publishing Company, a Division of Ginn and Company, 1967.) \$5.75.

To split a lichen into its two components, an alga and a fungus, and study them separately or to experiment on the complete thallus using radioactive tracers are the two main ways in which the physiology of this symbiosis may be examined. Professor Ahmadjian employs the first technique. The major part of this book gives an excellent account of his researches and those of his colleagues together with the results of earlier workers which are relevant to his approach. There are two chapters at the end of the book on the physiology of the complete plant and on lichen chemistry.

The book is beautifully produced, with a large number of photographs and drawings, although no scale is given on many of the latter. The drawings and photographs of lichen algae are undoubtedly the best published so far. The text is readable and informative, but there are a number of points which might be queried. In the first place, are heterocysts (page 22) any longer regarded as a method of vegetative multiplication of blue-green algae? Again, on page 31, the author cites the work of Kofler and Bouzon to support the statement that "the percentage spore discharge and germination among many temperate lichens are highest in early spring and fall when the most favourable climatic conditions for these processes

generally occur". As I understand it, the main aim of thes workers was to refute the theory of seasonal spore discharge and to show that discharge and germination wermore closely correlated with micro-climate and localimatic conditions than with time of year. (For example in one year they found that the maximum discharge and germination of *Umbilicaria* spp. spores was in July.)

In discussing lichen algae, Professor Ahmadjian has stated more than once that Trebouxia spp. are rarely if ever, found in the free living state (see, for example page 17 of this book). On page 67 he says, however, "that they do occur in small isolated groups among other algae" If this is the case then it would have been valuable to have a cited reference or further details. In view of the author's scepticism that Trebouxia is ever free living, is is surprising that he quotes work (see page 44) which found that "when pigments were extracted from strainform of T. decolorans and compared with those of a non-sensitive, non-lichenized Trebouxia" the pigments differed quantitatively but not qualitatively. Reference to the original paper shows that the non-lichenized species is given as Trebouxia humicola (Naeg.) Treboux, which is correctly Chlorococcum humicolum (Naeg.) Raben., acommon soil alga, and not a Trebouxia at all^{2,3}.

Except for a few criticisms of the type given above, the book is exceptionally useful; first, because it clearly describes a number of techniques for the culture and attempted resynthesis of the lichen symbiosis; second, because it poses many of the unsolved problems regarding the physiology of lichens; and last, because it contains a very comprehensive list of references. The book with its annotated bibliography gives an excellent survey of the present state of lichen physiology which will be welcomed by students and researchers alike.

D. H. S. RICHARDSON

¹ De Nicola, M. G., and de Benedetto, G., Boll. Ist. Bot. Univ. Catania, 3, 22 (1962).

² Puymaly, A. de, Revue Algol., 1, 107 (1924).

³ Fritsch, F. E., The Structure and Reproduction of the Algae, 146 (Cambridge University Press, 1948).

GAMBLING MATHEMATICS

The Theory of Gambling and Statistical Logic By Richard A. Epstein. Pp. xiii+492. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 80s.

The stated aims of this book are "to dissipate the mystery, myths and misconceptions . . . to overcome the appeal of fallacies . . . and to emphasize the advantages of utilizing objective probability in gambling". In so far as an objective mathematical analysis of gambling games is relevant, the book goes far to achieve its aims. Nevertheless, the author's impressive display of negative expectations is hardly likely to daunt the gambler any more than a documentary on cancer cuts the sale of cigarettes. We are to envisage a race of gamblers who are entirely rational, and "who would rather lose intelligently than win ignorantly". A secondary purpose of the book is to unify and bring together material otherwise to be found only in widely scattered sources.

The title of the book is unfortunate. Only about fifty of its 400 or so pages accord with the usual meaning of the words "theory of gambling", and I must confess to being little wiser concerning "statistical logic" after reading this book than before. In fact, by far the greater part of the text consists of an encyclopaedia of gambling games with their mathematical analyses.

The contents can be divided roughly into five categories (although not followed sequentially in the layout of the work). First (second chapter), the author summarizes the necessary prerequisites in probability, statistics and games theory. Although mostly satisfactory, there are occasional lapses in the mathematical exactitude, some of

which might be disastrous to the unwary reader (for instance, page 33).

The second section (third chapter) states and proves ten theorems constituting the author's theory of gambling. Most of these apply to the situation of repeated identical and independent games in which there are at most three outcomes. The first few theorems are contrived to pour increasing quantities of cold water on the so-called miraculous gambling "systems", while the others concern the probability of gambler's ruin and the chance of achieving a target fortune under various strategies of play. Although of direct relevance to simple gambling situations, the mathematician might understandably prefer the more sophisticated approach to be found, for instance, in Dubins and Savage's How to Gamble if You Must (McGraw-Hill, 1965).

Next comes a detailed and fascinating treatment of the probabilistic properties of coin tossing, dice rolling, card shuffling, and the like. Particular consideration is given to the possibilities of prediction and "cheating". leads directly to the fourth and largest category, being the encyclopaedic array of games mentioned here. These range from elementary games (which are solved completely) to games such as bridge (to which a whole chapter is devoted). While numerical solutions to these latter games are as yet inaccessible, the mathematically inclined addict will find much of interest here. Similar considerations are given to horse racing and stock markets, but the results are, of necessity, somewhat limited.

The excellent final chapter returns to the former theme of the book. It examines the widespread belief in luck and other gamblers' superstitions. It concludes with a general discussion of paranormal phenomena, including extrasensory perception experiments.

Anyone reading this book from cover to cover would be well advised to procure an a priori interest in the details of gambling games. To such, however, this book has an undoubted fascination. There is plenty of light relief in the text, and indeed the whole account is well presented and pleasingly written. The mathematical treatment is straightforward, and is thus accessible to a wide readership. J. B. COPAS

OBITUARIES

Professor E. B. Verney

E. B. VERNEY died on August 19 at the age of 73. After some clinical experience he chose an academic career, and in his early days worked under E. H. Starling and T. R. Elliott, both of whom quickly appreciated his worth. In 1926 he was appointed to the chair of pharmacology at University College, London, and in 1934 he moved to Cambridge, his alma mater, as Sheild reader, and in 1946 as the first Sheild professor of pharmacology. retired in 1961 and spent three years as research professor in Melbourne before returning to Cambridge in 1964. He received recognition in various forms and in many countries. Among other honours, he was elected FRCP in 1928 and FRS in 1936.

Verney had the utmost integrity and always thoroughly briefed himself before speaking on a subject or undertaking an experiment. Consequently his opinions were valued and carried considerable weight, and it was realized that his work was meticulous. He was also a first class surgeon and rarely did one of his complicated demonstrations or experiments fail. Preparations for demonstrations might begin at 6.30-7.00 a.m. in order that all should be perfect and ready by mid-morning, and his experimental preparations were on occasion completed by mid or late afternoon, when he would begin observations. Having worked with Starling and Elliott, he was interested in the cardiovascular system and had mastered the art of making heart-lung-kidney preparations. His first important observations added enormously to our knowledge of the pars nervosa of the pituitary gland, an organ about which there was little definite or accurate knowledge at the time he began. He showed beyond doubt that the pituitary released into the blood stream a substance which restored almost normal function to the isolated kidney. and that in the absence of this substance the urine produced was like that of a patient with diabetes insipidus. He further showed that the active substance could not be adrenaline and that its action was independent of renal innervation. He studied the effect of exercise in dogs, in which he collected urine separately from the two kidneys. and demonstrated that both the innervated and denervated organ responded similarly to a water load by diuresis, inhibition on exercise and then recovery. He went on to show that the antidiuretic effect of exercise depended on its emotional content and that one of the stimuli which brought about release of the pituitary substance was a rise in the osmotic pressure of the plasma passing to the region of the anterior hypothalamus. In other words, he showed conclusively that the pars nervosa contained an antidiuretic hormone which regulated the output of water from the kidney and was in turn regulated by the composition of the plasma reaching the hypothalamus.

Verney was also interested in the causes of renal hypertension and demonstrated in conscious dogs that even a brief bilateral occlusion of the renal arteries can lead to a rise in blood pressure. He also showed that hypertension of renal origin could be induced in almost totally sympathectomized dogs, so that in all probability the active substance must act directly on the blood vessels. Latterly Verney turned his attention to the adrenals and among other things showed that adrenocorticotrophic hormone was more effective in releasing glucocorticoids than aldosterone from the isolated perfused gland.

When he died Verney had ended his days of experimental work, but his advice and judgment were still available. They will be deeply missed, but even more his friends will miss Verney for his own sake.

MARY PICKFORD

Dr W. R. Chapman

DR WILLIAM RONALD CHAPMAN, who died on June 29. graduated from Sheffield in 1922 and gained a PhD in 1927. His working career was mainly in the field of coal preparation, and in this he was one of the world experts. and was co-author with R. A. Mott of one of the standard textbooks on the subject, The Cleaning of Coal. He was engaged in preparing a second edition of this book at the time of his death.

Chapman applied his knowledge in various parts of the world; he acted as consultant to the Kailan Mining Administration in China, where he was taken prisoner of war by the Japanese. During his captivity he turned from the cleaning of coal to the making of bread and the cultivation of yeast for this purpose.

After the war he returned to England and was chief coal preparation engineer to the East Midlands Division of the National Coal Board. He then went as adviser on coal preparation to the Turkish Government, returning to Great Britain as assistant director of research of the National Coal Board Coal Research Establishment. During this period he was given leave of absence for a period to advise the Indian Government on coal preparation. He was also an honorary lecturer in coal preparation and minerals engineering at the University of Birmingham. After leaving the National Coal Board he became a consultant to Messrs. Head Wrightson until the time of his death.

During his long career Chapman published many papers in various journals, and sat on many national and international committees. He was a founder member of the Coal Preparation Society and was its president for two years. He was also a member of the editorial advisory board of Coal Preparation.

University News:

Bradford

DR J. B. Helliwell, at present reader in gas dynamics in the University of Strathelyde, has been appointed to the chair of mathematics in the Board of Studies in Engineering, and Dr G. Brown, at present reader in physics, has been appointed professor of nuclear physics. Professor D. C. Johnson, visiting professor at the university, has been appointed to the chair of industrial technology. Professor H. T. Stamboliev, dean of the Faculty of Technology at the University of Skopje, has been appointed to the visiting chair of chemical engineering for the session 1967–68, and Dr M. Green, a director of Zenith Radio Research Corporation (UK) Ltd, has been appointed visiting professor of physical chemistry for a two year period commencing in October this year.

Dundee

Professor A. R. MITCHELL, formerly reader in applied mathematics in the University of St Andrews, has been appointed to the newly created third chair of mathematics.

Lond

THE title of professor of experimental pathology has been conferred on Dr Lynne Reid in respect of her post at the Institute of Diseases of the Chest.

Ulster

MR ALAN MILTON, vice-principal and acting principal of the University College of Rhodesia, has been appointed professor of education and director of the Education Centre.

Appointments

MR A. S. ASHTON, a director of Esso Petroleum Co Ltd, and Mr H. W. Morris, deputy chairman of ICI Fibres Ltd, have been appointed members of the Shipbuilding Industry Board, which was established under the Shipbuilding Industry Act, 1967.

Announcements

THE Ministry of Agriculture, Fisheries and Food has placed an order with Messrs Ferguson Brothers (Port Glasgow) Ltd for a new fisheries research vessel. The vessel, which will replace the present research vessel, Ernest Holt, is expected to be in service in late 1969.

DR LESLIE MULLINS, head of Kodak's technical services to marketing, delivered the Mehl Honor Lecture at the 1967 Conference of the American Society for Non-Destructive Testing. Dr Mullins, the first British scientist who has been invited to present this lecture, spoke on "The Development of Non-Destructive Testing".

THE Drummond Managing Trustees have awarded Mr A. P. Nandi Majumdar a Drummond Scholarship in nutrition for the year 1967-68. The scholarship will be held at the London School of Hygiene and Tropical Medicine.

Meetings

HEATING and Ventilation for a Human Environment, November 1-2, Institution of Mechanical Engineers (R. S. Glynn, The Institution of Mechanical Engineers, 1 Birdcage Walk, London, SW1).

PSYCHOSOMATIC Disorders, November 10-11, London (Dr A. H. Crisp, c/o Academic Psychiatric Unit, Middlesex Hospital, London, W1).

AIR Navigation Conference, November 14—December 13, Montreal (International Civil Aviation Organization, International Aviation Building, 1080 University Street, Montreal).

ERRATUM. In the communication "Transformation of the Histochemical Profile of Skeletal Muscle by 'Foreign' Innervation", by G. Karpati and W. K. Engel (Nature, 215, 1509; 1967), the two photographs appeared in reverse order, so that Fig. 2 was printed above the legend to Fig. 1 and vice versa.

Erratum. In the communication "Markovian Models of Dialogic Time Patterns", by J. Jaffe, S. Feldstein and L. Cassotta (Nature, 216, 93; 1967), the third sentence following equation (1) should read "One consequence of the model is that the probability of reaching any state j from any state i in exactly n steps is given by the ijth term . . .".

Erratum. In the communication "Electron Thermodynamic Nonequilibrium in p-n Junctions", by M. A. Melehy (Nature, 215, 1251; 1967), equation (7) should read $\alpha = (I/I^*)$, $p_e = (1 + \alpha) \ p_n$. The title of the second column of Table 1 should be p_n (cm⁻³).

CORRESPONDENCE

Origin of Plastids

SIR,—I am prompted to write to you briefly on reading, I think for the third time, in the pages of Nature the statement, this time from your Cell Biology Correspondent (216, 14; 1967), "Ever since the discovery that mitochondria and chloroplasts contain . . . there has been speculation that these organelles evolved from symbiotic bacteria . . ." This is an example of a tendency manifested especially by non-biologists now working in the biological field to imagine that the new methods now being applied to biological problems are providing answers and suggesting hypotheses which the older "classical" biologists have not and could not have conceived. I believe the idea that this speculation is a new one should be killed. The idea that both chloroplasts and mitochondria might be organelles evolved from symbiotic bacteria was very much in the air thirty or forty years ago and precisely for the same reason stated by your correspondent—namely, on account of the genetic continuity of both these organelles; and what is new now is not this speculation but the molecular basis on which it can be made, and this seems to me a trivial distinction. Reference to this speculation can be found for mitochondria on page 19 of the book *Protoplasm*, by W. Seifriz, published in 1936. I cannot for the moment lay my hands on a similar statement for chloroplasts, but I recall that in 1935 I attended the Saturday evening seminars organized for his students by Professor O. F. Curtis at Cornell University, during one of which we discussed the possibility that chloroplasts may be derived from symbiotic bacteria. This discussion had then been a feature of the seminars for several years past. coming to Leeds I found precisely the same speculation being placed before his students by Dr (later Professor) W. H. Pearsall and I have no doubt that the same speculation was rife in almost all botany departments in the world at that time.

This is itself a trivial matter, but it is one example in which less than justice is done to the older workers. I believe it is important to keep in mind that development in science is a continuous process; and that younger workers are well advised to keep a wary eye on what was done by their forebears. As a younger worker I was brought up short in this regard both by Professor G. van Iterson, jun., in Holland, and by Professor I. W. Bailey of Harvard. This is a lesson I have never forgotten.

Yours sincerely,

R. D. Preston

Astbury Department of Biophysics, University of Leeds.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 30

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr D. L. Thomas and Mr E. P. G. Wright: "The Impact of the CCITT No. 6 Signalling System on Telecommunications".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5.30 p.m.-Mr Arthur T. Gill: "Faraday and Photography".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5.30 p.m.—Dr N. A. Mitchison: "Control of the Immune Response".*

University of London Institute of Education (in the Great Hall, King's College, Strand, London, WC2), at 5.30 p.m.—Professor A. V. Judges: "The Idea of Equality in Education".*

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "The Implications of Going Metric".

Monday, October 30-Wednesday, November 1

Institution of Electrical Engineers (at Savoy Place, London, WC2)
-Conference on "Metering and Apparatus for Modern Electricity Supply Tariffs".

Monday, October 30-Thursday, November 2

INSTITUTE OF WELDING (at 54 Princes Gate, Exhibition Road, London, SW7)—Autumn Meeting on "Welding in Non-Ferritic Materials".

Tuesday, October 31

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr J. R. Tata: "Hormonal Control of Protein Synthesis". (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, SWI), at 6 p.m.—Discussion Meeting on "Requirements—How to Specify Them to Advantage".

University of Hull (in the Assembly Hall), at 8 p.m.—Professor M. V. Wilkes, FRS: "Digital Computers from their Early Beginnings to the Present Time" (The St. John's College, Cambridge Lecture).

Wednesday, November I

BOYAL INSTITUTION, HISTORY OF SOIENCE DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Mr Frank Greenaway: "Victorian Attitudes to the Application of Science".

BIOMETRIO SOCIETY, BRITISH REGION (at the Wellcome Building, Euston Road, London, NW1), at 2.30 p.m.—Professor J. F. Scott: "The Investigation of a Psychological Syndrome by Multivariate Analysis"; Mr G. M. Clarke: "Inverse Polynomial Response Surfaces Applied to Data from Plant Nutrition Experiments".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W1), at 3 p.m.—Symposium on "Triassic Rocks of the British Isles".

University of London (at the Institute of Dental Surgery, Gray's Inn Road, London, WC1), at 5 p.m.—Professor Sir John McMichael: "Science and Practice".*

University of London (at Bedford College, Regent's Park, London, NW1), at 5.15 p.m.—Sir John Wolfenden: "Universities and the State".*

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W1), at 5.30 p.m.—Mr H. P. Munro: "Uses of Critical Path Planning".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr A. G. J. MacFarlane: "State Space Methods in Dynamical Systems Analysis".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, TELECOMMUNICATIONS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 6 p.m.—Mr K. E. Martin and Mr B. E. Attwood: "Deflection Circuits for Colour and Monochrome Television Receivers".

Wednesday, November I—Thursday, November 2

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, SW1)—Symposium on "Heating and Ventilation for a Human Environment".

Thursday, November 2

ROYAL SOCIETY (at 6 Carlton House, Terrace, London, SW1), at 10 a.m.—Discussion Meeting on "Anomalous Aspects of Biochemistry of Possible Significance in Discussing the Origins and Distribution of Life" organized by Mr N. W. Pirie, FRS.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m.—Professor Ronald King: "The Atomic Nucleus and Nuclear Energy" (Civil Service Lecture).

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, WC2), at 5.15 p.m.—Mr J. R. Stebbing, OBE: "Possible Contributions of Nuclear Science to the Needs of Africa".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Sir Harry Melville, KCB, FRS: "The Position of Fundamental Scientific Research in the United Kingdom" (Third Appleton Lecture).

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5,30 p.m.—Mr D. P. Burkitt: "Study of Cancer Patterns in Africa". (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

University of London (in the Physiology Theatre, University College London, Gower Street, London, WC1), at 5.30 p.m.—Dr Britton Chance: "Electron and Ion Transport in Mitochondrial Membranes".*

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at 14 Belgrave Square, London, SW1), at 6 p.m.—Meeting on "The Biodeterioration of Plastics".

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, SE1), at 6 p.m.—Mr H. W. Miller and Mr T. P. Gordon Brown: "Recent Developments in Ground Freezing".

PHARMACEDTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, WC1), at 7 p.m.—Dr J. G. Dare: "A Brief Survey of Australian Pharmacy".

UNIVERSITY OF LONDON (at Withersdane Hall, Wye College, near Ashford, Kent), at 8.15 p.m.—The Right Hon. Lord Annan: "Where are the Universities Going?"*

Friday, November 3

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarie Street, London, W1), at 1 p.m.—Professor D. Bryce-Smith: "Some Recent Developments in the Photochemistry of Benzine".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (joint meeting with the Manchester Section, Agriculture and Food Groups, at the Literary and Philosophical Society's House, 36 George Street, Manchester), at 1.30 p.m.—Symposium on "Food Production in the Year 2,000 A.D.".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 9 p.m.—Professor J. Maynard Smith: "The Genetics of Ageing".

Saturday, November 4

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum. London Road, Forest Hill, London, SE23), at 3.30 p.m.—Mr Richard Tapper: "On Migration with the Shahsavan Tribesmen of Persia".*

Monday, November 6

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 1.15 p.m.-Professor Ronald King: "Faraday—Father of Electrical Engineering".

FARADAY SOCIETY (in the Department of Chemistry, The University, Edinburgh), at 4.30 p.m.—Professor Dudley Herschbach (Harvard University): "Molecular Beam Kinetics" (Bourke Lecture).*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at $5.30~\rm p.m.$ —Lecture on "AC and DC Current Comparators".

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "What Can We Do Without in a Nuclear Power Station?"

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT ECTION (at Savoy Place, London, WC2), at 6.30 p.m.—Mr F. F. Mazda: "Thyristor Control".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, SW1), at 6.30 p.m.—Dr M. W. Davles: "The Chemistry of Iron and Steel Making".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

Sentor Demonstrator (with an honours degree in microbiology, extensive experience in the organization and operation of practical courses, and preferably experience in research) in Microbiology at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (London and Brisbane, November 3).

LECTURER of ASSISTANT LECTURER (with interests in fungal physiology or morphogenesis) in Mycology—The Registrar, The University, Leicester (November 4).

LECTURER (with a good honours degree in physics or electronic engineering and relevant teaching and research or industrial experience) in Physics—The Principal, Rutherford College of Technology, Ellison Place, Newcastle upon Tyne, 1 (November 4).

CHAIR OF EXPERIMENTAL PHYSICS—The Secretary (NAT), University of Stirling, Stirling, Scotland (November 6).

SENIOR LECTURER IN PATHOLOGY—The Registrar, The University, Sheffield (November 10).

CHAIR OF CONTROL ENGINEERING in the DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, University of Salford, Salford 5, Lancashire (November 14).

JUNIOR LECTURER/LECTURER/SENIOR LECTURER IN HORTICULTURE at Massey University, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (New Zealand and London, November 15).

LECTURER or ASSISTANT LECTURER (preferably with qualifications in parasitology and/or entomology) in Zoology at the University of Botswana, Lesotho and Swaziland—The Inter-University Council, 33 Bedford Place, London, WC1 (November 15).

FARM Demonstrator (with a degree in agriculture or an honours degree in pure science followed by an adequate postgraduate qualification)—The Secretary, School of Agriculture, University of Cambridge, Cambridge (Sambridge (November 18).

Assispant Lecturer or Lecturer in Theoretical Physics or atomic contributions.

Der 18).
ASSISTANT LECTURER OF LECTURER IN THEORETICAL PHYSICS (candidates should have worked in theoretical problems in solid state physics or atomic spectroscopy)—The Registrar (Room 38, O.R.B.), The University, Reading (November 20).
LECTURER (Smallfact in Canada and Cana

(November 20).

LECTURER (qualified in one or more of the following fields: blometry, agricultural statistics, blomathematics, mathematical statistics, an agricultural or biological discipline with supplementary experience in its mathematical or statistical aspects) in BIOMETRY in the DEPARTMENT OF ACRICULTURAL BOTANY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SWI (Australia and London, November 24).

CHAIR OF PHYSIOAL CHEMISTRY at Massey University, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SWI (New Zealand and London, November 30).

HEAD (physiologist with a postgraduate qualification) of the Crop Physiology Department in the Botany Division, to study the growth of herbage

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plants with particular reference to the natural environment—The Secretary, Grassland Research Institute, Hurley; Maidenhead, Berkshire (December 1).

READER or SENIOR LECTURER IN MICROBIOLOGY at the University of Tasmania—The Association of Commonwealth, Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, December 4).

IMMUNOLOGIST or BIOCHEMIST (with immunological experience) to collaborate in the development of isotopic methods in the immune assay of

IMMUNOLOGIST OF BIOCHEMIST (with immunological experience) to collaborate in the development of isotopic methods in the immune assay of 'lysosomal enzymes—The Administrative Secretary, Strangeways Research Laboratory, Wort's Causeway, Cambridge.

LECTURER IN BIOCHEMISTRY: a LECTURER IN MICROBIAL GENERICS; a LECTURER IN ZOOLOGY (specializing in physiology); and a LECTURER IN BOTANY—The Principal, Barking Regional College of Technology, Longbridge, Road, Dagenham, Essex.

LECTURER (with a basic knowledge of physics, and preferably research experience in the field of sediment studies on the Continental Shelf, particularly in the geological aspects) in GEOPHYSICS—The Registrar (S), Bath University of Technology, Bath, Somerset, quoting Ref. 67/116.

REESBARCH ASSISTANT (with a first-or second-class degree in some branch of engineering or physics) in the DEPARTMENT OF APPLIED MATTEMATICS for research in rheology—The Registrar, University College of Wales, Aberystwyth.

for research in rheology—The Registrar, University Courge of manes, Aberystwyth.

Technician for work involving the manipulation of radio-active materials, including labelling procedures, and the use of a range of different types of counter—The Secretary, Institute of Nuclear Medicine, Middlesex Hospital Medical School, London, Wi.

Technician (preferably with previous experience in bacteriology or biochemistry) for the Microbiology Section of the Department of Botany, to be responsible for laboratories used both for teaching and research purposes—The Registrar, The University, Nottingham.

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Almost a Good Bargain

THE University Grants Committee has done well, in all the circumstances, to negotiate a reasonably generous agreement on the quinquennium starting in 1968. Only a few months ago, it seemed as if there would be all kinds of deprivation in the period immediately ahead. Not merely did it seem as if the economic pressures on the British Government would make it hard actually to provide the money which the universities will need if they are to continue to grow. There was also a period during which it seemed as if the Government was flirting with the notion that a period of stagnation in the universities would do no serious harm. That at least was the impression created by much of the oversanguine talk about the future development of polytechnics which accompanied the first statements about the potential benefits of the binary system now emerging for British higher education. In the event, it seems as if the universities will be able to grow steadily although not exuberantly in the five years ahead. That is something to be glad about.

In terms of the recurrent grant, there will be an increase of 28 per cent in the five years ahead, and an accompanying increase of roughly 22 per cent in the student population, which suggests that the universities should be able fairly comfortably to keep their heads above water. Indeed, this simple comparison may considerably underestimate the strength of the position of the universities, for there is some evidence of slack in the university system as things are at present. There is, for example, scope for making much better use of the skills and energies of university teachers. The expansion of the past five years has also enabled the universities to recruit people to their staffs more quickly than they are able to deploy them in the teaching force. With skill and good housekeeping, the universities as a whole should be able to keep ticking over comfortably in the next quinquennium.

But this is only half the story. Nobody's interest will be served if the universities grow bigger but stay the same in the quinquennium ahead. And by now it is clear for all to see that these five years will see the introduction of a great many innovations in the pattern of university teaching. One consequence is that the universities will—or should—find themselves spending more heavily on new kinds of lecture rooms, new kinds

of ancillary aids to the teaching process and even on new kinds of teaching. This will, and should, be comparatively expensive, which suggests that the comparatively modest grants—£16 million next year rising to £18 million in five years—which the UGC is now being given for equipment and furniture may be the first component of the new budget to show obvious signs of strain. And although there is obviously no point in fighting battles before they are engaged, the universities would do well to make early preparations for a thorough study of the ways in which educational innovations may change the character of university teaching and the scale of their demands on the public purse.

The other side of this coin is that the quinquennium ahead should see important steps towards a much more economical use of the capital equipment now locked up in the universities. It is now several years since it became possible to talk safely, in university common rooms, of schemes for adding an extra term to the university year, for example, or for making changes in the ratio of teachers to undergraduates which has had the status of a magic number (one to nine for undergraduates and one to three for postgraduates) ever since the Robbins Committee reported. So far, however, talk has tended to become a substitute for change. There is, however, good reason to believe that moderate reforms along these and other lines could bring important economies and greater freedom for the universities as a whole. It would also help towards the same objectives if the universities were to take more vigorous steps towards regional planning among themselves. Britain is now so thickly sprinkled with universities that the attempt to create from scratch virtually identical educational establishments in cities separated by thirty miles or so is often extravagant and sometimes absurd. But there is also a case for asking that the universities collectively should somehow encourage a greater diversity among themselves. The attempts of the UGC to push towards this worthy objective have not been markedly successful, which is not much of a surprise. The universities themselves could win great benefits by this means, however, and this is something which they ought to work towards in the quinquennium now ahead.

Who Should Keep the Books?

So the British Government has decided not to build a new British Museum Library, or any other, at Bloomsbury. Instead, it intends to go back to square one and to think quickly about the functions a national library should fulfil and the best means of achieving them with existing libraries. Although the way the Government announced this decision is deplorable, the decision itself may well prove to be right. That entirely depends on what sort of national library is eventually created, where it is built, and whether or

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not the Government's action becomes a cause for still further delay.

Unfortunately it is not possible to regard the Government action simply as a belated recognition that it could do a more thorough job in the forward planning of British libraries. Mr Gordon Walker seems to have reached his decision in a manner almost calculated to insult the trustees of the British Museum. It is true that most of them have been long in public life, and have developed broad backs as a result, but is it really wise that the Department of Education and Science should show such little respect to its voluntary servants? Is it sensible to allow distinguished men to work away at plans for a new library for the best part of two decades, only to tell them just before contracts are to be let that there is no building to be done? It is entirely possible that the proposed building in Bloomsbury would have done more architectural damage to the neighbourhood than sensible men would like to see, and there are all kinds of other grounds on which the plans could have been argued against. But the department's failure properly to consult the trustees is not merely discourteous but potentially dangerous how easy will it be for the minister to recruit advisers in the months ahead?

That said, it was entirely sensible that the Government should ask-before it became inextricably committed to building the proposed new British Museum Library—whether simple rehousing of the museum library would be the best way to provide a national library service. Under the existing plan, the British Museum Library would simply have moved into a bigger building. It was never planned as a national library-indeed, the trustees have always been careful never to refer to it as such. But the report of the UGC committee under Dr Thomas Parry made it clear that it is the only institution which could hope to fulfil even part of that role. Under the same plan, the National Reference Library for Science and Invention-essentially the stock of the Patent Office Library with additions from the British Museum-was formally instituted in 1966 and placed under the British Museum. This was to have been housed separately on a site on the South Bank of the Thames, near Waterloo Bridge. That, however, has at last gone the way of all Cheshire cats.

So, with the Government not committed to any site or building, the committee which it is to set up will at least have a chance to propose a radical reorganization to plan for a complete national library service. The two most important decisions to be made are whether or not the national library should comprise several broadly specialized units separately housed or one comprehensive library and whether these should be autonomous, administered in part or wholly by the British Museum.

The recent tendency has been to separate libraries by subject—the National Reference Library for Science and Invention and the National Lending Library for Science and Technology are indicative—and the UGC Committee on Libraries proposed similar national

lending and reference libraries for social sciences and the humanities. But the case for giving up separation by subject and building a single great comprehensive national reference library is strong. First, as recent events prove, the more sites that have to be found and buildings planned, the less the chances of any actually being started. Second, a multiplicity of buildings will inevitably create duplication of some facilities. Third, the boundaries between disciplines are already obscure and bound to become more so; any division based on subject is bound to lead to anomalies and overlap. Fourth, it is not obvious why it should be easier to co-ordinate the activities of several units when they are scattered about London rather than under one roof, or why a single library if properly organized is less able to deal with the enormous growth of literature in all subjects. The Parry report is hard to follow on this point.

Whether or not the decision is for a single comprehensive library or for two or three broadly specialized libraries, it is vital that they should be organized to make the greatest use of computers for cataloguing, data processing and retrieval. The use of computers in libraries is still in its infancy, but twenty years from now it is hard to see how these tasks can be accomplished any other way. The British Museum Library is investigating the possibility of automatic data processing to maintain its catalogue and automate as many library functions as possible. The National Reference Library for Science and Invention and the British Museum Library use different cataloguing systems, and the only hope of co-ordinating these lies in the development of a computerized catalogue. But can this easily be achieved within the existing framework of the British Museum? There is at least a case for thinking it would be better to set up a new body, independent of the British Museum, to organize and run the national library—even if this did mean that the traditional links between the British Museum and its library were broken. But even if in the end the Government decides to leave the relationship between the museum and its library much as it is, the National Reference Library for Science and Invention should be separately administered by an organization which understands the needs and problems of science, technology and industry. There is no reason why some outside body such as the Royal Society should not be persuaded to do this. If Mr Gordon Walker's abruptness was really a sign that the Government had at last recognized that placing the National Reference Library for Science and Invention under the British Museum was a mistake, he may yet win back some supporters.

In parallel with the national library, which must obviously be a reference library, there is a need for a comprehensive system of national lending libraries. The National Lending Library for Science and Technology, established by the Department of Education and Science in 1962, has been operating successfully from Boston Spa in Yorkshire and could serve as a model for two new national lending libraries for the social sciences and humanities which should not of

course be built in London. Why not have all three at Boston Spa co-ordinated as the National Lending Library, and providing a focal point for all the lending library services in the country? At the moment the resources of the National Central Library in London, which is severely limited by lack of money, are pitifully inadequate to meet a mounting demand for lending material in these fields, and as a result the National Lending Library for Science and Technology has already started to collect social sciences periodicals.

But where should all these buildings be? The Government may regret the decision to abandon the Bloomsbury site. It has thrown away a unique opportunity of having the national library and the great national collection of antiquities side by side, which could only have increased the efficient use of both. Although a national library has more important functions than providing research libraries for the departments of the British Museum, these would not have been impeded by having the two institutions on one site.

Bloomsbury Cinderella

THE Secretary of State for Education and Science, Mr Patrick Gordon Walker, brought consternation to the trustees of the British Museum, and to a good many other people as well, by his announcement on October 26 that the British Government has decided not to proceed with the scheme for building an extension to the British Museum in Bloomsbury put forward in detailed form a year ago. That scheme would have entailed the development, on a site opposite the frontage of the present museum, of a new building to hold the overflow from the library of the museum, now bursting at the seams. In his statement in the House of Commons, Mr Gordon Walker gave as his principal reason for rejecting the development scheme, on which the trustees of the museum have been working for the best part of two decades, the view that the Camden Borough Council is right to protest at the destruction of the 500 dwellings that would have to be knocked down before the new library could be built. But it is plain that Mr Gordon Walker has also welcomed this opportunity to take another look at the development of central library services in Britain, which is why he has decided to set up an independent committee to give advice on the development of central libraries.

The committee will particularly concern itself with the question of the storage needs of the libraries.

The trustees of the British Museum have reacted strongly to this announcement, and the chairman, Lord Radcliffe, has denied Mr Gordon Walker's suggestion that he had been consulted in advance. The independent committee has not yet been appointed, but the minister has promised that it will report within twelve months. This further review comes at a time when the trustees of the museum are seeking to appoint a new director in succession to Sir Frank Francis, who is retiring this year, which may have suggested to the Government that the time is ripe for a further examination of the relationship between the library and the other collections of the British Museum. The issue is particularly important to scientists because the National Reference Library for Science and Invention is a dependant of the British Museum, and has not yet

But did the Government feel that the autonomy of a national library would be jeopardized if it were too close to the British Museum? It must have realized that by refusing to develop Bloomsbury it was creating enormous difficulties for itself. Mr Gordon Walker confidently asserts that a new site can be found in time for building to begin on schedule in the early 1970s. It must. There have been too many delays already, and providing national library facilities is a matter of utmost urgency. The cost of delays rises steadily. Merely hiring storage space will cost the British Museum alone an average of more than half a million pounds annually between now and 1985, when the planned library was to have been completed. The cost to the nation caused by the inefficient use of libraries because of overcrowded and totally inadequate facilities is incalculable. If the Government is to dispel the scepticism and despair that fill both library users and keepers, it must live up to its promise that its committee will reach a decision within twelve months.

been provided with a site on which to unite its two divisions, one at the Patent Office Library and one in Queensway.

Money for Dons

MR PATRICK GORDON WALKER last week announced how much money would be made available to the British universities over the next five years. The figures show a less dramatic increase than has been customary over the last five years, but there are several reasons for this. The major period of expansion after the report of the Robbins Committee is now over, for one thing, and expansion in the next five years is likely to be less swift. For another, the provision made for equipment has now been listed as a separate item, in line with the new equipment grants which have caused so much rancour between universities and the UGC.

As the table shows, the equipment grants will begin in 1968-69. The recurrent grant for this year was flad million

The Secretary of State also announced that a further £1.65 million would be made available this year for the purchase of furniture and equipment. This extra grant will go some way towards filling the gap between what the universities wanted and what the UGC has so far been able to supply. The UGC says that it will be increasing rations to some selected universities. The number of students in British universities is now running ahead of the Robbins predictions; this year the number will probably exceed 200,000, against the Robbins target of 197,000. By 1971–72, the Robbins Committee estimated the total university population at 204,000, but the grants announced last week assume that the totals will in fact have risen to between 220,000 and 225,000 by that date.

	RECURRENT	EQUIPMENT		
	GRANTS	GRANTS		
1967-68	£150-8 million			
196869	£153·6 ,,	£16.5 million		
1969–70	£159·5 "	£19·0 ,,		
197071	£166·0 ,,	£18·25 "		
1971–72	£172·5 ,,	£18·25 "		

The grants will not be allocated to the individual universities until approximately November 18; Vice-Chancellors generally consider therefore that it is too early to decide whether or not they are adequate. Dr C. L. Bosanquet of Newcastle University considers that the proposals are at first sight reasonably encouraging and Dr D. G. Christopherson of Durham University welcomes the £1.65 million allocated to the purchase of university furniture and equipment for the current financial year. Each university is aware of its own needs but is ignorant of the needs of others and it may be some time therefore before an overall picture of the national situation is built up.

Money for Schools

Grants to students cost local authorities just over £58 million in 1965–66. The vast majority of the expenditure was made up by the full value awards, which cover fees and a maintenance element subject to parental income. There were 167,547 of these awards in 1965–66, and they cost £56 million. The other £2 million is made up by a further 21,000 lesser value awards, made by local authorities either for non-scheduled courses, or to students who do not qualify academically for a full award.

The figures are given in the latest bulletin of statistics from the Department of Education and Science, Statistics of Education, Volume 5 (HMSO, 8s 6d). The volume covers finance and awards, and shows that at the end of 1966 a total of £124 million had been approved for building schools and colleges of education.

Science for Primary Schools

This week the Nuffield Foundation brought out four books and three pamphlets in the Nuffield Junior Science Project, intended for teachers in primary schools. All are published by William Collins Sons and Co. Ltd. and they mark an important new development in Nuffield Science teaching.

The Nuffield approach of learning by doing is particularly suitable for primary schools, which have shown themselves more willing than most to abandon the traditional approaches to teaching. The books and booklets just published are intended to give the primary school teacher both a general idea of what Nuffield teaching means and practical examples which have been proved in the classrooms. One of the books is devoted to apparatus, and contains some remarkable examples made by children of average ability at an urban primary school. They include quite elaborate electrical circuits, including a buzzer working on a simple "make and break" circuit which went through no less than four development stages before reaching a Development of the buzzers brought final model. both miniaturization and increasing efficiency, and the final model incorporated three electromagnets and a threaded adjustor to control the amount of vibration.

The other books in the series describe the work which can be done with animal and plants in the classroom, and two guides for teachers with examples of projects which were done by classes in the early stage of the project. One of the three booklets describes how the changing seasons can be used to stimulate children's interest in science, and the other two describe experiments with mammals in the classroom, and, perhaps

oddly, how science and history can be taught simultaneously. The introduction puts history firmly in its place—"Many people have been taught to regard history as consisting almost exclusively of politics... if history is to make sense to children, they must surely be encouraged to put themselves in the position of the men who made the discoveries which contributed to our present understanding of the world."

Whether the Nuffield Junior Science Project will redress this supposed imbalance remains to be seen. Its own fault is its tendency to elevate curiosity to the status of discovery.

Causes of Death

TEN years ago the World Health Organization produced a study of cardiovascular diseases and resulting mortality in twenty-four countries. Following the observation of low death rates from these diseases in Latin America, a further study was begun with the intention of providing a comprehensive account of the causes of mortality of adults in widely separated populations. The final results of the survey have just been published by the Pan American Health Organization.

Eleven cities with annual death rates of about 2,000 and efficient death registration systems were selected for study. A twelfth, Ribeirão Prêto, with only 500–600 deaths per year, was included because of the high incidence of Chagas infection. The cities, each an established medical centre, were Bogotá and Cali (Colombia), Carácas (Venezuela), Guatemala City (Guatemala), La Plata (Argentina), Lima (Peru), Mexico City (Mexico), Ribeirão Prêto and São Paulo (Brazil) and Santiago (Chile). Bristol and San Francisco were included for comparison. The aim was to investigate 2,000 deaths in each city for each of two consecutive years, by means of a standard questionnaire.

In Latin American countries birth rates and child mortality rates are high, and death rates must be adjusted to take this into account. Up to the mid-forty age group mortality rates in Latin American cities are double those of Bristol, while in the rural areas of South America they are probably about three times the Bristol level. Thus in Guatemala the mortality rate of young adults is six times that in Britain (Bristol figures being close to the national average). Cities in South America are more favourable than the country, but the reverse is true in San Francisco. For the 35–54 age group, death rates for the city are above the national average.

Two diseases emerge as leading causes of death. Diseases of the heart were the leading cause for males in eight cities, and for females in three. Cancer led in three cities for men and in nine cities for women. Cirrhosis of the liver, chiefly caused by alcohol, caused most male deaths in Santiago. Chagas disease was responsible for a large number of deaths in Ribeirão Prêto but did not appear significantly in any other city. La Plata had the highest number of deaths from cancer for both sexes; Bristol, not far behind, had the same number of male lung cancer victims. The figure for male cancer deaths in Mexico City was about half that for the other cities, but the reason is not known. This city had the highest death rate from diabetes mellitus. In the respiratory diseases, death from bronchitis was

particularly common in Bristol. Motor accidents caused a significant number of all the deaths from external sources.

The survey concludes with possible numbers of deaths which could have been prevented. Males are more likely than females to die from alcoholism and external causes, and over half the deaths for men under 35 are thought to be preventable. For women under 35 the figure is just about half. In the 35–54 age group these figures fall to around 40 per cent for both sexes and in the 55–74 group they fall again to 20–30 per cent. A significant number of female deaths are attributable to pregnancy and childbirth, and, of these, abortions form a substantial, and preventable part. The situation in country districts of South America, where shortage of medical facilities is thought to be responsible for the high mortality rates of young adults, is one which can be changed.

Less Discrimination

RACIAL discrimination in Britain was described in a report sponsored by PEP (Political and Economic Planning) and published in April this year. Further legislation was thought to be necessary and the Race Relations Board has now sponsored a complementary report which would be a guide for Government action in extending the 1965 Race Relations Act. Professor Harry Street, Mr Geoffrey Howe and Mr Geoffrey Bindman have made a thorough investigation of anti-discrimination legislation in countries other than Britain, assessed its effectiveness and considered what types of legislation would be most suitable for Britain. Their findings (Anti-Discrimination Legislation, The Street Report) are published this week by PEP.

Much has been learned from legislation in the United States on discrimination which has often been too little and too late. To prevent inequality becoming entrenched in British society, the Street Report proposes widespread extension of the present act to cover all aspects of public life and a large proportion of private tracts, insurance and all services from shops and repairs to those of professional people should, it says, be added to the present list of public buildings, trans-Crown and government port and entertainment. employment should be included except where national security questions have to be considered. Small units of housing or employment where family relationships are involved are the only cases recommended as exceptions to the law. Owner occupied houses are thought to be such a fundamental part of family life that legislation against discrimination in sales of such properties is thought to be a political question, and the report, without a unanimous decision, hands the matter to the Government.

Discrimination on factors of race, colour and ethnic or national origin is the basis of the legislation, but religious discrimination should not be included. It is emphasized that legislation alone will not be enough. Wholehearted government backing must be given to ensure that the law is enforced efficiently, and that justice is seen to be done. Education programmes are essential to show the public the importance of an integrated society. The report suggests that the structure of the Race Relations Board and its local conciliation committees should be strengthened to put

these proposals into effect, and the powers of the board should be extended. The board should be able to issue subpoenas, but their orders should only be enforceable through the courts. Conciliation is the key word in discrimination proceedings, but a tribunal should be set up for cases where it fails. There should be no question of criminal penalties for unlawful discrimination, but failure to comply with decisions could result in proceedings for contempt of court.

Heat and Sound

PROFESSOR EIGEN, who shares the Nobel Prize for Chemistry, has worked closely in collaboration with L. deMaeyer at Gottingen and has introduced and developed a technique for following the kinetics of very rapid reactions in solution and has worked on both the theory and practice of reactions ranging from 0·1 sec, down to almost the limit of chemical reactions.

The principal difficulty in studying fast reactions is bringing the reactants together in a sufficiently short time. That is, the mixing time must be less than the reaction time. Professor Eigen's solution is to take a system in equilibrium and to subject it to a disturbance in the external parameter such as temperature, pressure or electric field strength. changes the position of the equilibrium but sometimes the position is not readjusted as soon as the external conditions have been changed: that is, there is a The displacement from equilibrium is time lag. measured spectrophotometrically or by conductivity. It is a first order process and the reciprocal of the rate constant is the relaxation time. The latter is a composite function of the rate constants of the elementary steps occurring in the reacting system. By analysing chemical relaxation (the difference between "internal" and "external" conditions) under different conditions, intimate details of chemical reactions in the system can be studied.

There are two particular techniques. is a stationary method of analysis using, for example. ultrasonics: the second is a transient method using temperature jump. The latter is the more versatile and best developed but it does not measure the fastest techniques. Chemical relaxation is important because it has opened up a vast new range of chemical reactions for kinetic studies. It was originally used for very fast inorganic studies such as proton transfer and metal complex formation in water. In the last few years, however, attention has been focused on reactions of biological importance; for example, enzyme-substrate reactions, muscle contraction and protein synthesis. It can also be used to follow DNA replication reactions—one base pair replication of DNA must occur at least every 10-3 sec. Eigen's main interest now lies in this biophysical field and he is to have the use of a new Max-Planck institute which will be opened in three years.

Flashing Lights

THE award of a half-share in the Nobel Prize for Chemistry to Professors R. G. W. Norrish and George Porter is reckoned to be a fitting recognition of twenty years of careful work by two talented experimenters. Professor Norrish retired two years ago as professor of physical chemistry at Cambridge, but remains actively

at work at the laboratories. Professor Porter was a research student at Cambridge after the Second World War, and it was there that he and Professor Norrish began work on the development of flash photolysis as a technique for the study of extremely fast chemical reactions, usually by the intermediary of unstable molecular species. In 1955 he moved to Sheffield as professor of chemistry and then, a year ago, to the Royal Institution as director.

It is probably fair to say that Nobel Prizes are of two kinds-those awarded for discoveries of an immediate character, and those awarded for the opening up of a new field of investigation, which usually entails the development of a new technique. This is a Nobel Prize of the second kind, and is in this sense comparable to the award of Nobel Prizes in the past for the development of techniques such as the use of photographic emulsions for the measurement of cosmic rays and the study of nuclear reactions or for the application of X-ray diffraction analysis to the definition of tertiary structure in proteins. Not merely is flash photolysis as such a difficult technique, but its full value is only realizable now that there is a sufficient body of information available for the interpretation of results to be fairly straightforward and unambiguous. What Professors Norrish and Porter have done may well be described as the founding of a novel kind of chemistry.

Over the years, the duration of the pulses of light energy used for the irradiation of a sample of material has decreased from a millisecond to a microsecond, and the arrival of lasers has made it likely that there will now be a further compression of the time-scale. At the same time, energy output has steadily increased, with the result that 1,000 Joules may be fed through a light source in one pulse and that chemistry laboratories are made to seem even more like thermonuclear research establishments, with banks of capacitors accumulating charge. Perhaps the most tangible benefit so far is that it is now possible to observe directly the properties of some of the unstable molecular species which are assumed to play a part in chemical reactions. Now that Professor Porter has turned his interest to the study of flash photolysis in liquids and solutions, the harvest of innovation will no doubt be even more diverse.

Nuclear Reactions

Professor Hans Bethe, who is awarded the Nobel Prize for Physics for 1967, is one of the most productive of Grand Old Men. He has been a prolific source of innovation in theoretical physics since the early thirties, and although the Nobel Committee has singled out for special mention his identification (in 1938) of the nuclear reactions most probably responsible for the output of stellar energy, this represents only a very small part of his output.

Professor Bethe, who is 61, left Germany for the United States in 1935, and was by then distinguished for his versatility. His review articles on the theory of metals and of nuclear reactions—the latter published in 1937—are models of their kind (and still topical enough to be re-edited and republished). His first pieces of theoretical physics were concerned with the motion of electrons in solids, and were carried out in collaboration with Professor A. Sommerfeld. In the

early thirties he made a monumental contribution (with Heitler) by calculating the electrodynamics of electron-positron pair production. His contributions to nuclear physics proper began in 1934 with some calculations of nucleon-nucleon scattering, and by 1936 he was deeply involved in the scattering of nuclear particles from multi-nucleon nuclei. Bethe was one of the first to make calculations with the meson field as a means of mediating nuclear interactions.

Bethe's paper on the thermonuclear energy of stars may seem almost to be a by-product of the remainder of his activity. By the late thirties, the problem was to identify which nuclear reactions could realistically be supposed to contribute to the energy production in Bethe picked out two possible sequences of reactions by which hydrogen may be converted into helium—that which involves the successive additions of protons to a proton to form helium-3 followed by the interaction of two helium-3 nuclei to form one of helium-4, and that which involves a shuttling of protons between the isotopes of carbon and nitrogen. At the time it seemed as if the carbon-nitrogen cycle would be the chief source of thermonuclear energy in stars. Now it is more probable that the direct sequence of events is the more important.

Bethe has been a public figure as well as a physicist. After his stint at Los Alamos, he returned to Cornell University and has since been unafraid to involve himself in the Oppenheimer hearing (where, it is true, he had no option) and in public discussion about such issues as the feasibility of technical methods of disarmament—the detection of underground explosions, for example.

Striking a Balance

SCIENCE and technology have not yet swept the board in the schools with innovations of the curriculum. Members of the Group for Educational Services in Museums have now launched a service designed to bring young people to a closer understanding of their place in history.

The service will function in several ways. First, it will provide facilities for teachers and students to make use of collections in museums. These will cover art, geography, history and natural history. Second, it will organize regular loan distribution of museum objects for schools. Finally, the service will organize miscellaneous activities such as lectures and clubs. Two patterns of organization are in operation. One is based on county boroughs or borough museums administered by a committee of the authority and financed by grants from the local education authorities. The other, the county service, may or may not be based on a county museum, but is financed chiefly or wholly by the county education committee. Sometimes a service may also have an advisory committee consisting of teachers or schools inspectors. All the services are in the charge of a school officer who is responsible to the chief officer of the museum or education committee for detailed administration of the departments.

Organization will depend to a large extent on the particular area. Thus rural areas will have a greater need of a loan service than will better equipped urban areas, and the cost will be higher. The character of an area will also be reflected in the line of study: for

example, near the coast studies may be expected to be predominantly marine. The size of area covered by one service will vary, but there is inevitably an optimum above which it will be necessary to modify the sims of a service to produce maximum efficiency.

The scheme is already established in three places—Bristol, Leicester and Derbyshire. Staff will be expected to have the usual teaching qualifications but in addition may have to study for the Museums Association diploma. Teachers who question the necessity of the scheme are reminded by the Museum Group that the experience gained by a child in becoming directly acquainted with an object is the chief justification for the time and money spent. The group is particularly emphatic in its belief that museum collections can help children to see their environment in a clearer perspective of time.

Traffic in Towns

PROFESSOR R. J. SMEED, first occupant of the new chair of traffic studies at University College London, took the opportunity in his inaugural address last week to describe the academic and practical merits of his young subject. About 8 per cent of all male deaths occur on the roads, and 16 per cent of the national income is spent on road transport. The problems are worth investigating. By discussing one aspect of the subject, traffic congestion in town centres, Professor Smeed was able to illustrate his point.

Formulae have been devised to relate the variables of traffic movement, so that calculations can be made of such things as speeds, journey times, and road space requirements. As traffic increases, each extra car adds more congestion than the last. Under light traffic conditions a one mile journey could take 2.7 minutes, while at 50 per cent of maximum traffic the same journey could take 3.7 minutes and at 98 per cent of the maximum it could take 20 minutes. There is, however, a limit to congestion, because people will not spend more than a certain amount of time in a traffic jam, but will change their transport, work or home to avoid Motorways would increase traffic flow more efficiently than an equivalent ordinary road, and better junctions would give dramatic increases in town road capacity. Ring roads improve the conditions inside towns, but produce intersection difficulties and lengthen journey distances. An average bus carries the equivalent of six car loads, but sending all commuters by bus instead of allowing cars would only improve the journey time of the original bus users, not those of everyone.

The problem of congestion is a simple one—too many vehicles—but the most obvious suggestion of more road space would not solve it, because more people would immediately take to their cars and return the congestion to its former levels. Since the amount of traffic in Britain is likely to double in the next 10-20 years, the road capacity to get traffic moving fast would have to be vastly increased. It is calculated that capacity required in London would be 20 times its present level, an unlikely amount, even with redesigned vehicles which will travel closer together. Congestion seems to be the inevitable result of car-owning commuters. The only cure at present suggested is to remove people, home, work and all, to less crowded places, or else charge them prohibitive amounts for entering any central zone.

Kind Words for Concrete

THE annual report of the Cement and Concrete Association for 1966 makes one point clear—in the association's opinion, too few concrete roads are being built. Mr L. Russell, director-general of the association, points out that concrete is not necessarily more expensive than other forms of construction, even at first cost. Improvement in design and the use of modern equipment has made concrete fully competitive even without taking into account subsequent maintenance costs, as has been demonstrated by a number of recent Apart from direct cost comparisons, every motorist is aware that maintenance and repair work cannot be carried out without serious congestion and great risk of accident. It is becoming essential to place greater emphasis on lower maintenance characteristics in future road construction—and the association believes that the concrete road is immeasurably superior in this respect.

The Lofthouse report had suggested that the ministry might need to revise its present conceptions about the balance between different types of road construction and it considered that the maximum benefit would not be achieved if the proportion of concrete paving remained as small as it had been in recent years. The association looks to the ministry to ensure that concrete is given the opportunity to compete on level terms for all future road construction contracts.

In the report Mr Russell draws attention to developments in the field of skid resistance of concrete roads, in which the association has been collaborating closely with the Road Research Laboratory during the past three years. The type of texture required to achieve maximum skid resistance at high speeds has been established and also the best method of imparting the texture during construction. Tests have shown that, if properly applied, the texture will last for at least ten to fifteen years, after which economical methods of restoring the texture are available.

Fewer Locusts

DURING 1966 the Anti-Locust Research Centre expanded its activities, and because of continued recession in locust activity was able to turn its attention to other pests and a larger number of projects This follows the policy decided by the overseas. Advisory Committee on Anti-Locust Research at its 1965 meeting, with the accompanying proviso that at first pests should be studied to which anti-locust methods could be applied. The Ministry of Overseas Development appointed an Overseas Pest Control Research Committee to replace the Tropical Pesticides Research Committee and, by giving it wider terms of reference, put the ALRC under its wing. As a result the advisory committee, in operation since 1946. was no longer necessary and its activities were wound up in October 1966.

The annual report of the centre which has just been published (HMSO, 7s.) describes the position and frequency of nine species of locusts and grasshoppers. Research work at the centre covers various fields, ranging from taxonomy and insecticides to biogeographical analyses of locust occurrence and the application of meteorology to locust problems. Biological research included the setting up of cultures

of two agricultural pests other than locusts, these being cotton stainer (*Dysdercus intermedius*) and the army worm species *Prodenia litura*. Four new species of Orthoptera were also added to the cultures. Morphometry, behaviour and maturation studies of locusts were continued, together with the physiology of feeding receptors. Chemical attractants in grasses were studied in collaboration with the Tropical Products Institute.

During the year the field research section was working in three continents, and the ALRC continued to increase its international co-operation. Four long-term projects were initiated, studying various aspects of locust populations in the Niger Republic, Saudi Arabia and Australia, and investigating the army worm in eastern Africa. Records for the desert locust were brought up to date by the publication of a further 522 reports. Annual expenditure for the centre during 1966 was £150,000.

Australian Fisheries

Tuna tagging is not the only pursuit of the CSIRO Division of Fisheries and Oceanography. According to two important schemes initiated this year, laboratories are to be constructed, one in Western Australia and one in Queensland, to house teams engaged in co-operative research programmes. Each will be financed by the government concerned. The Western Australian laboratory will be built at Perth to study in particular the physiology and behaviour of crayfish. The Queensland government has agreed to build a laboratory near Brisbane and the research team will undertake a general study of the biology of the main prawn species in southern Queensland waters. As in the case of the Western Australian crayfish, physiological and ecological research will be aimed at improving techniques of study of natural populations and to investigate possibilities of resource manipulation.

Several other research programmes sponsored by the division are in progress. For example, an atlas is being prepared of water conditions around Australia using nearly 20,000 observations of surface temperature and salinity. Work has continued on the processing and interpretation of accumulated data on tuna and Australian salmon, and data are being collected on the development and distribution of species of crayfish, prawns and bottom organisms. An investigation of the occurrence of sperm whales off Western Australia has shown that there has been an overall decrease of between 20 and 40 per cent since 1962. Five cruises were made by oceanographers during the year and deep sea investigations in conjunction with onshore laboratory studies of various aspects of oceanographic methodology were continued. New work in biochemistry, hydrology physics and physical chemistry was undertaken to supplement existing information and the amount of dissolved organic matter produced by phytoplankton during photosynthesis was measured during a cruise in the eastern Indian Ocean in June

The division received assistance from many sources during the year: ships collected oceanographic information, fishermen tagged fish and canneries provided information on tuna, salmon and crayfish measurements.

Parliament in Britain

Cervical Cytology

SINCE 1964 the rate at which cervical cytology smears are being examined has increased by a factor of three. Mr Julian Snow, Parliamentary Secretary at the Ministry of Health, gave the figures in reply to a question in the House of Commons. In June 1967 the laboratories responsible for examining smears were doing so at the rate of 19,000 a year. Mr Kenneth Robinson, Minister of Health, said that he was considering the possibility of extending the service to women under 35. (Written answer, October 23.)

Medical Schools

MRS SHIRLEY WILLIAMS, Minister of State at the Department of Education and Science, confirmed that a new medical school would be established at the University of Southampton. When this and other projects were complete, the number of places for medical students in the United Kingdom would have been increased by 650, she said. (Written answer, October 23.)

Concord

No further increases in the cost of the Concord supersonic airliner were admitted by Mr John Stonehouse, Minister of State at the Ministry of Technology, when he answered a question on October 24. The cost would be £500 million at 1966 prices, and another £100 million might be needed in the seventies to cover costs in advance of receipts. Will Concord make a profit? Mr Stonehouse was not to be drawn—"The return on Government expenditure on Concord will depend on the success in selling the aircraft." (Written answer, October 24.)

Aldabra

No decision has yet been made whether or not to use the island of Aldabra for defence purposes. This was virtually the only hard fact which emerged from the reply by Mr Merlyn Rees, Under Secretary of State for Defence for the Royal Air Force, to an adjournment debate opened by Mr Tam Dalyell. Mr Dalyell had started by suggesting that a Select Committee on Defence should be established to examine questions like these, and that the first witness should be Sir Solly Zuckerman. Mr Dalyell doubted the figure of £20 million which had been quoted as the cost of establishing a base on Aldabra—he thought the cost could well be as much as £100 million by the end of the day. The Royal Society, he said, was convinced that any development of Aldabra would inevitably destroy the greater part of its unique biological features. In any case, he doubted the strategic necessity for the base, or the staging post, whichever it was to be.

Mr Rees replied briefly, without committing the Government to an attitude towards the island. But he did admit that a number of other islands had been considered—Farquhar, the Cosmoledos, Assumption, Astove, St. Pierre, Mahé and so on. Mr Rees said that it had been decided that it would be possible to make a change to the East Channel without damming it, and this, he hoped, would lessen the effect on the ecology of the island if Aldabra were indeed selected. He refused to comment on the suggestion that it would be necessary to build a wall around the airfield to keep out the giant tortoises. As for the frigate birds, the hazard they present to aircraft would be one of the things considered before a decision was reached.

NEWS AND VIEWS

Artificial Intelligence

THE Science Research Council has done well to make a grant of £221,000 to the Department of Machine Intelligence and Perception at the University of Edinburgh to support the work now planned there for the next five years. For one thing, it is imaginative of the council to have backed so generously a group of talented people who have chosen to band together to work in a new and difficult field, often in the process turning their backs on comfortable and established reputations in other fields. It is also potentially a great benefit that on this occasion the Science Research Council should have been prepared to support the rapid growth of what cannot fail to be a commanding centre of excellence, as the phrase now goes, at least for several years ahead. It is true, of course, that the University of Edinburgh will be the first university in Britain to be equipped with a multiple-access computer, and that such a device is almost as important to those who work in artificial intelligence as is a radio-telescope to radioastronomers, but considerations like these are notand have not always been in the past-sufficient reasons for avoiding a wasteful dispersal of resources. (It is worth asking, in passing, when some other British university will have access to a multiple-access computer.) But the most important cause for pleasure with what the Science Research Council has done is that the field on which the group at Edinburgh is embarked has the most exciting promise. Enough has already been done at other British laboratories—the National Physical Laboratory, for example—to demonstrate the great interest of attempts to use computers for tasks other than wooden and repetitive arithmetic. In the United States there has been quite a boom in artificial intelligence for some years now. A good deal of what is spent on these activities is justified by their potential practical importance, in applications such as character recognition, for example. More immediately, however, there is the real prospect that everybody will be stimulated and improved by work of the kind now being undertaken.

Variable X-ray Star

THERE has been wide interest this week in the discovery of a markedly variable X-ray star in rocket flights from Woomera in Australia. According to newspaper reports, Professor K. G. McCracken of the University of Adelaide and Dr A. G. Fenton of the University of Tasmania have announced the disappearance of an X-ray star which was a bright object of its kind when first discovered earlier this year. Three separate groups have been concerned with the description of the event. By means of launchings of Skylark rockets from Woomera on April 4 and April 20 last year, the Australian group was able to single out the existence of a bright source of X-rays in Centaurus (declination 60° south, right ascension 13 hours 20 minutes). A British rocket flight from Woomera on April 10 seems to have taken place by good luck more or less at the peak of X-ray activity. By the end of May, when an American rocket was launched from Hawaii, however, the intensity of the X-ray source had fallen by a factor of six. Now, it seems, there has been a further reduction by a factor of ten.

The importance of this discovery lies in the unmistakable character of the variation of X-ray output from the star. Hitherto it has been hard to ensure that measured variations of output from X-ray stars could accurately be attributed to inherent variation. Friedman's group at the US Naval Research Laboratory has, however, reported a fourfold decrease of the intensity of the Cygnus source called R1 in a period of just over a year, and there are other reports of similar variability in other stars. Friedman and his colleagues suggest, for example, that this source and that known as XR2 are both markedly variable, with periods of the order of a month. So far, however, it has not been possible unambiguously to follow the earlier X-ray stars through a complete cycle of variation. Friedman and

his colleagues follow Shklovsky in the view that many X-ray sources may be double star systems in which a compact neutron star is the chief source of radiation and in which there is an eclipsing star which can periodically interrupt the passage of X-rays. Elsewhere—as among the group associated with the British experiments at the University of Leicester—the theory that X-ray stars are novae has firm supporters.

More News from Venus

THERE is now some confusion about the interpretation of the reports that the Russian spacecraft Venus IV demonstrated the absence of nitrogen from the atmosphere of the planet. In Moscow on October 30, Academician Keldysch described some of the preliminary results of the experiment, and confirmed that the devices with which the spacecraft was provided for the analysis of atmospheric gases on Venus included analysers for carbon dioxide, water, oxygen and nitrogen. He did not, however, confirm earlier reports that nitrogen was found to be entirely absent. It also became plain that the atmosphere of Venus was sampled at two points only, at heights corresponding to pressures of 520 mm and 1,500 mm. The pressure at the surface of the planet was estimated to be within 2 atmospheres of 20 atmospheres, and the pressure is still one atmosphere at a height of 26 kilometres. In the circumstances, it will not be surprising if the lower layers of the atmosphere of Venus are eventually found to have a somewhat different composition from that now measured directly.

Measurements of water and oxygen in the atmospheric gases were carried out with simple absorbers of phosphorus pentoxide and phosphorus, respectively. The accuracy of the carbon dioxide measurement is

given as ten per cent. The concentration of oxygen appears to have been within 0.4 and 0.8 per cent, and the total of water vapour and oxygen was given as 1.5 per cent. At the levels in the atmosphere at which these analyses were carried out, the temperatures were comparatively high-40° C at 520 mm and 80° C at 1.500 mm.

According to Academician Keldysch, there is no doubt that the Russian spacecraft actually reached the surface of Venus. Heights above the surface were determined by means of radio altimeters, probably at a speed (calculated from the Doppler shift) of three metres per second. At various stages during the descent, the deceleration of the instrument capsule was as great as 300g. According to the same account by Professor Keldysch, there is no ionosphere worth talking of on Venus—the concentration of positive ions never exceeded 1,000 particles per cubic centimetre. magnetic field, measured with a directional magnetometer, is less than three ten thousandths of the dipole magnetic field of the Earth. It also appears that the spacecraft used a resistance thermometer for the measurement of temperature and an aneroid barometer for the measurement of pressure.

Automatic Pipettes

A "Which" type of investigation has been used to assess thirty-five automatic dispensing pipettes that are at present available in Britain. Results of the assessment—carried out by Mr P. M. G. Broughton of the University of Leeds, Mr A. H. Gowenlock of the University of Manchester, Mr G. M. Widdowson of the Queen Elizabeth Hospital, Birmingham, and Mr K. A. Ahlquist of the Christie Hospital and Holt Radium Institute, Manchester-appear in the third scientific report of the Association of Clinical Biochemists. Each instrument was tested in at least one of four laboratories and its ease and speed of use, accuracy and precision were assessed.

The instruments tested were of two types, those with a fixed volume determined by the manufacturer and those with an adjustable volume. Instruments were further divided into four groups. Group A included instruments based on a pipette, group \vec{B} consisted of syringe pipettes, group \vec{C} contained double action pipettes and group D contained electrically operated automatic pipettes. Instruments based on a pipette were found to be fragile and the rate of delivery was no faster than with conventional glass pipettes. Syringe pipettes, on the other hand, are said to be faster, more adaptable and more accurate than conventional ones, but many were fragile, expensive and difficult to use. Double action pipettes were fairly fast and usually precise but some were fragile and expensive. Electrically operated pipettes in group D were considerably more expensive and seemed best suited to mechanized or semi-automatic mechanized systems.

Of the thirty-five instruments, nine had faults when received or developed faults during use. Only six were considered sufficiently precise and easy to use. These were the "Zipette", "Quickfit Dispenser", Schuco Automatic Pipette (adjustable model), "Autospenser", "Volusence" and "Auto-Dispenser". Prices were, respectively: £4 9s. 6d. (10 ml. size), £6 15s. (10 ml.), £13 (10 ml.), £25 (5 ml.), £34 7s. (10 ml.) and £36 (10 ml.). In view of these unsatisfactory results, it is

hardly surprising that only three instruments were specifically guaranteed by the manufacturers.

Russian View of Phylogeny

by Mary Lindley

Professor A. L. Takhtajan of the Komarov Botanical Institute of the USSR Academy of Sciences was formally admitted a foreign member of the Linnean Society at the meeting on October 26. Professor Takhtajan, a distinguished taxonomist, then delivered a communication on classification and phylogeny with

special reference to the flowering plants.

He began by putting systematics in its place at the centre of biology; it is not an auxiliary discipline devoted to producing a catalogue of living organisms, but a synthetic science capable of drawing all biological knowledge into an ordered system. Considering classification, Professor Takhtajan said that there are two types—general purpose and special purpose—and only the former is needed today. To be useful, a general purpose classification must convey as much information as possible about the organisms it classifies. Because the phyletic system can convey so much information about the flowering plants and their relationships, upon which it depends, this system will be much more valuable than any others.

In a group lke the flowering plants, with such an inadequate fossil record, a complete phylogeny such as there is for man and the horse cannot be possible. This is α-taxonomy, when the fossil record shows clearly what evolutionary changes were involved in the development of contemporary species. (Professor Takhtajan was not optimistic about the suggestion that a useful ancient angiosperm might be found in Lower Cretaceous deposits.) Phylogenetic relationships, however, can be worked out within taxa, based on the relative primitiveness and advancement of the plants concerned. This a-taxonomy, based on information from comparative morphology, cytology, seriology and now to some extent on DNA hybridization studies, reveals horizontal relationships between members of groups. From these horizontal relationships it may be possible to deduce vertical relationships so that hypothetical lineages can be constructed. As more botanical knowledge is made available more of these relationships will be revealed.

Professor Takhtajan discussed some of the problems involved in finding phylogenetic relationships. Parallelism and convergence may have allowed common characters to evolve in distantly related organisms; in the first case from similar origins, and in the second case from different origins, both as a result of adaptation to similar environmental conditions. should not cause much difficulty as long as there are sufficient independent characters. Another problem arises when a taxon contains members with characters at different stages of evolution, because different characters have evolved at different rates or have reverted to a more primitive condition. This phenomenon, called heterobatmy by Professor Takhtajan, is expressed to a greater extent in primitive taxa such as the Magnoliaceae and Winteraceae. In such cases it is usually impossible to produce a phyletic series. In spite of these difficulties, Professor Takhtajan feels that as more botanical knowledge becomes available—only comparatively few groups have been studied sufficiently

yet—an α -phylogeny will be possible for the flowering plants.

Commonwealth Veterinary Association

from a Correspondent

REPRESENTATIVES from Gambia, Ghana, India, Kenya, Lesotho, Malta, Malaysia, Nigeria, Pakistan and Uganda were able to attend the 85th Congress of the British Veterinary Association held at Southport during the week commencing September 17, thanks to grants from the Commonwealth Foundation. These representatives met others from Australia, Canada, Malawi, New Zealand and the United Kingdom. Sir Dawda Jawara, Prime Minister of the Gambia, and himself a veterinary surgeon, chaired six meetings held during congress week, at which the possible formation of a Commonwealth Veterinary Association was discussed.

All the delegates were in favour of establishing the association, particularly in view of the world shortage of protein. In 1962, the Food and Agriculture Organization estimated that in the United Kingdom, the United States and France alone some £1,500,000,000 worth of food was destroyed as a result of animal disease. That was 15 per cent of the total production of food. In the less developed countries the figure rose to something like 30 per cent. It was felt that reducing this loss might be even more important to mankind than the direct work on human health, and all delegates agreed that the veterinary profession could help, through the Commonwealth Foundation, in improving human health and nutrition both by a direct contribution to food production and by the control of zoonotic diseases

As a result of the six meetings held during the week, a draft constitution was agreed unanimously. Two of its aims are to promote the closest possible links between member associations within the Commonwealth and to facilitate the dissemination of professional and scientific knowledge and information. The representatives of the sixteen countries agreed to recommend the draft constitution to all Commonwealth countries, including those not represented at Southport. They agreed also that a Commonwealth Veterinary Association should be set up as soon as possible, and that it should have a permanent central secretariat established in a Commonwealth member country.

Whatever the future of a Commonwealth Veterinary Association may be, there can be no doubt that the current series of meetings were pervaded by the spirit of goodwill and co-operation which has been characteristic of Commonwealth meetings and did much to increase the understanding of problems of animal health and disease throughout the Commonwealth.

New Place for Plants

from a Correspondent

Two new plant houses at the Royal Botanic Garden, Edinburgh, were officially opened by HRH Princess Margaret on Wednesday, October 25. The buildings, designed by the Ministry of Public Building and Works, have a unique structure with no internal framework. The glass is suspended from an intricate external array of steel tubes and cable. The design frees the interior

from any supporting framework and minimizes the corrosion risk that is common to ferrous metals in plant houses.

The scheme, which cost £263,000, consists of a new exhibition plant house and an exhibition orchid house. The plant house, 420 ft. by 60 ft., is divided by glass screens into five compartments. Each has a different environment with temperature controlled by fully automatic heating and ventilating equipment. The different sections will be occupied by cacti and succulent plants, warm temperate aquatic plants, temperate plants, tropical aquatic plants, and temperate ferns. Planting has been designed to achieve a natural landscaping effect, and wherever possible has a geo-graphical basis. The plant collections are intended to give as representative a picture as possible of the groups concerned, and will be supplemented with specimens of botanical interest collected by staff on expeditions abroad. An observation window, built below water level in the tropical aquatic plant pool, allows visitors to study the underwater parts of plants. The orchid house, 100 ft. by 50 ft., connects with the main new exhibition house and the existing palm house. The new buildings replace dilapidated glasshouses that were built at the beginning of the century. The temperate palm house and the 140-year-old tropical palm house are being renovated and retained.

The Royal Botanic Garden, Edinburgh, founded in 1670, is now a department of the Ministry of Public Building and Works. It covers 75 acres and is visited annually by more than half a million people. Research in the taxonomy of both flowering and non-flowering plants is the primary task of the institution. Studies in the laboratory at present include cytotaxonomy, anatomy, palynology, developmental morphology and various aspects of the experimental taxonomy of fungi.

Localization of Enzymes

from our Cell Biology Correspondent

Analyses of isolated cell organelles have shown that the intracellular distribution of many enzymes is very restricted. Indeed, the enzyme content of organelles is so characteristic that the presence of a particular enzyme is often used as a marker during the isolation of them. How is such precise localization of enzymes determined? Does it arise simply because of the structure of the enzyme molecule or are there specific localizing factors? In 1961, Paigen discovered that a mutation in the structural gene of β glucuronidase, an enzyme which is localized in lysosomes and the endoplasmic reticulum in mouse liver cells, alters the distribution of the enzyme between the two sites.

It is not therefore surprising that the structure of an enzyme can affect its intracellular localization, but this is not the complete explanation. Ganschow and Paigen ($Proc.\ US\ Nat.\ Acad.\ Sci., 58, 938; 1967$) have now discovered a nuclear gene which controls the localization of β glucuronidase in the endoplasmic reticulum. They find that in liver cells of mice, strain YBR, glucuronidase is present in the lysosomes but not in the microsomes (isolated endoplasmic reticulum) and, as far as they could tell, this was the only significant difference between YBR microsomes and those of other strains. A series of genetic crosses and back

crosses with YBR and another strain CH3/HeHa proved that the structural gene of glucuronidase in YBR was not mutated and showed that YBR lacks a specific factor essential for the insertion of the enzyme into the endoplasmic reticulum. This factor is also required for the insertion of glucuronidase in C3H/HeHa and the gene which specifies it, the E gene, is in a different linkage group to the glucuronidase structural gene.

This is the first proof that a nuclear gene can specifically regulate the intracellular localization of an enzyme. This factor could work either by altering the structure of native glucuronidase so that it can fit into the reticulum or, more probably, by providing a specific binding site for the enzyme; it may even be a structural component of the reticulum membrane. If so, the implication is that other enzymes associated with the reticulum are localized by similar nuclear genes and the localization of enzymes in other organelles may also be genetically controlled. Certainly this would explain the highly specific distributions found in practice.

In mitochondria, where the correct assembly of enzymes is critical for co-ordinated function, Woodward and Munkres (1966) found evidence that the precise conformation of malic dehydrogenase, which is specified by a nuclear gene, is determined by interaction with mitochondrial structural protein, which is specified by the organelles DNA. Specification of enzyme binding sites could be one of the major functions of plastid DNA, whereas in organelles which do not contain DNA this could be effected by nuclear genes like the E gene discovered by Ganschow and Paigen.

Plant Virus in Vectors

from our Microbiology Correspondent

Since 1960 several research groups have demonstrated by electron microscopy the presence of insect-borne plant viruses within their insect vectors. Dr Karl Maramorosch and his colleagues Hirumi and Granados have concentrated their efforts on the wound tumour virus (WTV) and studied its distribution and multiplication in leafhoppers. The location of WTV in a relatively efficient vector, Agallia constricta, and in an inefficient vector, Agalliopsis novella, is described in two recent publications (J. Virol., 1, 430; 1967; and J. Invert. Pathol., 9, 147; 1967), and the observations made are related to the transmitting efficiencies of the leafhoppers. In the inefficient vector, wound tumour virions were distributed systemically in the midgut, musculature, blood, fat bodies, tracheoblasts and ventral ganglia. This is the first direct evidence of plant viruses invading the nervous tissues of insect vectors and corroborates the earlier antigenic analyses of Sinha (Virology, 26, 673; 1965). However, Sinha's experiments did not distinguish between complete viruses, sub-viral constituents and soluble viral anti-

The accumulation of WTV in all organs of A. novella was slight and, significantly, the low titre or complete absence of virions in the salivary glands suggests an immediate reason why this species is a poor vector for this virus. Although systemic infection of these leafhoppers by WTV is clearly indicated, the establishment of a disease condition has not been verified. The authors rightly point out that the killing of

occasional vectors could easily be missed and suggest that it is improbable that such systemic viraemia could remain completely harmless. Invasion of the insect nervous system was examined fully in A. constricta, the more efficient transmitter of the disease. Three types of viral formation occurred in nervous tissues: isolated virions (V₁) found predominantly in the ganglion cells; tubular formations (V2) in which single files of virions lie within membranous envelopes; and aggregate formations (V3) bounded by limiting membranes. These latter inclusion bodies were distributed mainly in the glial cells, perineurium and tracheo-blasts. Wound tumour virus produces an intense cytopathogenic effect on nerve cells and, most notably, the cytoplasm of degenerating ganglion cells was converted into an electron dense matrix containing fibrous material and ribosomes. These matrix areas surrounded by WTV particles closely resemble those in situ aggregations of pox-viruses, reo-viruses and polio-viruses which are thought to represent centres of virus synthesis. A reasonable inference from the present study is that the V₁-type virions associated with polysomes are early stages of virus development and that the dense matrix areas indicate active sites of WTV multiplication. In contrast, the V₃-aggregations which are rarely associated with the matrix areas or polysomes are considered to denote virus storage. significance of the tubular symmetry in virus development is not known. They have been detected before in non-nervous tissues of the vectors and in host plants while similar structures are common to other insectborne plant virus systems. Maramorosch's group are at present using leafhopper culture cells to pursue this problem further and their future reports will be awaited with interest.

Hydroxyproline in Cell Walls

from a Correspondent in Cell Biology

Hydroxyproline, an imino-acid which is found in only a few proteins such as animal collagen, also occurs in a protein of plant cell walls. This protein, sometimes called extensin, is linked to the cellulose microfibrils of the wall. One of its properties is to increase the tensile strength of the wall but it also seems to be associated with cell growth. Free hydroxyproline inhibits cell growth at 1 mM, and even does so in the presence of indole acetic acid (IAA)—a plant hormone, which greatly stimulates cell enlargement. A great deal of work published this year attempts to pin-point the site of hydroxyproline inhibition in the hope that this will give a clue to the mechanism of cell growth regulation.

IAA is thought to enhance cell growth through the stimulation of RNA and protein synthesis. Cleland (Plant Physiol., 42, 1165; 1967), using oat seedling coleoptiles, has shown that free hydroxyproline does not affect these two processes. Instead, he proposes that hydroxyproline blocks the utilization of a particular protein rich in hydroxyproline, possibly by feedback inhibition, needed for wall growth. Earlier, Cleland had shown (Plant Physiol., 42, 271; 1967) that the effect of hydroxyproline, on growth stimulated by IAA is not immediate. This suggests that a pool of this protein is present and is used up before inhibition takes place. Experiments with cycloheximide and p-fluorophenylalanine suggest that the protein is being synthes-

ized in cells capable of enlargement even before they are stimulated to greater enlargement by IAA. However, hydroxyproline had no effect on the subsequent ability of the cells to enlarge.

There may be a further mechanism of hydroxyproline inhibition. Using sycamore callus tissue, Holleman (Proc. US Nat. Acad. Sci., 57, 50; 1967) showed that the hydroxyproline in the wall protein arose from hydroxylation of previously incorporated proline (as in collagen). In the presence of an inhibitor of hydroxylation (aa'-dipyridyl) hydroxyproline added to the cells was still incorporated into the cell wall protein. Holleman suggests that this incorporated hydroxyproline has forced its way into the protein which is then rendered non-functional, and in this way causes the cell growth inhibition. But what is the function of this protein? Although it is implicated with cell enlargement, Cleland and Karlsnes (*Plant Physiol.*, 42, 669; 1967) also implicate it with the cessation of enlargement. Their conclusion is based on the finding that in cell walls of growing pea epicotyls, the greatest increase in the protein which contains hydroxyproline occurs when cell elongation has finished. The two systems may not be incompatible but certainly there are no clear-cut answers yet. More data are needed on the types of protein required for cell growth and on the mechanism by which IAA influences these, and also how this hydroxyproline-containing protein fits into the cell wall during enlargement.

This protein, extensin, has often been likened to collagen and indeed collagen has now been reported by De and Ghosh (*Exp. Cell Res.*, 47, 637; 1967) to be present in plant cells. But surprisingly it is in the nucleus.

Sulphur in RNA

from our Molecular Biology Correspondent

The discovery of sulphydryl groups in transfer RNA has increased the number and variety of "unusual" bases recognized in these species. Their function, though the subject of some slightly remote speculation, is unknown. The very unexpectedness of the thiolated bases and their chemical reactivity, which offers the possibility of generating covalent cross-links, makes them, however, a source of considerable interest.

There appear to be several such bases, but one of them, 4-thiouracil, is the most prevalent. In total tRNA from E. coli there appear to be rather fewer than two sulphur atoms per molecule. Lipsett has now shown (J. Biol. Chem., 242, 4067; 1967) that, as expected, mild oxidation converts the thiol groups into disulphide bonds, and from a complete nuclease digest of tRNA a 4-thioUMP disulphide has been isolated. No mixed disulphides with any other thiolated nucleotides are found. In a further study Lipsett and Doctor (ibid., 4072) have examined a single tRNA, with a greater than average thiol content. This is E. coli tyrosine tRNA—one of the easier species to prepare, since it appears at one end of the countercurrent distribution profile. Titrations with thiol reagents show that two sulphydryl groups are present, and both turn out to be 4-thioUMP. On reduction, a disulphide bridge is formed as before, and it is surprising to find that the sedimentation coefficient is not increased. It follows that the disulphide bridge is intramolecular. This is a useful (as well as an unexpected)

result, for it indicates that none of the dimeric, and other aggregated, species which have been observed by other workers are likely to involve intermolecular disulphide bonds.

This internal disulphide formation is accompanied by a small but significant increase in absorbance and a decrease in thermal stability. It therefore seems likely that the new bond is achieved at the expense of some base-pairing. At the same time the amino-acid acceptor activity survives intact, despite the evident disturbance of the conformation. (It has not so far been established whether other functions, such as the capacity to attach to the ribosome, are extirpated).

The 4-thioUMP has an absorption band at about 330 m μ , which is therefore resolved from the main nucleotide absorption centred at 260 m μ . Although its concentration is low, and the absorption therefore weak, there is clear indication of hypochromicity in this band, which supports the view that the 4-thioUMP forms part of the double-helical structure. After oxidation the effect is lost, although a shifted long-wavelength absorption can still be seen.

That the 4-thiouracil can indeed pair with adenine in a helical structure has just been demonstrated by Scheit (Biochim. Biophys. Acta, 145, 535, 1967), who has prepared copolymers of UMP and 4-thioUMP, and complexed them with poly A. Hypochromism is observed both at 260 and 330 mµ—where the thionucleotide absorbs. Only three-stranded complexes appear to be formed at room temperature, but temperature-absorbance profiles show two phases, which by analogy with the intensively studied poly (A+2U)system suggests that in a certain temperature range the two-stranded form is stable. It is clear, however, that the A-thioU pairing is much weaker than A-U; this is consistent with the observations of Lipsett and Doctor on the apparent readiness with which basepairs involving 4-thioUMP are broken to allow the formation of internal disulphide bonds.

One would hesitate at this juncture to hazard any guesses at the possible purpose of thionucleotides in in tRNA. It is nevertheless perhaps interesting to store the information alongside the recently demonstrated effects of sulphydryl-blocking reagents on the function of *E. coli* ribosomes (see, for example, Traut and Haenni, *Europ. J. Biochem.*, 2, 64; 1967).

They Wobble

from our Cell Biology Correspondent

In the latest two papers from Khorana's laboratory, Söll, Cherayil and Bock, and Söll and RajBhandry (J. Mol. Biol., 29, 97 and 113; 1967), summarize their results on the coding specificities of yeast and E. coli tRNA. Their data are in striking agreement with Crick's (1966) Wobble hypothesis.

Because the genetic code is highly degenerate, Crick predicted that one species of $t{\rm RNA}$ can recognize more than one codon. An unambiguous demonstration of this requires homogeneous $t{\rm RNA}$ and a technique to study interactions. But now between codons and anti-codons $t{\rm RNA}$ species can be isolated in a sufficiently pure form to allow meaningful experiments, and Nirenberg and Leder (1964) have found that the interaction can be detected by binding $t{\rm RNA}$ to ribosomes programmed with tri-ribonucleotides (single codons). The synthesis, in Khorana's laboratory, of

ribopolynucleotides of known base sequence has provided another method, more reliable, for the determination of codon specificities, for it makes it possible to find what amino-acids are incorporated into a cell free system programmed with polymers of known sequence.

Söll et al. deduce three general principles of tRNA specificity from their binding experiments. First, if two codons specifying one amino-acid begin with different bases, they are recognized by two tRNAs. Second, one species of tRNA can only recognize three codons if these have the first two bases in common and U, C or A as the third base (for these pair with inosine in the anticodon); two codons are recognized by one tRNA only if the first two bases are the same and the the third either A or G or U or C, but not if one codon ends in a purine and the other a pyrmidine. Third, tRNAs specific for only one codon always recognize codons ending in G. These results agree with the Wobble hypothesis that there should be strict Watson-Crick pairing between the first two bases of the codon and anticodon, and that in the third there may be pairing between bases not included in the strict Watson-Crick prescription-G with U, for example. Wobble theory also predicts that anticodons which recognize three codons contain inosine. In support of this, Söll et al. find that of the tRNA species for thirteen amino-acids tested, three from yeast but only one from E. coli recognize three codons and Hall (1963) reported yeast tRNA is richer in inosine than tRNA from E. coli.

Formyl methionine tRNA which recognizes GUG and AUG codons at chain initiation is the sole exception to these rules. So either two species of Fmet tRNA exist, one for each codon, which have not yet been separated or, because Fmet tRNA binds to the peptidyl site in ribosomes, instead of the amino-acyl site different Wobble rules apply. (Wobble of the first base pair instead of the third, for example.)

In the second paper, Söll and RajBhandry report experiments with ribopolynucleotides which verify the coding specificities of seven species of tRNA determined by binding experiments and provide even more convincing evidence for multiple codon recognition and wobble. Yeast phenylalanine tRNA translated both UUU and UUC. Wobble theory predicts an anticodon GAA, not AAA, for this molecule. The primary sequence of yeast phenylalanine tRNA does not contain the sequence AAA but does contain the sequence 2'0-methyl GAA which must be the anticodon (RajBhandry et al., Proc. US Nat. Acad. Sci., 57, 751; 1967). As predicted by Wobble theory, G in the third anticodon position must pair with C or U in the third position in the codon because both UUU and UUC are translated.

Both binding and incorporation experiments also indicate the redundancy of several tRNA species; one codon is recognized by two different tRNAs. Such redundancy provides safeguards against mutation and offers a neat explanation of the origin of suppressor tRNA

Theoretical Astronomy at Cambridge

by F. L. WEST WATER



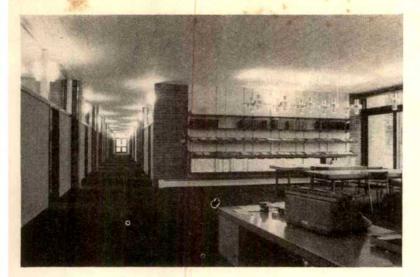
The Institute of Theoretical Astronomy at Cambridge will be formally opened next Tuesday by Sir Isaac Wolfson

THE institute under its director, Fred Hoyle, is equipped with its own computer and should not only provide a focus for theoretical astronomy in Great Britain but is certain to attract visits from other workers in the United States and elsewhere.

Although the need for such an institute was recognized as far back as 1959, and Government support would almost certainly have been made available, progress was seriously hampered because of disagreement over the location of the institute. Hoyle has always supported Cambridge, not because of his personal association with the university but more because he felt that the strong mathematics faculty with its great traditions would always attract as students the brightest young mathematicians in the country. Furthermore, the

amenities and reputation of Cambridge provide an inducement for visitors from abroad. There were others, however, who felt that one of the new universities should be chosen and there was also at this time a lack of enthusiasm for the idea in Cambridge itself.

In 1964, thanks to the interest taken by Lord Todd and the late Sir John Cockcroft, offers of financial help were promised by the Nuffield Foundation and the Wolfson Foundation. Further support from the Government was provided by the Science Research Council and agreement was reached that the institute should be at Cambridge on the understanding that the university also lent its support. In the event, the Wolfson Foundation has provided the buildings at a cost of about £180,000, and finance to run and equip the institute for five years has been provided by contributions of £250,000 from the Nuffield Foundation, £25,000 from the SRC and £125,000 from the University of Cambridge. The intention is that the bulk of the SRC contribution should be devoted to paying for the installation of the computer—an IBM



360/44. The remainder of the grant will suffice to pay for routine maintenance and single-shift operation for the period of five years.

The SRC has raised no objection in principle to the computer being used by other scientists in Cambridge, provided the needs of the institute are first fully met, but the additional costs involved must, of course, be provided by the users. One of the organizations to take up this offer is the Microbiological Unit of the Medical Research Council, which has been promised 1,000 hours a year for two years. Other users include the Cavendish Laboratory, the Chemical Laboratory and the

Department of Geophysics.

Discussions on the relationship of the institute with the university and the various faculties were protracted, but it was finally agreed that the institute should not be attached to any faculty and should come under the supervision of the general board of the university. For this reason, the institute staff is not permitted to give undergraduate lectures unless specially requested. spite of this restriction—or, as some may think, advantage—it is the wish of the university that other astronomers in Cambridge should be associated with the institute as closely as possible. General control of the institute is vested in a committee of management on which the Wolfson Foundation, the Nuffield Foundation, the Science Research Council and various university faculties are represented.

It will be for the institute to demonstrate its value during the five year period, after which new arrangements will have to be made. One must hope that it will be possible for the university, through the University Grants Com-

mittee, to continue the project.

The final administrative details were completed in the late summer of 1966 and the institute was established by statute on August 1. A site next to the existing observatories on the Madingley Road was obtained and work started in August 1966. The computer block was completed in February 1967 and the main institute buildings were ready for occupation in August 1967-surely a remarkable achievement. Present research staff numbers twelve, but this is likely to be increased to twenty within the next 2 years. All are post-doctoral appointments and are held for 3 years. A limited number of research students up to a maximum of sixteen can be accepted.

It is clear that a research institute in such a subject as theoretical astronomy cannot function properly without an interchange of ideas with workers in other countries and for this reason provision has been made to enable members of the staff to carry out visits to other centres. Finance is also available to invite and pay a number of visiting fellows from other countries, for periods varying from a few weeks to a year. The high degree of collaboration which already exists between Hoyle and the staff of the California Institute of Technology has been a considerable asset, and during the past summer, even before the institute was complete, five visiting fellows from California spent most of the summer in Cambridge.

With the completion of the buildings and the computer now working in two shifts, the activities of the institute can be expected to build up steadily during the current academic year. A series of graduate lectures has been arranged for the present session and these are being well supported. Contact with the undergraduate body is being maintained by a series of lectures given by Hoyle who, of course, retains his position as Plumian professor of astronomy and experimental philosophy in the university. It is hoped that it will be possible to arrange one or possibly two Foundation Lectures each year to be given by distinguished workers in astronomy or allied fields. Consideration is also being given to holding symposia on special subjects to which experts will be invited at the institute's expense. Plans are also being prepared to enable the institute to play its part when the International Astronomical Union visits England in 1970.

Technology in Scotland

by NIGEL HAWKES

Is there a technological revival in Scotland? And will it bring prosperity?

For almost forty years, Scotland languished as a technological backwater. Ironically, it was probably the industrial success of the nineteenth century which set the scene for the disasters of the twentieth. Until the end of the First World War, Scotland was unsurpassed as a centre of engineering excellence, and for a time imperial expansion was a guarantee of new markets. After the war, things began to go wrong; the old industries-coal, engineering and shipbuilding-found that their products were harder to sell. The new industries were all in England. As a result, the depression hit Scotland particularly hard. During the thirties, unemployment never fell below 11 per cent, and at its worst reached an unprecedented 34 per cent. Graduates from Scottish universities either went on the dole or moved to England for work. The situation graphically demonstrates what can happen when industry becomes detached from technological progress. Since the Second World War, the situation has begun to right itself; or, to be more accurate, the Scots themselves have set about righting it. Much of the credit for this must go to the Scottish Council, the first regional development council, which has originated many of the policies now followed by other councils in other development areas. The council has been aided by the policies of central government, which has encouraged the settlement of new industries in development areas by investment grants, and sometimes by the less subtle expedient of refusing to let companies expand where they were. Mistakes have undoubtedly been made, but the result of these policies has been a good one, and the scientific climate in Scotland is now in many ways more encouraging than that in England.

The greatest success story of all has been the establishment in Scotland of a substantial electronics industry. The major element in this is the Ferranti Company, which has invested £3 million in developments in Scotland, and employs 5,000 people there. Ferranti first went to Scotland in 1943 and established a factory for the manufacture of gunsights. Since the war, expansion of Ferranti activities has been continuous, and the Scottish group includes several research laboratories, an important element in industrial development. The progress of Ferranti has encouraged other electronic companies to come to Scotland, including two companies from the United States. An even more encouraging sign is the development of small companies spawned by the presence of the others, and a conscious effort is being made to encourage an atmosphere like that around the technological centres in the United States. At the department of engineering at Edinburgh University, a new postgraduate retraining course in micro-electronics begins this week. Intended for designers in industry whose training finished before the new micro-electronic techniques were invented. the course is staffed from industry, and consists of six three-week courses separated from each other by five week breaks in which the designers being retrained will return to their firms. Thus, although the course lasts 11 months, engineers need be away from their firms for only 18 weeks. Although the course deserves to be oversubscribed, so far industry is treating it gingerly. The university, at least, cannot be accused of dragging its heels—the plan was put before the university court in March this year, and by the beginning of November the buildings were up, in part at least, and ready for their

Another industry of some growth potential is the supply of desalination equipment. Weir Westgarth, of Glasgow, has so far supplied half the world's capacity in large scale desalination equipment. Recently the company opened a new research and development centre at Troon, where desalination techniques, particularly flash distillation, will be studied. Flash distillation is a simple process; brine, pre-heated by low grade steam, passes through a series of chambers at decreasing pressure, so that it boils in each one. In each chamber the pure water boiling off is collected on condenser tubes. Essentially the problem is to improve the heat transfer and the efficiency of the process, and much of the work at Troon will attempt to achieve this. Other methods of desalination may also be tried, although there is only one—reverse osmosis-which seems to offer a serious rival to flash distillation. One of the company's minor difficulties is that of enforcing its patents in the United States—a problem shared, incidentally, by a number of British and Continental companies. Patent litigation takes so long to come to court in the US, and the results are so unpredictable, that British companies are often happy if they can negotiate offset agreements allowing them to use US patents in exchange for their own. Recently Weir Westgarth has become involved with the Atomic Energy Authority in some joint studies of combinations of desalination plant

with nuclear power stations. The danger of this approach is that prospective customers will come to feel that they can only buy a desalination plant if they are also prepared to fork out for an advanced gas cooled reactor at the same time; a complete misconception, as Weir Westgarth is at pains to point out.

Not all Scotland's industry looks as well placed as this. Privately, staff from the Scottish Council will agree that it was a mistake to force British motor companies to expand in Scotland; the motor component industry, which it was hoped would follow BMC and Rootes to Scotland, has not appeared. Shipbuilding is another headache, made worse by the reluctance of the large shipyards to take on anything but full-blooded ships. If more of the yards were ready to build large engineering constructions, like the oil rigs which some are already building, they would find it easier to get work. John Browns, which is building the Queen Elizabeth II, is said to be particularly stiff-necked. And coal mining, of course, is bound to decline even further as the prospects of getting another coal fired generating station in Scotland fade. But the Scottish Council is making sure that Scotland's claim for one of the new aluminium smelters does not go unheard; the preferred site is at Invergordon.

All this development needs better communications between Scotland and the rest of the world. One of the crucial factors in the developments of the last ten years has been the arrival of the airlines, or more precisely the airline, since British European Airways has a monopoly on many of the routes. In Scotland as elsewhere it is easy to find people who believe that the BEA monopoly is wrong, and that competition would help both to bring down prices and to make seats on the aircraft easier to come by. At the moment it is often necessary to book several weeks in advance for flights to London, which business men find very inconvenient. For all this, there is no doubt that BEA has transformed life in the Highlands and the Islands, which are now within ready reach of Glasgow and Edinburgh.

In science, Scotland has potentially the greatest growth point in the UK. Aberdeen could well become the British centre for oceanography and marine science. Already it has the Torry Research Station, the Marine Biological Laboratory, and a Unilever laboratory which is investigating methods of farming fish. On the agricultural side, Aberdeen boasts the Rowett Research Institute and the Macauley Soil Institute. This is a concentration of excellence which it would be hard to equal anywhere else, and the staff of the laboratories are full of ideas for improving Scotland's fisheries and farms. One of these is the hope of developing new fisheries on the west coast of Scotland, hitherto unfished because there is no port of sufficient size. There are apparently substantial amounts of valuable shellfish simply waiting for exploitation, and the first step would be to establish a factory for deepfreezing the fish before transport to the south. Unilever is experimenting with the cultivation of lobsters and prawns, because expensive fish offer the best chance of commercial success in fish farming.

All this makes a pleasing contrast with the condition of Scotland between the Wars. But in future the job is likely to be even harder—for one thing, the rest of the United Kingdom is going to have to woo advanced industry in the way demonstrated by Scotland, which is bound to make the Scottish problem worse. in Scotland is that the process has now gone far enough to be a self-generating one, but this may be over-optimistic. Undeniably, though, some of the Scottish Council's statistics are impressive. In 1959, only one in sixteen of the workers in Britain in high technology industries worked in Scotland; now the figure is one in eight. Scotland's exports are up by 50 per cent in the five years from 1961. Even the squeeze seems to have been less severe over the border—unemployment is up by 50 per cent, but in England the increase has been 84 per cent.

first students.

Underwater Vision of the Sea Otter

by R. L. GENTRY R. S. PETERSON Division of Natural Sciences, University of California, Santa Cruz

A sea otter, Enhydra lutris, was trained to select the larger of two disks simultaneously presented under water, and its ability to discriminate between disks of nearly the same size was tested. results provide an index of visual acuity, and suggest that Enhydra has slightly poorer underwater vision than Zalophus or Phoca.

As the ancestors of modern aquatic mammals moved from land to water, adaptation of sensory organs may have been critical. Variations in the underwater effectiveness of visual systems may therefore accurately suggest the degree of adaptedness of different species to the aquatic

Schusterman et al.1 measured the ability of California sea lions (Zalophus californianus) to discriminate visually under water between disks of different sizes, and Feinstein and Rice2 used the same technique to test harbour seals (Phoca vitulina). Both species could make very fine size discriminations under water, and their underwater visual acuity must be excellent. This finding is consistent with

their high degree of aquatic adaptation.

In lacking pectoral limbs modified for swimming, and in lacking a streamlined body form, Enhydra seems less adapted to the open sea than do pinnipeds (for discussion compare ref. 3). Kenyon showed that sea otters are usually found in the littoral zone, although they make short trips on to land and to the open sea4. Otters and pinnipeds are morphologically and behaviourally dissimilar, so that sensory differences may be expected. We have tried to reveal existing differences in underwater visual discrimination between Enhydra, Zalophus and Phoca.

We tested the only sea otter known to us to be in captivity, a male at Point Defiance Aquarium, Tacoma, Washington. Captured in Alaska 14 months before testing, the animal was between 4 and 5 yr old and weighed 75 lb. Its daily food ration, 15 lb. of fish and squid, was maintained during testing, and was dispensed in three portions each day during the experimental procedures. Training and testing from March 13-26, 1967, were carried out in the $2 \times 4 \times 8$ ft. (0.6×1.8) × 2.6 m) wooden tank in which the animal had been held since capture.

We used a test apparatus (Fig. 1) similar to that used for testing the underwater vision of Zalophus and Phoca^{1,2}. This device presented two targets 20 in. (50 cm) apart separated by an 18 in. (45 cm) divider. For test targets we used a size graded series of nine white painted metal disks of the following sizes: 67, 86, 96, 101, 108, 114, 122, 137 and 174 cm2. After 4 h of training, the animal would dive, swim the length of the tank under water, and strike a training target (the 108 cm2 or standard target of the series) to receive a reward of a 10 g piece

To determine whether the subject had a pre-existing preference when faced with two targets of different sizes, we simultaneously presented the largest and smallest targets in the series for thirty-five trials, rewarding responses to either target. The animal selected the larger target in twenty-one of the trials, suggesting a slight preference. In all subsequent experimentation we rewarded responses to the larger target only. To establish this response firmly, we then ran 360 additional presentations. At the end of these training trials, performance had reached virtually 100 per cent correct and testing was initiated.

The method of constant stimuli (ref. 5, page 118) was used in these tests. Each of the eight targets in the series was paired with the standard (108 cm2) target for ten trials in each test session. The position of the larger target was alternated randomly using a standard Gellerman series and the sequence of the target pairs was also randomized from one session to the next. The subject received usually three sessions of eighty trials each day during three days of testing.

We estimated the discrimination ability of the otter by the percentage of correct responses it made in ninety trials with each of eight pairs of targets (Fig. 2 and

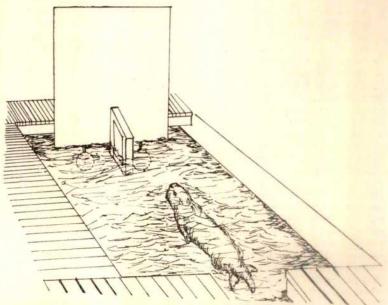
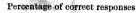


Fig. 1. Sea otter making an underwater approach to targets suspended from the test apparatus.



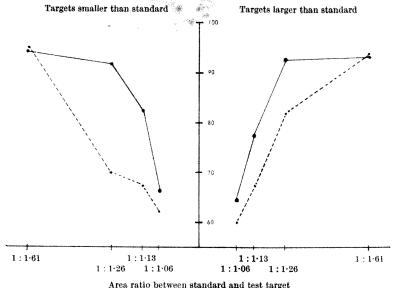


Fig. 2. Comparative performance of Zalophus (——) and Enhydra (---) on a size discrimination task.

Table 1). When two very different targets were presented (area ratio of 1:1.61), the animal selected the larger target about 95 per cent of the time. When the two targets were nearly identical (area ratio of 1:1.06), the percentage of correct responses was approximately 60 per cent.

Table 1. SIZE DISCRIMINATION TESTS ON SEA OTTER

Ratios between areas of targets	No. of sessions of ten trials	Mean performance level	Standard deviation
(Targets smaller tha	n standard)		
1:1.61	9	9.5	0.498
1:1.26	9	7:0	1.764
1:1.13	9	6.7	1.397
1:1.06	9	6-2	1.812
(Targets larger tha	n standard)		
1:1.06	8	6-0	1.323
1:1.13	9	6.7	1.315
1:1.26	9	8.4	0.956
1:1-61	9	9.4	1.066

The table shows relative performance levels at different sessions.

Data given by Schusterman et al.1 for two sea lions show mean levels of performance similar to those of the sea otter at very high and very low area ratios. The performance levels of the two species diverge at intermediate ratios, however. The amount of divergence can be measured by finding the 75 per cent performance level, a commonly used approximation of the threshold at which discrimination ability becomes useful to an animal. At that level, Zalophus seemed able to make finer discriminations in area than Enhydra.

The data also suggest that the otter performed better when comparing targets with a large absolute size than when comparing those of smaller size, despite the fact that area ratios were exactly the same (see particularly the two points for area ratio 1:1.26, Fig. 2). It is possible that absolute size, as well as area ratio, may be involved in the otter's ability to make discriminations in the size range tested, although data on Zalophus and Phoca indicate no such dependence.

The optics of the eyes of amphibious mammals are poorly understood. The morphology of the eye of Zalophus and Phoca is quite different from that of otters. The pinniped eye functions normally (is emmetropic) under water and possesses some special adaptations for aerial vision. Its lens, adapted for use under water, is

not used in air, but instead the iris is closed to a tiny aperture which serves as a "pin hole lens". Conversely, the eye of the otter (Lutra) is emmetropic in air and has some special adaptations for vision under water. While submerged, well developed ciliary and sphineter muscles in the otter's eye strongly distort the anterior part of the air adapted lens, giving it a focal length similar to that of a spherical lens. It would not be surprising, then, to find that Zalophus and Phoca can see somewhat better under water than can Enhydra, as our data suggest, because the lens of the pinniped eye appears highly specialized to function under water, while that of the otter is amphibiously adapted.

It is possible that retention of sharp air vision has been ecologically more important to Enhydra than the evolution of adaptations for acute underwater vision. For example, terrestrial predators might have been more significant in the ecology of this littoral zone inhabitant than for a more pelagic species. Field studies have shown that Zalophus is slow to respond to threatening visual stimuli, such as an approaching man, in air6. R. J. Schusterman (personal communication), however, has

tested the ability of this species to make size discriminations in air, and found it very similar to the underwater performance. We could not test aerial vision on the Tacoma sea otter, unfortunately; the animal did not respond when the targets were presented above the surface of the water.

If strong accommodation accounts for the otter's underwater visual abilities, we wondered whether a typical land mammal, such as man, could accommodate as well over short distances while submerged. We repeated the test procedures using two students as test subjects. When the pairs of disks of most nearly the same size (area ratio of 1:1.06) were presented, the humans achieved performance levels of approximately 80 per cent in forty trials at a distance of 3 ft. This was a decrease of 20 per cent from their performance in air. These results cannot be compared directly with results of tests on marine mammals because of testing differences such as the longer response latency of humans. trials show, however, that land mammals can probably accommodate as well at short underwater distances as sea otters can. The tests also show that comparison of disks for approximating visual acuity can reveal acuity differences of the magnitude of those experienced by a human entering water from air. This method is therefore useful for finding visual differences among some aquatic But when the differences in acuity become relatively small, as between Zalophus, Phoca and Enhydra, more sensitive measurements (such as Landolt's broken ring test) seem warranted.

We thank the US Fish and Wildlife Service and the Tacoma Zoological Society for permission to study their captive otter and for their assistance. We thank Čecil A. Brosseau, Dr M. L. Johnson, Karl W. Kenyon and Dr Victor B. Scheffer for their help. Ronald J. Schusterman lent the targets, and Victor Downing and Thomas Snyder were the human test subjects.

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Geomagnetic Reversals and Pleistocene Chronology

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Lamont Geological Observatory, Columbia University, New York The palaeontology and palaeomagnetic stratigraphy of several Atlantic and Pacific cores has been determined to establish the age relationship of various palaeontological boundaries which have been used to define a Pliocene—Pleistocene boundary in deep sea sediments.

STUDENTS of the Quaternary have turned to the slowly accumulating pelagic sediments of the deep sea in their quest for a complete and concise record of this latest chapter of Earth history, but, because foraminiferal oozes are deposited at a rate of about 2.5 cm/1,000 yr (according to radiocarbon dating), the cores which are 5–15 m long usually fail to penetrate completely the

pelagic Quaternary sequence. Because of post-depositional disturbances such as slumping, however, truncated and discontinuous early Quaternary and Tertiary sequences have been recovered in long cores.

Ericson, Ewing and Wollin^{1,2} constructed what they believed to be a complete Pleistocene stratigraphic section by piecing together and correlating overlapping sequences recorded in twenty-six cores. They based their reconstruction on climatic curves (determined from the relative number of Planktonic foraminifera from cold and warm water, Fig. 1) and changes in the direction of coiling of Globorotalia truncatulinoides (Fig. 2). In addition, they established the following faunal criteria for defining a Pliocene-Pleistocene boundary in deep sea sediments: (1) extinction of Discoasteridae, the organisms responsible for secreting the minute star-shaped objects called discoasters; (2) extinction of Globigerinoides sacculifera fistulosa above the boundary; (3) appearance of Globorotalia truncatulinoides in abundance above the boundary; (4) change in the direction of coiling of the members of the Globorotalia menardii complex from 95 per cent coiling to the right below to 95 per cent coiling to the left above the boundary; and (5) marked increase in size and wall thickness of the tests of members of the G. menardii complex above the boundary.

The thickness of the zone, within which these changes occur, differs somewhat in the various cores. In order to have a common level of reference in all the cores containing the boundary, Ericson et al. defined their boundary by the change in coiling direction of the G. menardii complex. By extrapolating beyond 175,000 yr they established a time scale for their Pleistocene stratigraphic section and arrived at an

age of 1.5×10^6 yr for their Pliocene-Pleistocene boundary (Fig. 1). They had, however, no method of determining the relationship of this boundary to faunal boundaries observed in Pacific and Antarctic sediments and they could not correlate their boundary with the officially adopted Pliocene-Pleistocene boundary³ at the base of the Calabrian in Italy.

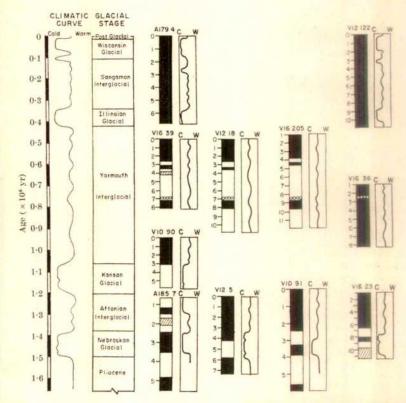


Fig. 1. The Pleistocene stratigraphic section of Ericson et al. showing ten of the twenty-six cores originally used by them. The generalized climatic curve on the left is based on variations in abundance of various temperature-sensitive planktonic foraminifera. The correlation between this curve and continental glaciation is also shown. The time scale, based on sedimentation rates determined from radiocarbon and protactinium-lonlum measurements and extrapolated down through their Pleistocene section, is shown to the extreme left. The correlation between the cores and the climatic curve is indicated by the position of the cores in the figure. The extinction of Globorotalia sp. 1, according to Ericson et al., is indicated by a horizontal line of open circles across cores V16-39, V12-18. V16-205 and V16-36. The palaeomagnetic stratigraphy of each core, according to this paper, is designated by black (normal) and white (reversed).

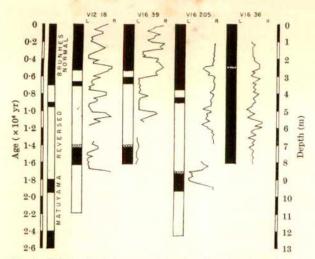


Fig. 2. Relationship between variations in coiling direction of Globorotalia truncatulinoides² and magnetic stratigraphy. The relationship between changes in coiling direction of G. truncatulinoides and the magnetic stratigraphy shows almost perfect correlation between cores V12-18 and V16-39. Correlation between these two cores and V16-205 is fairly good. There is, however, no correlation between these three cores and V16-36, indicating that it is misplaced in the Pleistocene stratigraphic section of Ericson et al. In addition, the horizontal line of open circles indicates the last occurrence of Globorotalia sp. 1. In V12-18, V16-39 and V16-205, this species becomes extinct immediately above the Olduvai event. In core V16-36, however, it disappears in the middle of a long normal interval, again indicating that this core is misplaced in the stratigraphic section of Ericson et al.

Palaeomagnetic studies of volcanic rocks on land have established and dated by potassium—argon a series of reversals of polarity of the Earth's magnetic field^{4,5}. It is known that the Earth's field has had its present or normal polarity for the last 700,000 yr and that for approximately 1·8×10⁶ yr before that it had an opposite or reversed polarity, except for two periods of short duration (<200,000 yr). The reversed and normal intervals of long duration are called epochs and the shorter intervals are called magnetic events (Fig. 3). It has recently been shown that the remanent magnetism of deep sea sediments is sufficiently strong and stable that these polarity reversals can be used to date and correlate

geological events recorded in these sediments throughout the world during the past four million years^{6,7}.

In order to date the Pliocene-Pleistocene boundary of Ericson et al. and to determine the relationship between their boundary and presumedly equivalent faunal boundaries in the Pacific and Antarctic Oceans, the palaeomagnetic stratigraphy of several cores from the stratigraphic section of Ericson et al. and several Pacific cores was determined.

In order to establish the magnetic stratigraphy, each core was sampled every 10–50 cm. The intensity, relative declination and inclination of each sample were measured on a 5 c/s spinner magnetometer⁸ after the sample had been partially demagnetized in an alternating field of 50–100 oersted in order to remove unstable components. Reversals are indicated by simultaneous changes in the sign of the inclination and 180° shifts in declination (Fig. 4).

We were able to determine the palaeomagnetic stratigraphy of thirteen of the thirty-four cores used by Ericson et al. to establish their Pleistocene stratigraphic section (Table 1, Figs. 1 and 3). The upper part of the sequence based on dozens of nearly identical climatic curves had been deposited entirely during the present period of normal polarity. The magnetic stratigraphies showed that several of the cores assigned to the earlier Pleistocene by Ericson et al. had been miscorrelated. Three of these, however (V12–18, V16–34 and V16–205), which they regarded as being of middle Pleistocene age, actually contain a reasonably clear and complete record of magnetic reversals and faunal changes during the past 2–2·5×10° yr.

Although most of the cores which contain their Pliocene-Pleistocene boundary show evidence of depositional hiatuses at or near the boundary, it is evident that sediment accumulation across the boundary was continuous in two—that is, cores A185-7 and V10-91—of the five in which meaningful magnetic measurements could be made.

By correlating the palaeontology with the magnetic stratigraphy (Fig. 5) we were able to date several palaeontological boundaries used by various authors to define a Pliocene-Pleistocene boundary in Atlantic and Pacific sediments. Discoasters and planktonic foraminifera,

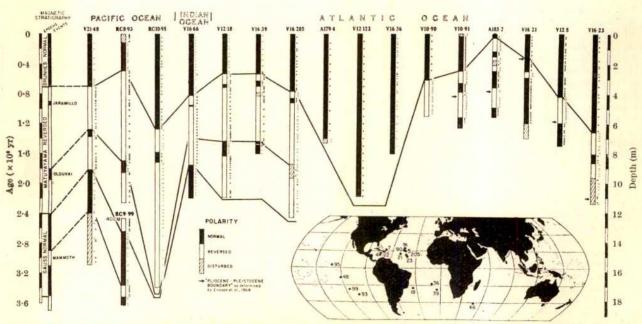


Fig. 3. Correlation of deep sea magnetic stratigraphy with magnetic stratigraphy dated on land by potassium-argon. Positive polarity is normal in the northern hemisphere and reversed in the southern hemisphere. Disturbed sections, resulting from coring, are often present at the top or bottom of a core.

			Table 1			THE WIND DOWNSON
Core	Lat.	Long.	Location	Depth (m)	Length (cm)	Sedimen- tation rate cm/ 1,000 yr
A179-4 A185-7 RC8-93 RC9-99 RC10-95	16° 36′ N. 21° 07′ N. 29° 22′ S. 24° 36′ S. 3° 31′ N.	74° 48′ W. 72° 51′ W. 105° 14′ W. 115° 27′ W. 129° 45′ W.	Caribbean North Atlantic S.E. Pacific S.E. Pacific Equatorial	2,965 2,470 3,157 2,570 4,473	690 555 1,131 610 1,705	~ $\frac{2 \cdot 4}{0 \cdot 7}$ + $\frac{0 \cdot 5}{0 \cdot 8}$ ~ $\frac{0 \cdot 7}{0 \cdot 8}$
V10-90 V10-91 V12-5 V12-18 V12-122 V16-21 V16-23 V16-36 V16-39	23° 02′ N. 23° 23′ N. 21° 12′ N. 28° 42′ S. 17° 00′ N. 17° 16′ N 13° 15′ N. 19° 22′ S. 24° 43′ S.	47° 03′ W. 46° 24′ W. 45° 21′ W. 34° 30′ W. 74° 24′ W. 48° 25′ W. 40° 40′ W. 11° 26′ W. 4° 45′ W.	Pacific North Atlantic North Atlantic North Atlantic South Atlantic Caribbean North Atlantic North Atlantic South Atlantic South Atlantic	4,030 3,540 3,000 2,935 2,730 3,795 4,885 3,485 4,510	580 630 735 1,080 1,090 702 1,140 830 802	~ 0.4 ~ 0.4 ~ 0.6 0.4 2.8* — 0.95 > 1.1 0.4
V16-36 V16-205 V21-48	42° 39′ S. 15° 24′ N. 9° 31′ S.	45° 40′ E. 43° 24′ W. 126° 22′ W.	S.W. Indian Ocean North Atlantic Equatorial Pacific	2,995 4,045 3,922	1,108 1,257 1,533	0-6 0-48 0-5

* Based on carbon-14 measurements from Ericson et al.*

† Based on the lengths of what we assume to be the Jaramillo and Olduvai
events.

Globigerinoides sacculifera, thought to define the Pliocene-Pleistocene boundary in Atlantic and Pacific sediments^{1,8}, became extinct at the Olduvai event (Fig. 5) about 1·8 × 10⁶ yr ago. (The occurrence of rare or scattered discoasters and G.s. fistulosa in younger sediments is probably the result of the reworking of old sediments into younger sediments by bottom currents and benthonic organisms.) In core V21-48 G.s. fistulosa first appears in sediments of the Gauss normal magnetic epoch and thus seems to have a range of about 1 × 10⁶ yr or less.

Globorotalia truncatulinoides first appears just below the Olduvai event both in Pacific and Atlantic cores. Banner and Blow¹⁰, working in the Calabrian type section in Italy, have shown that G. truncatulinoides evolved from G. tosaensis at the Pliocene-Pleistocene boundary. Thus for the first time deep sea sediments can be correlated with the type Pliocene-Pleistocene section in Italy. Because G. truncatulinoides first appears immediately below the Olduvai event, the Pliocene-Pleistocene boundary must have an age of about 2×10^6 yr.

Bed I in the Olduvai Gorge in Tanzania, which has been dated at 1.8×10^6 yr (ref. 11), contains upper Villafranchian fauna, which supports an age of about 2×10^6 yr for the Pliocene–Pleistocene boundary, because the Villafranchian is accepted as the continental equivalent of the Calabrian stage³. In addition, Selli¹² by extrapolation of sedimentation rates, has arrived at a date of 1.8×10^6 yr for the Pliocene–Pleistocene boundary in the Calabrian section in Italy.

The Globorotalia menardii complex changes coiling direction about midway between the Gauss-Matuyama boundary and the Olduvai event in Atlantic sediments, but in Pacific sediments (core RC9-99) this change occurs near the Mammoth event (Fig. 5). Thus the boundary of Ericson et al. has an age of about $2 \cdot 1 \times 10^6$ yr in Atlantic sediments.

Sphaeroidinella seminulina and S. subdehiscens last appear in abundance at the top of the Mammoth event in Pacific cores (V21-48 and RC9-99). Sphaeroidinella dehiscens makes its first appearance at this point and is abundant in most cores up to the Matuyama-Brunhes boundary where it becomes rare. This decrease in abundance seems to remain through the Brunhes normal epoch.

The extinction of the radiolarian Pterocanium prismatium, used to define a Pliocene-Pleistocene boundary

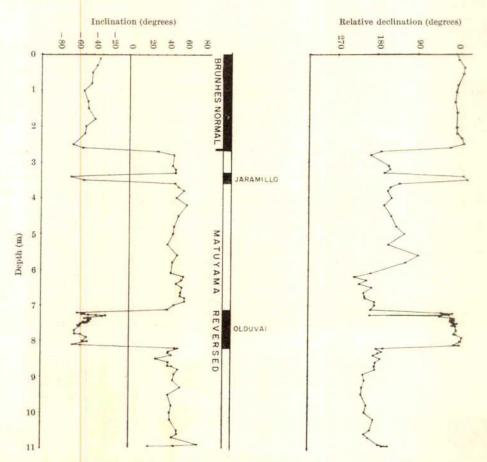


Fig. 4. Correlation between inclination, relative declination and magnetic stratigraphy in core V12-18. Magnetic reversals are indicated by change in sign of the inclination and 180° shifts in declination. Because the core is from the southern hemisphere, samples with a negative inclination are normal and samples with a positive inclination are reversed.

in Pacific sediments13, has been correlated with the last magnetic reversal14. The extinction of this radiolarian. however, has also been correlated with discoaster extinction13 and we have shown that discoasters became extinct during the Olduvai event. Thus it would seem that P. prismatium must have become extinct near the Olduvai event rather than at the Matuyama-Brunhes reversal. This conclusion is supported by core RC10-95 in which P. prismatium becomes extinct approximately simultaneously with discoasters and both extinctions seem to be close to the Olduvai event. (Although the magnetic data are not clear in the bottom of this core, extrapolation of sedimentation rate from the Matuyama-Brunhes reversal indicates that the bottom of the core is near the Olduvai event. This conclusion is supported by the presence of discoasters in abundance up to about

Core V16-205 contains all the faunal changes cited by Ericson et al. as occurring at or near their boundary. According to the magnetic reversals the evolutionary changes in the G. menardii complex took place about $2\cdot 1 \times 10^6$ yr ago. The first appearance of G. truncatulinoides in this core occurred 2.0 × 106 yr ago, and was followed by the extinction of G.s. fistulosa about 1.8×10^6 yr ago. The Discoasteridae seem to have died out between 2.0 and 1.8×10^6 yr ago.

Several of the palaeontological changes defining the boundary of Ericson et al. occur in core V12-18. These changes have the same relationship to the magnetic stratigraphy indicated in core V16-205. In addition, McIntyre¹⁵ finds a continuous evolutionary change in the coccolith assemblage between 700 and 850 cm in this core which he has also observed across the boundary of Ericson et al.1 in other cores.

Now that it is known where the Pliocene-Pleistocene boundary of Ericson et al. occurs with respect to the magnetic stratigraphy, several cores in which they identified their boundary can be examined.

In core V16-23 S. dehiscens is rare if not absent in the normal zone, in the upper half of the core, and abundant in the reversed zone below (Fig. 3), thus indicating that the normal zone of this core is the Brunhes normal epoch. The short normal zone at about 8 m is probably the Jaramillo event. The occurrence of the boundary of Ericson et al. at the bottom of this core must therefore be the result of a hiatus with about 650,000 yr missing.

Sphaeroidinella dehiscens is also rare in the normal zone at the top of core V10-91 and more abundant in the reversed zone below, indicating that the normal zone is the Brunhes normal. The normal zone at about 3 m might be either the Jaramillo or the Olduvai event, but the presence of discoasters in abundance part of the way through this zone indicates that it is probably the top of the Gauss normal. There is therefore a hiatus present in this core also, but it is probably above the Olduvai event. There is no evidence of discontinuity across the Pliocene-Pleistocene boundary of Ericson et al.

The complex magnetic stratigraphy of core A185-7 indicates several hiatuses and the palaeontology indicates some reworking. The short normal zone at the top of the core is probably Brunhes normal because S. dehiscens is rare if not absent in it and abundant in the reversed zone below. Discoasters extend up to just below the normal zone between 245 and 360 cm indicating that this normal zone is the Olduvai event. G. truncatulinoides, however, which is usually abundant down to the Olduvai event, only occurs in abundance down to about 150 cm, and is rarely observed down to where discoasters become extinct. S. semulina and S. subdehiscens are present up to a depth of 470 cm and S. dehiscens is present from there to the top of the core. This boundary occurs in the Gauss normal epoch in cores V21-48 and RC9-99 from the Pacific. There must therefore be a large hiatus in the bottom of this core. There must also be a hiatus between the Olduvai and Jaramillo events, and the abbreviated Brunhes normal section indicates a hiatus in the top of the core. The position of the boundary of Ericson et al. within the reversed zone and just below the Olduvai event, however, together with a layer by layer correlation of faunal changes in the boundary zone with the same faunal changes in core V10-91, indicates that the core is continuous across the boundary.

Core V16-21 is completely normal except for a short reversed zone between 250 and 285 cm. The decrease in abundance of S. dehiscens at 100 cm indicates that the top part of the core, at least above 100 cm, is the Brunhes normal. Because discoasters and G.s. fistulosa become extinct at about 150 cm and G. truncatulinoides first appears at about 150 cm, the boundary of Ericson et al. must represent a hiatus between Brunhes normal and Gauss normal sediments with the entire Matuyama section missing. The transition from S. subdehiscens to S. dehiscens at about 500 cm again indicates that the bottom part of the core is within Gauss normal sediments.

The decrease in abundance of S. dehiscens above 420 em in core V12-5 indicates that the normal zone at the top of the core is the Brunhes normal. The boundary of Ericson et al. occurs at a reversal which probably represents a hiatus with an incomplete Matuyama reversed

section lying on the top of the Gauss normal.

Three cores (V16-205, A185-7 and V10-91) contain a continuous record of faunal changes defining the Pliocene-Pleistocene boundary of Ericson et al. In cores A185-7 and V10-91, the time interval represented by this sequence is of the order of 0.2×10^6 yr. In core V16-205, however, it seems to be 0.3×10^6 yr, that is from 2.1 to 1.8×10^6 yr ago. The time interval, therefore, represented by the faunal changes defining the Pliocene-Pleistocene boundary of Ericson et al., is probably somewhere between 0.25 and 0.30×10^6 yr.

Hays¹⁶ defined a boundary, $\varphi - X$, in Antarctic cores based on the extinction of two radiolaria (Clathrocyclas bicornis and Eucrytidium calvertense) which he tentatively correlated with the boundary of Ericson et al. Opdyke et al.6 determined the palaeomagnetic stratigraphy of several Antarctic cores containing Hays' boundary and found that it occurs just below the Olduvai event about 2×10^6 yr ago. Core V16-66 from the Indian Ocean contains both the Pliocene-Pleistocene boundary of Ericson et al. and the $\varphi - X$ boundary of Hays. Because this core is from a high latitude, only two of the criteria for defining the boundary of Ericson et al. are present (extinction of discoasters and appearance of G. truncatulinoides). Both of these changes occur at approximately the same level as the extinction of C. bicornis and E. calvertense (Fig. 5) which defines the boundary of Hays.

Arrhenius¹⁷ defined a "Pliocene-Pleistocene boundary" in Pacific sediments on the basis of carbonate content. He placed his boundary at a level above which there was a marked increase in carbonate, on the supposition that abundant biogenous carbonate represented times of rapid oceanic circulation resulting from climatic deterioration. Riedel et al.13 found that discoasters and the radiolaria, Pterocanium prismatium and Eucrytidium elongatum peregrinum, became extinct in Pacific sediments approximately at the boundary of Arrhenius. We have shown that in the Pacific discoasters and P. prismatium became extinct at the Olduvai event. Thus the $\varphi - X$ boundary of Hays in Antarctic sediments and the Pliocene-Pleistocene boundary of Arrhenius based on carbonate content in Pacific sediments are approximately equivalent to the Pliocene-Pleistocene boundary of Ericson et al. in Atlantic cores.

The dates for the generalized climatic curve of Ericson et al. (Fig. 1) for the past 0.5×10^6 yr are assumed to be correct because the same sequence is found in several cores that have been dated by the radiocarbon², protactinium-ionium¹⁸, protactinium¹⁹ and thorium-230 (ref. 20) methods. This climatic curve is based on the variations in relative numbers of species of planktonic foraminifera the geographical ranges of which are known to be limited by

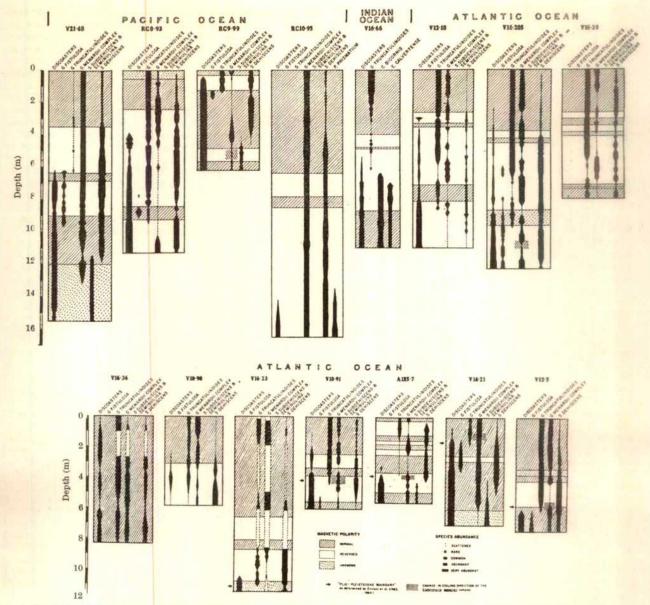


Fig. 5. Correlation between palaeontological changes and magnetic stratigraphy in ten Atlantic, one Indian Ocean and four Pacific cores. Where the biogenous component is not abundant the species abundance is shown by dashed lines.

temperature. Most of the cores in the remainder of their Pleistocene section are misplaced, however, and therefore their climatic curve below 0.5×10^6 yr is probably incorrect. We have shown, however, that three of the cores (V12–18, V16–39 and V16–205) from their Pleistocene stratigraphic section have an almost complete record of sedimentation for the past 2–2.4 × 10 6 yr. By correlating their climatic curves for these cores with the magnetic stratigraphy, a generalized climatic curve for the past 2.4 × 10 6 yr was obtained (Fig. 6). The climatic zones are designated by letters according to the system begun by Ericson et al. 21 .

Recorrelating the cores in accordance with the sequence of magnetic reversals increases the durations of the three earliest climatic events, Q, R and S. The S zone, considered by Ericson et al. to be equivalent to the Kansan Ice Age, now seems to include nearly as much time as the T zone, which Ericson et al. equated with the Yarmouth Interglacial.

Conolly and Ewing²² defined an ice-rafted boundary in Antarctic sediments which they believe indicates the initiation of glaciation in the southern hemisphere. The date of this boundary is 2.5×10^8 yr according to Opdyke et al.⁶ In addition the generalized climatic curve obtained from cores V12–18, V16–39 and V16–205 shows a cold zone extending back to 2.4×10^8 yr (Fig. 6). Thus, it seems that climatic deterioration began at least 500,000 years before the beginning of the Pleistocene.

Uffen²³ suggested that during reversals of the geomagnetic field the Earth's surface was subjected to an increase in cosmic-ray bombardment which caused a higher mutation rate which in turn affected evolution. The paper by Opdyke et al.⁶ showed that there was a striking correlation between radiolarian boundaries in Antarctic cores and geomagnetic reversals, thus giving some support to Uffen's hypothesis. It is obvious (Figs. 5 and 6) that several foraminiferal boundaries are also correlated with reversal boundaries, as well as the extinction of discoasters and Pterocanium prismatium, thus giving further support to Uffen's hypothesis.

In conclusion, we have shown that discoasters, G.s. fistulosa, Globorotalia sp. 1, and Pterocanium prismatium all became extinct near the top of the Olduvai event (1.8 × 106 yr ago). Sphaeroidinella dehiscens evolved from

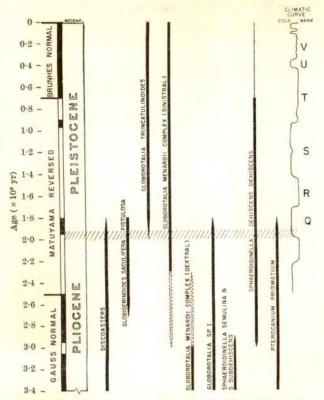


Fig. 6. Correlation of species range, climatic fluctuations and the Pliocene-Pleistocene boundary with magnetic reversals. The Pliocene-Pleistocene boundary based on the first appearance of G. truncatulinoides occurs at the base of the Olduvai event about 2-0×10° yr ago. Discoasters. G.s. fistulosa. Globorotalia sp. 1 and Pterocanium prismatium became extinct near the top of the Olduvai event (about 1-80×10° yr ago). Sphaeroidinella dehiscens evolved from S. subdehiscens at the Manmoth event (3×10° yr ago) and decreased in abundance at the Matuyama-Brunhes boundary. The generalized climatic curve is based on the interpretation of Ericson et al. and has been modified to accord with the sequence of geomagnetic reversals.

S. subdehiscens at the Mammoth event (about 3 × 10° yr ago) and decreased in abundance near the Matuyama-Brunhes boundary (Fig. 6).

The Pliocene-Pleistocene boundary of Ericson et al.1,2 defined by the change in coiling direction of the Globoro-

talia menardii complex occurred about 2·1×106 yr ago in the Atlantic Ocean, instead of 1.5 x 106 yr ago as suggested by Ericson et al., and it was followed by several other faunal changes which define a zone representing a time interval of about 0.25-0.3 × 106 yr.

The Pliocene-Pleistocene boundary, based on the first evolutionary appearance of Globorotalia truncatulinoides, occurs at the base of the Olduvai event about 2.0 × 106 yr ago (Fig. 6). A cold zone, however, in Atlantic cores extends back to 2.4 × 106 yr (Fig. 6) and ice-rafting recorded in Antarctic cores began at least 2.5 × 106 yr ago. Climatic deterioration therefore preceded the Pleistocene epoch by at least 500,000 yr.

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Plutonium-244 in the Early Solar System and Concordant Plutonium/Xenon and Iodine/Xenon Decay Intervals of Achondrites

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Concordant plutonium/xenon and iodine/xenon decay intervals have been obtained for a dozen meteorites, mostly achondrites. Plutonium-244 and iodine-129 abundances in the early solar system suggest that these extinct nuclides were synthesized in the galactic nucleosynthesis process, which lasted several billion years.

THE study of the extinct nuclides which existed in the early solar system is of great scientific interest, because knowledge of their relative abundances yields a crucial test for or against various theories concerning the galactic synthesis of the chemical elements, and because it enables us to establish a new dating method for the early history of the solar system.

Since the plutonium-244 hypothesis was first presented in 1960 (ref. 1), much experimental evidence in support of the theory has been obtained in this laboratory and elsewhere²⁻⁹. Kuroda et al.⁸ attempted to determine the initial plutonium-244/iodine-129 ratio at the time of cessation of galactic nucleosynthesis assuming that the plutonium/xenon and iodine/xenon decay intervals for several achondrites are concordant at about 300×10^6 yr. Two difficulties arose in this work: (a) some of the achondrites seemed to yield concordant plutonium/xenon and iodine/xenon intervals considerably shorter than 300×10^6 yr, and (b) a number of meteorites (notably chondrites) seemed to contain far greater quantities of excess radiogenic xenon-129.

Kuroda et al.⁸ left (a) unexplained and attempted to explain (b) as resulting from the fact that excess quantities of xenon-129 were produced from iodine-129, which was formed in the solar synthesis process, such as suggested by Fowler et al.^{10,11}.

It is far more difficult to interpret xenon isotope ratio data than to interpret lead isotope ratio data, not only because (i) xenon has nine stable isotopes, while lead has four, and precise measurements of the isotope ratios of the former are much more difficult than for the latter, but also because (ii) the meteoritic xenon isotope abundance ratios are often severely altered by the addition of the isotopes at masses 124, 126, 128, 129, 130, 131 and 132 produced by cosmic rays. The only isotopes of xenon which are not affected by the cosmic-ray irradiation are the isotopes at masses 134 and 136.

The chief difficulties encountered in the previous works were principally caused by (ii), because the xenon isotope ratio data were normalized to xenon-130 and the abundance of this isotope, especially in calcium-rich achondrites, can be quite severely altered by the cosmic-ray irradiations.

In the present work, we have re-examined all the xenon isotope ratio data obtained in this laboratory and elsewhere, and re-calculated the plutonium/xenon and iodine/xenon decay intervals of many achondrites. In this new calculation, we take advantage of the fact that xenon-134 and xenon-136 are the only stable xenon isotopes which remained unaffected under the cosmic-ray irradiations. The initial abundances of the two important extinct nuclides plutonium-244 and iodine-129 at the time of cessation of nucleosynthesis can be determined by this method in a much less cumbersome manner than before. A surprisingly excellent concordancy was obtained between the plutonium/xenon and iodine/xenon decay intervals of about a dozen achondrites studied in this laboratory and elsewhere.

For the plutonium/xenon decay interval, because the production of xenon-134 and xenon-136 caused by cosmic-ray bombardment is negligible, we may write

$${\binom{134 \text{Xe}}{136 \text{Xe}}}_{Met} = {\frac{134 p \text{Xe} + 134 f \text{Xe}}{136 p \text{Xe} + 136 f \text{Xe}}} = m$$
(1)

where superscripts p and f represent primordial and fission components respectively, and subscript Met refers to the meteorite.

We may also write that

$${\binom{134 p \times e}{136 p \times e}} = p \ (= constant)$$
 (2)

We have chosen the Murray carbonaceous chondrite to represent primordial xenon. Because $(^{134}\text{Xe})^{136}\text{Xe})_{\text{Murray}} = 1\cdot19 \simeq 1\cdot18 = (^{134}\text{Xe})^{136}\text{Xe})_{\text{atm}}$, the presence of a slight atmospheric component in the meteoritic xenon will not seriously affect our calculation.

If the fission component is from one single source, namely plutonium-244, then

$$\frac{^{134}fXe}{^{136}fXe} = f \text{ (= constant)}$$
 (3)

From equations (1)-(3) we have

$$\frac{^{136}f\text{Xe}}{^{136}\text{Xe}} = \frac{p-m}{p-f} \tag{4}$$

where ${}^{136}Xe = {}^{136}pXe + {}^{136}fXe$.

If plutonium and uranium were not fractionated in the meteorites, we may write that

$$(^{136f} Xe)_{244} = \alpha \cdot {}^{238} U \cdot \exp(\lambda_{\alpha_{238}} (T + \Xi)) \cdot Y_{136} \cdot \left(\frac{\lambda_f}{\lambda_{\alpha} + \lambda_f}\right)_{244} \cdot \exp(-\lambda_{\alpha_{244}} \Xi)$$

$$(5)$$

where α is the plutonium-244/uranium-238 ratio at the time of cessation of nucleosynthesis; λ_{a238} is the alpha decay constant of uranium-238; T is the age of Earth and meteorites $(4.55 \times 10^9 \text{ yr})^{12}$; Y_{136} is the plutonium-244 spontaneous fission yield for mass chain 136; λ_{a234} and λ_{f244} are the alpha and spontaneous fission decay constants of plutonium-244; and Ξ is the plutonium/xenon decay interval which is the interval between the cessation of nucleosynthesis and formation of the meteoritic body and its subsequent cooling to temperatures at which the meteoritic body could retain xenon (sometimes termed the formation interval).

Because

$$\exp(\lambda_{\alpha_{238}}(T+\Xi)) \simeq \exp(\lambda_{\alpha_{238}}T)$$

we have

$$(^{136}fXe)_{244} = ^{238}U \cdot \exp(-\lambda_{a244}\Xi)$$
 (a constant) (6)

The contribution from the uranium-238 spontaneous fission is

$$(^{136f}\text{Xe})_{238} = ^{238}\text{U} \cdot Y_{136} \cdot \left(\frac{\lambda_f}{\lambda_\alpha + \lambda_f}\right)_{238} \cdot \\ \left[\exp(\lambda_{238}(T + \Xi)) - 1\right] \quad (7)$$

Using $T=4.55\times 10^9$ yr, $Y_{136}=6$ per cent and if $\Xi\cong 3\times 10^8$ yr we obtain

$$\left(\frac{^{136}fX_{\Theta}}{^{238}U}\right)_{238} = 3.1 \times 10^{-6} \text{ cc stp/g of uranium}$$
 (8)

As pointed out by Rowe and Kuroda³, this amounts to approximately one-tenth of that from plutonium-244 spontaneous fission in the case of the meteorite Pasamonte. It follows from equations (4), (5) and (6) that

$$\frac{p-m}{p-f} = \left(\frac{^{236}\text{U}}{^{136}\text{Xe}}\right)_{Met} \cdot \exp(-\lambda_{a224}\Xi) \cdot \text{constant} \quad (9)$$

which means that if (p-m) is plotted against the uranium-238/xenon-136 ratio, a straight line should be obtained for one or more meteorites, which were formed at the same time and the slope should correspond to the value E. Fig. 1 shows such a plot. The results indicate that these meteorites were not formed at the same time, as was previously assumed⁸. It is also clear from Fig. 1 that there exist real differences in the formation interval in the same class of meteorite as well as in different classes This information, when more accurate of meteorites. measurement of gas content, isotope ratios and uranium measurement in the same aliquot are available, could be of vital interest in shedding light on problems of cooling rate, the depth of these meteorites in the parent body and the number of parent bodies.

The absolute value of Ξ for these meteorites cannot be determined until the value of α becomes known, while differences in the values of Ξ for other meteorites relative

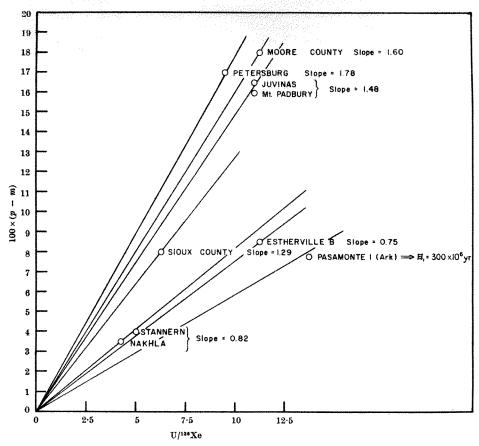


Fig. 1. A plot showing correlation between fission xenon-136 and uranium in various achondrites. The logarithms of slopes have direct proportionality to plutonium/xenon decay interval.

to Pasamonte (or any other meteorite) can be calculated from the slopes in Fig. 1. Let us by way of experiment use the expression for a given by Kuroda² in 1961, for the purpose of calculating tentative values of Ξ :

$$x = \frac{P_{244}}{P_{238}} \cdot \frac{\lambda_{238} - \lambda_{*}}{\lambda_{244} - \lambda_{*}} \cdot \frac{\exp(-\lambda_{*}\Delta) - \exp(-\lambda_{244}\Delta)}{\exp(-\lambda_{*}\Delta) - \exp(-\lambda_{238}\Delta)}$$
(10)

where P_{244} and P_{238} are the production rates of plutonium-244 and uranium-238, respectively, and are assumed to be equal in the r process, λ_* is the rate constant for the nucleosynthesis (the nucleosynthesis rate is assumed to decay exponentially since the mean time of beginning with the decay constant λ_*), and Δ is the duration of nucleosynthesis. Using $\lambda_* = 0.1 \times 10^{-9} \text{ yr}^{-1}$, $\Delta = 6.0 \times 10^9 \text{ yr}$, $\lambda_{238} = 0.154 \times 10^{-9} \text{ yr}^{-1}$ and $\lambda_{244} = 8.45 \times 10^{-9} \text{ yr}^{-1}$, the above equation yields a value of $\alpha = 2.35 \times 10^{-2}$.

In terms of cc str/g of uranium, we now have the plutonium-244 spontaneous fission produced ¹³⁶ Xe

$$\left(\frac{^{136}\text{Y.e}}{\text{U}}\right)_{Met} = 3.2 \times 10^{-4} \exp(-\lambda_{244}\Xi) \text{ (cc stp/g)}$$
 (11)

From equations (5), (8) and (11) we secure a value of $\Xi = 300^{+4}$ million years for Pasamonte. The plutonium/ xenon decay intervals based on this value are tabulated in Table 1.

In the cases of some meteorites (Lafayette, Estherville, Cumberland Falls and Pena Blanca Springs), the values of (p-m) are small, and the calculation of Ξ by this method becomes uncertain. The E values for these meteorites were calculated by the method described by Kuroda et al.8 and are included in Table 1. In calculating the values of Ξ , we estimated ± 30 per cent errors in determining the slopes, most of which are caused by uncertainties in the measurements of total gas contents

($\sim \pm 25$ per cent). This error limit corresponds to an uncertainty in Ξ of +45 and -35×10^6 yr.

The calculation of the iodine/xenon decay intervals is somewhat more complicated as xenon-129 consists of

Table 1. PLUTONIUM/XENON DECAY INTERVALS OF VARIOUS ACHONDRITES

Name	Classifi- cation*	p.p.b. 10 per cent	$^{136}\mathrm{Xe}_{\times~10^{-12}}_{\mathrm{cc~STP/g}}$	134 Xe 136 Xe ± 1.4 per cent	$\frac{U}{iss Xe}$ $\pm 30 \text{ per}$ cent	S:44 ×10* yr
Sioux County	Eucrite	130†	20.53 ¶	1.11¶	6.2	205
Mount Pad- bury	Mesisoderite	109†	10.0¶	1.03¶	10-9	190-45
Juvinas	Eucrite	200†	18.19¶	1.025 ¶	11.0	190-35
Moore County	Eucrite	130‡	11.87¶	1.01 ¶	11.2	180^{+45}_{-35}
Petersburg	Eucrite	130‡	15·28¶	1.02¶	9-4	165-44
Stannern	Eucrite	180†	36.7¶	1.15¶	4.9	260^{+45}_{-35}
Nakhla	Nakhlite	40†	9·26¶	1.157¶	4.3	250^{+45}_{-25}
Estherville B	Mesisoderite	100§	8.93**	1.105**	11.3	270^{+45}_{-85}
Pasamonte I (Ark.)	Eucrite	132	10.36¶	1.11¶	13.8	300-45
Lafayette††	Nakhlite	40†	20.3¶	1.18¶	2.9	190-45
Estherville A††	Mesisoderite	100§	20.9**	1.18**	4.8	270-45
Cumberland Falls††	Aubrite	16.3†	13-6¶	1.17**	1.2	22045
Pena Blanca Springs††	Aubrite	15-4†	13-4**	1.17¶	1.1	125 - 75

* Classification according to ref. 15.
† See ref. 16.
‡ Average encrite value according to ref. 16.
§ Average of value by Clark et al. 16 and private communication from Murthy, V. R.

|| Nix, J. F., and Kuroda, P. K. (unpublished work).
¶ See ref. 5.

^{**} See ref. 5. †† Ξ_{244} evaluated by the method of Kuroda et al.*.

		Table 2.	IODINE/XENON DECAY	INTERVALS OF VAR	IOUS ACHONDRITES		
Name*	I† p.p.b.	x = 1.00 f = 0.95 Ξ_{129}	$ \begin{array}{c} x = 1.00 \\ f = 0.96 \\ \Xi_{120} \end{array} $	x = 1.00 f = 0.97 \mathcal{Z}_{189}	x = 1.00 f = 0.98 Ξ_{120}	x = 1.00 f = 0.99 S_{111}	z=10±04 f=1400 ≤
Sioux County Mount Padbury Juvinas Moore County Petersburg Stannern Nakhla Estherville B	14·0 96 35 140 50 828 180	220 × 10 ⁸ yr 240 200 235 — — 230 245	210 × 10 ⁴ yr 230 190 230 230 225 235	200 × 10* yr 225 185 225 215 — 225 225	190 × 10 ⁶ yr 220 180 220 205 820 220 220 220	180 × 10 ⁸ yr 215 175 215 200 300 220 210	175 ± 2 × 10* yr 210 ± 3 170 ± 2 210 ± 2 190 ± 3 290 ± 10 220 ± 3 205 ± 10
Pasamonte I (Ark.) Lafayette † Estherville † Cumberland Falls † Pena Blanca Springs †	110 100 30 460 22		315 190 ± 30 × 10 ⁴ yr 280 ± 30 × 10 ⁴ yr 250 ± 30 × 10 ⁵ yr 160 ± 30 × 10 ⁵ yr	280	265	250	245±2

three components: primordial (p), cosmic-ray-produced (c) and radiogenic (r). Fortunately, however, an error by a factor of two introduces an uncertainty of $\pm 17 \times 10^6$ yr in the value of Ξ .

Let
$$(^{134}Xe)^{(130}Xe)_{Obs} = a$$
 $(^{136}Xe)^{(130}Xe)_{Obs} = b$ $(^{134}pXe)^{(130}pXe) = p_1$ $(^{136}pXe)^{(130}pXe) = p_2$ $(^{120}pXe)^{(130}pXe) = p_2$

It follows from equations (1) and (3) that

$${\binom{134 f \text{Xe}}{136 f \text{Xe}}} = f = {\binom{134 \text{Xe} - 134 p \text{Xe}}{136 \text{Xe} - 136 p \text{Xe}}}$$
(13)

From equations (12) and (13) we have

$$\frac{^{130}\text{Xe}}{^{130}p\text{Xe}} = \frac{p_1 - f \cdot p_2}{a - f \cdot b} \tag{14}$$

and

$$\frac{^{1297}\mathrm{Xe} + ^{1296}\mathrm{Xe}}{^{130}p\mathrm{Xe}} = \left(\frac{^{129}\mathrm{Xe}}{^{130}p\mathrm{Xe}}\right)_{Met} - \left(\frac{^{129}\mathrm{Xe}}{^{130}\mathrm{Xe}}\right)_{P} \qquad (15)$$

where superscripts r and c refer to radiogenic and cosmicray-produced components, respectively, and subscripts Met and P refer to the ratios in the meteorites and in the primordial xenon (Murray), respectively.

The cosmic-ray-production ratios 124eXe: 126eXe: 128eXe: 1306Xe have been determined by a number of investigators 5, 5, 9, 12, 13 and are reasonably well known. The relative production ratio, however, for 1226 Xe is unknown, except that Rowell estimated that

$$\frac{^{129}cXe}{^{126}cXe} = x \le 0.84$$

We have used x=1 in our calculations. At f=1.00 we try to show the effect of large variation in x (± 0.4) on Ξ₁₂₉ in Table 2.

The value of f in equation (14) is also unknown. Pepin⁵ calculated the value of f to be 0.98, while according to Hohenberg et al. f = 0.96. Because of this uncertainty we have calculated the values of Ξ for f = 0.95, 0.96, 0.97, 0.98, 0.99 and 1.00. The results are shown in Table 2. In the case of four meteorites (Lafayette, Estherville A, Cumberland Falls, Pena Blanca Springs), Z values were calculated by the method described earlier by Kuroda et al.8.

The initial 120 I/127 I ratio (β) used in the calculation was again taken from the report by Kuroda²

$$\beta = \frac{P_{129}}{P_{127}} \cdot \frac{\lambda_*}{\lambda_{129}} \cdot \frac{1}{\exp(\lambda_* \Delta) - 1}$$
 (16)

Using $\lambda_{129} = 40.3 \times 10^{-9}$ yr⁻¹, $P_{129} = P_{127}$, $\lambda_{*} = 0.1 \times 10^{-9}$ yr⁻¹ and $\Delta = 6.0 \times 10^{9}$ yr, we obtain

$$\beta = 3.0 \times 10^{-3}$$

This gives in terms of cc str/g of iodine

$$\frac{\binom{1297\text{Xe}}{\text{I}}_{\text{Met}}}{1}_{\text{Met}} = 5 \cdot 3 \times 10^{-1} \exp\left(-\lambda_{129}\Xi\right) (\text{ce str/g of iodine})$$
 (17)

The results are plotted in Fig. 2 in such a manner that the mean value of Ξ_{129} obtained from Table 2 is placed on the solid line whose slope corresponds to the iodine-129 half-life (17 million years). The corresponding values of ¹³⁶ Xe/U are plotted vertically above or below the Ξ_{128} points. The dotted line drawn through the values of ¹²⁶fXe/U thus plotted has a slope corresponding to the half-life of 82 million years. Plutonium-244 and the initial value at $\Xi = 0$ corresponds to 3.2×10^{-4} cc str/g.

Fig. 2 shows that the plutonium/xenon and iodine/ xenon decay intervals of these meteorites are concordant within the error limits. It also shows that the cosmological

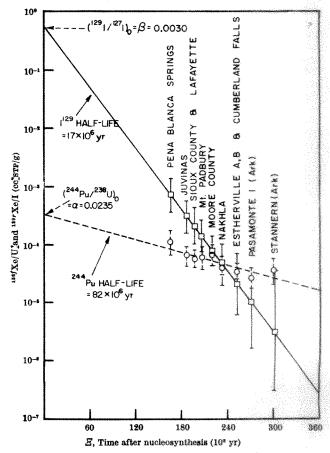


Fig. 2. A comparison of plutonium/xenon and iodine/xenon decay intervals for various meteorites.

^{*} For classification see Table 1.
† Values by Clark et al., see ref 16.
‡ Z₁₂₉ evaluated by method of Kuroda et al.*.

model used in deriving equations (10) and (16) is quite reasonable.

It should be pointed out here that the concordancy cannot be improved much by changing the cosmological model for galactic nucleosynthesis, and a concordancy between the plutonium/xenon and iodine/xenon decay interval cannot be obtained at all if the single event (big bang) model of nucleosynthesis is used.

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Latent Viruses in Chimpanzees with Experimental Kuru

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National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland Forty-seven strains of virus have been isolated from sterile long maintained tissue explants from chimpanzees suffering from experimental kuru. Many are new simian viruses, and none have been demonstrated to be a cause of the kuru syndrome.

The experimental transmission of kuru from humans to chimpanzees1 and the serial transmission of the disease from chimpanzee to chimpanzee² have provided a system from which it is hoped successful isolation of the actiological agent of kuru will be forthcoming. Chimpanzee brain suspension is infectious at 10-5 dilution and the agent is filterable through gradacol membranes of average pore diameter of 220 mu and, in recent experiments, probably 100 mu. The agent is therefore presumably a virus. It is known that the chimpanzee, like other primates, serves as host to endosymbiotic viruses and the presence of such latent agents makes the isolation and identification of the agent causing kuru a difficult problem. The isolation and characterization of a remarkable number of such virusesforty-seven strains so far-from chimpanzee tissues are described here. We have no reason to believe that any of these is aetiologically related to kuru.

The tissues from which these agents were isolated were aseptically removed at autopsy from nine chimpanzees (Pan satyrus) experimentally inoculated with kuru 1-3 yr previously. The chimpanzee virus strains (CV) have been obtained from nine different tissues: brain, spinal cord, kidney, spleen, sympathetic ganglion, optic nerve, lymph nodes, thymus and thyroid. Twelve of the strains were obtained from different areas of the brains of five chimpan-Viruses were recovered using the technique of long term maintenance of tissue fragments in explant cultures and the transfer of undiluted explant fluids to primary human embryo kidney (HEK) cell cultures. Such subculture has usually been carried out when moderate cytopathic effect appeared in the primary explant cultures. Blind passage to HEK cultures of fluids from apparently normal explants, however, has also yielded viruses. In contrast, direct inoculation of suspension of the same chimpanzee brain into many types of tissue cultures, including HEK, has yielded no viruses.

From these virus strains, seven virus types have been differentiated; these have been designated Pan viruses.

The criteria we have used to define a new Pan virus type are: (a) demonstration of antibody to the agent in the serum of the chimpanzee from which isolation was obtained; (b) demonstration of antibody to the agent in the sera of rabbits hyperimmunized with fluids or cells from the HEK cultures; (c) failure of the same immune rabbit sera to neutralize other Pan virus types; and (d) a specific pattern of presence or absence of antibody to the agent unlike that to other Pan virus types in the sera of a large number of chimpanzees from our colony, both kuru-affected and kuru-free.

Eight of these chimpanzees were in advanced kuru disease at the time of death. The disease appeared 11–30 months after inoculation of brain suspension from kuru patients or from chimpanzees with experimental kuru. The ninth animal showed the neuropathological changes observed in kuru³, but was without clinical signs of the kuru syndrome at the time it was killed because of acute gastroenteritis, 10 months after inoculation with brain tissue from a kuru chimpanzee.

In most instances, tissues for explant were obtained within 2 h of death by exsanguination under 'Sernylan' anaesthesia. ('Sernylan': C-395 (phencylidine hydrochloride) or 1-(1-phenylcyclohexyl) piperidine hydrochloride; Parke, Davis and Co.) Representative tissues were removed and dropped immediately into growth medium (Gro-BAF). The vials were then held at room temperature until return to the tissue culture laboratory. (Growth medium was 60 per cent F-10/HAM (ref. 4); 20 per cent foetal calf serum unheated; 10 per cent bovine amniotic fluid; 4 per cent NCTC 109 (ref. 5); 5 per cent Hanks lactalbumin hydrolysate 0.5 per cents; 1 per cent antibiotic mixture and fungizone. The pH of the medium should be 6.6 to 6.8.)

All explants were carried in plastic 30 ml. flasks (Falcon Plastics), incubated at 35° C. Tissues were cut into 0.5–1.0 mm cubes which were then placed in the flasks and spread with the tip of a capillary pipette. Originally the tissue fragments were clotted in place with chick embryo extract

Table 1. CHIMPANZEE VIRUS STRAINS ISOLATED FROM EXPLANTS OF TISSUE FROM KURU AFFECTED CHIMPANZEES

Tissue	
CV 2 A 1 Mesenteric lymph node 19 <17	n No.
CV 2 A 1 Mesenteric lymph node 19 <17 Reo 5-3 3 CV 3 A 1 Brain; frontal cortex 93 107 6 Reo 6-4 4 CV 4 A 1 Brain; cerebellum 84 103 6 Reo 6-5 3 CV 5 A 1 Brain; parietal cortex 19 17 CV 6 A 1 Brain; corpus striatum 19 26 CV 7 A 1 Brain; cocipital cortex 19 32 1-0 CV 8 A 9 Kidney 69 18 4-0 CV 9 A 9 Thymus 69 69 18	1
CV 3 A 1 Brain: frontal cortex 93 107 6 Reo 6·4 ** CV 4 A 1 Brain: cerbellum 84 103 6 Reo 6·5 ** CV 5 A 1 Brain: parietal cortex 19 17 CV 6 A 1 Brain: corpus striatum 19 26 CV 7 A 1 Brain: occipital cortex 19 32 1·0 CV 8 A 9 Kidney 69 18 4·0 CV 9 A 9 Thymus 69 69 18	į
CV 4 A 1 Brain: cerebellum 84 103 6 Reo 6*5 CV 5 A 1 Brain: parietal cortex 19 17 CV 6 A 1 Brain: corpus striatum 19 26 CV 7 A 1 Brain: occipital cortex 19 32 1·0 CV 8 A 9 Kidney 69 18 4·0 CV 9 A 9 Thymus 69 69 18	į.
CV 5 A 1 Brain: parietal cortex 19 17 CV 6 A 1 Brain: corpus striatum 19 26 CV 7 A 1 Brain: cocipital cortex 19 32 1-0 CV 8 A 9 Kidney 69 18 4-0 CV 9 A 9 Thymus 69 69 18 4-5	j.
CV 6 A 1 Brain: corpus striatum 19 26 CV 7 A 1 Brain: occipital cortex 19 32 1.0 CV 8 A 9 Kidney 69 18 4.0 CV 9 A 9 Thymus 69 69 18	
OV 7 A 1 Brain: occlpital cortex 19 32 1.0 CV 8 A 9 Kidney 69 18 4.0 CV 9 A 9 Thymus 69 69 18 4.5	
CV 8 A 9 Kidney 69 18 4-0 CV 9 A 9 Thymus 69 69 18 4-5	
CV 9 A 9 Thymus 69 69 18 4.5	
017 10 4 0 Sulgari 60 18 2-5	
CV 12 A 2 Kidney 49 14 Foamy 3.0	
CV 13 A 2 Thymus 41 49 14 Foamy 4.5	ži tiči i
CV 14 A 2 Inguinal node 49 11 Foamy 4.0	\$ 100
CV 15 A 2 Brain: corpus striatum 33 49 14 1. U	
CV 16 4 2 Brain midbrain 41 49 7 Foamy 2.5	å :
CV 17 A 5 Kidney 65 40 22 1.0	
OV 18 4 5 Thymns 39 59 22 Foamy 4.5	L
CV 19 A 5 Inguinal node 65 59 27 1.0	
CV 20 A 5 Brain: thalamus 67 59 22 1-0	
CV 21 A 5 Brain stem 48 40 24 3.5	
OV 22 A 7 Kidney 63 63 33 3-5	J 346
CV 23 4 7 Mesenteric node 126 63 14 Adeno 6:5	à.
CV 24 A 15 Kidney 32 57 7 3-0	
Gr 25 A 15 Spleen 57 12	
CF 26 A 15 Thymns 185 57 12	
CV 27 A 15 Sympathetic nerve 38 57 12 1.0	
OF 28 4 15 Carvical node 57 16 1.0	
CF 29 A 15 Lumbar cord 57 16 1.0	115656
CV 30 A 16 Kidney 77 100 32 Foamy 3-5	1
CF 31 4 16 Spleen 77 100 26	
© 7 Adeno 5.5	0
CV 33 A 16 Inquirial node 100 15 Adeno 6.5	7
CV 84 A 16 Thymns 100 15 Foamy 3.5	Ł
CV 35 4 16 Lumbar cord 100 15 Foamy 4-8	Ł
CV 36 4 16 Cervical cord 100 32 Foamy 3-8	1
CV 37 A 16 Thalamus 100 15 Foamy 4-5	1

and chicken plasma, but latterly they have been spread in the flask without a clotting matrix. After 1.5-12 h at 35° C, 1.0 ml. of slightly acid growth medium was added. When the longer period of incubation was contemplated, the pieces of tissue were moistened with a few drops of growth medium contained in the tip of the pipette. Flasks were not handled for several days unless, by inspection, the medium had become definitely alkaline. If this occurred, it was gently decanted and replaced with 1.0 ml. of fresh Gro-BAF. Occasionally this was repeated several times. Only rarely did cellular outgrowth occur if the medium remained alkaline. When the fragments were firmly adherent to the flat face of the flask an additional 2.0 ml. of Gro-BAF was added without danger of floating the tissue. Subsequent feedings were in 3.0 ml. volumes. When the outgrowing cells had been well sheeted, the originating tissue fragments usually became detached. A few of these were successfully restarted as late as 42-69 days after the original explant by gently emulsifying them with a pipette and distributing into new flasks.

Outgrowth was usually visible in 4-6 days. Flasks were examined microscopically at weekly intervals and the cultures were fed with Gro-BAF at 10-21 day intervals. At irregular intervals, generally when explant cultures were being fed, subcultures into human embryo kidney cells were made, using the spent fluids and cellular debris as inoculum. Subculturing was, at times, performed in the absence of the cytopathic effect in the explants; in some such cases the effect appeared later, at times even after it had already been noted in the HEK subcultures. No attempt was made to split the explants. Those in which viral cytopathic effect was noted were frozen at -70° C when the effect reached 3+ or 4+, while others are still living after more than a year. From nine explanted tissues of one additional animal, no viral agent has been isolated and seven of the cultures are still surviving after more than a year.

Hyperimmune antisera were prepared for each of the newly isolated viruses in rabbits by intravenous injection

of 1.0 ml. of undiluted tissue culture fluid containing the agent on days 0, 7, 14 and 21. Animals were generally bled out about 14 days after the last dose of antigen, that is, on day 35 of the immunization procedure.

The first thirty-seven viruses isolated are summarized in Table 1, with the day of passage to HEK cell cultures and the day of first appearance of cytopathic effect in both the primary explants (if it occurred) and in the HEK indicator cultures. The appearance of the cytopathic effect of the strains classified thus far and the virus titre attained in HEK cultures on first or second passage are also recorded. Ten additional virus strains from explanted tissue of two chimpanzees (A 14 and A 21) are not included in Table 1, because they were only recently isolated. (All chimpanzee A numbers used in this paper refer to animals previously reported in the literature with summaries of the inocula they received, the incubation periods and the durations of disease2.)

Chimpanzee virus (CV) strains are listed serially in Table 1 in the order of isolation. Each new isolate is immediately given a CV strain designation. Distinct virus types defined from these strains are tentatively called Pan viruses, from the generic name of the chimpanzee, and carry a serial number designation until such time as they are identified with known viruses. Thus, the Pan designation does not designate a new virus type but is our code for successively distinguished viruses that we have found to be latent in the chimpanzee. When each Pan virus is better characterized, it is given an appropriate name in the viral group to which it belongs, or it is identified as a known virus. Thus, Pan 1 has been given the name Simian foamy virus 6, and Pan 2 simian foamy virus 7. Pan 3 is reovirus type 2, and Pan 4 reovirus type 1. Pan 5, 6 and 7 are adenoviruses, but cannot yet be designated by type.

So far, seven chimpanzee virus strains (CV 1, CV 18, CV 30, CV 34, CV 35, CV 36, CV 37) have been designated as Pan 1; five strains (CV 11, CV 12, CV 13, CV 14, CV 16) as Pan 2; two strains (CV 2, CV 4) as Pan 3; and one strain each as Pan 4 (CV 3), Pan 5 (CV 23),

Numbers refer to the experimental animals reported by Gajdusek, Gibbs and Alpers².
 Blanks indicate that explants were observed for over 120 days without showing any CPE; some are still growing.
 Blanks indicate a type of CPE without identifying characteristics.
 Titre expressed as -log₁₀ of that dilution which contains a TCID₅₀/linoculum.

Table 2. ANTIBODIES TO LATENT CHIMPANZEE VIRUSES IN CHIMPANZEE, HUMAN AND HYPERIMMUNE RABBIT SERA

			V-04 Yet 47 TA 1	a mann	IL SMA	n.	
	Pan 1	Neuti Pan 2	ralizati Pan 5	on*	Dan 7	tion inh	glutina- ibition†
	(CV	(617	1011	FAHO	rau /	Pan 3	·
	1)	(CV)	(CV)	(CV	(CV)	(CV 2	Pan 4
Chimpanzee sera:	1)	11)	23)	32)	33)	and 4)	(CV 3)
Kuru affected:							
A 1	> 1,280	0	+	0	+	>1,280	640
A 2	0	> 640	+	ŏ	±	> 1,280	> 5,120
A 2 A 4 A 5 A 6	Ô	0	+	ŏ	÷	71,200	10
A 5	160	ŏ	÷	ŏ	+	40	2,560
A 6		1.28ŏ	+	ŏ	+	40	
A 7	ŏ´	0	+	ő			320
A 8	v	U	7	U	+	320	320
à Š	+	0				40	320
15	+						
A 16		0					
Normal	+	0	+	+	+		
13							
		> 640		0	+		
25	0	0		0	0		
28	0	+	+	0	+		
29	0	+	0	0	0		
30	0	+	0	0	Õ		
31	0	0	-	õ	ŏ		
32	+	+	+	ŏ	+		
33	+	<u>;</u>	+	Ť	+		
34	+	+	Ó	ò	ō		
35	<u> </u>	+	Ť	ŏ			
36	ó	+	ŏ	ŏ	+		
39	ŏ	ŏ	U	U	U		
40	±	ŏ					
Human sera:	I	U					
Kuru patients:							
Kigea				_			
6 others	0	0		0	+	40	40
Ouners	. 0	0					
Normal Fore nat		_					
6 subjects	0	0					
Immune rabbit sera		_					
Pan 1	320	0	0	0	0	0	0
Pan 2	0	320	Ó	Õ	ŏ	ŏ	ö
Pan 3	0	0	Ó	Õ	ŏ	> 5,120	ŏ
Pan 5		•	4.	ŏ	ň	- 0,120	v

* Titres expressed as the reciprocal of the highest dilution of serum that neutralized 50 to 300 $TCID_{50}$ in HEK cell cultures. Titres less than 10 are indicated by 0, titres \geq 10 are indicated by +.
† Titres expressed as the reciprocal of the highest dilution of serum that inhibited haemagglutination of human group O erythrocytes by about 4 $_{\rm U}$ of antigen. Titres less than 10 are indicated by 0, titres \geq 10 are indicated by +.

Pan 6 (CV 32), and Pan 7 (CV 33). Pan 1 has been isolated from three chimpanzees, whereas Pan 2, the other foamy virus, from only one animal. Three different virus types have been obtained from chimpanzee A 1 (Pan 1, Pan 3 and Pan 4) and from chimpanzee A 16 (Pan 1, Pan 6 and Pan 7).

Table 2 summarizes determinations of neutralizing antibody to Pan viruses 1, 2, 5, 6 and 7, and haemagglutination inhibiting (HAI) antibodies to Pan 3 and 4 in sera from normal and kuru affected chimpanzees, sera from human kuru patients and normal Fore natives in the kuru region, and sera from four hyperimmunized rabbits. Serum from each homologous chimpanzee neutralizes the virus isolated from its tissues: serum from chimpanzee A 1 neutralizes Pan 1 (CV 1); serum from chimpanzee A 2 neutralizes Pan 2 (CV 11). Sera from chimpanzees A 5 and A 16, from which other strains of Pan virus 1 were isolated, also neutralize the CV 1 strain of Pan virus 1, as well as the homologous CV strains isolated from each animal. These chimpanzee sera neutralize only the chimpanzee foamy virus type which their explants yielded. This is fortuitous because sera from kuru affected chimpanzees whose explants yielded no strains of Pan virus 1 or 2 have neutralized one or the other of these viruses, as have sera from some normal chimpanzees. Human kuru patients and normal Fore natives show no antibody to either Pan 1 or Pan 2, but the one patient whose serum was examined more fully shows antibody to Pan 3 (reovirus type 2), Pan 4 (reovirus type 1) and Pan 7 (adenovirus). The four hyperimmune rabbit sera are specific for the immunizing virus. Tests with Pan 5 hyperimmune rabbit serum are incomplete; sera against Pan 4, 6 and 7 are in preparation.

Simian Foamy Viruses from Chimpanzees

Pan viruses 1 and 2 in HEK cell cultures produce a vacuolated syncytium which eventually degenerates and leaves a "moth eaten" appearance. This cytopathic

effect is slow in developing, rarely appearing before 7 days, and is often delayed for 2 to 3 weeks. Tissue culture fluids contain $10^{2.5}$ – $10^{4.5}$ $TCID_{50}/0.1$ ml. of inoculum. Both viruses are stable on storage at -70° C, ether sensitive, inhibited by 5-iodo-deoxyuridine (IUDR), and are inactivated at 56° C in 30 min. No inclusion bodies have been seen.

The ill defined group of simian foamy viruses consists of viruses isolated from monkey or ape tissues which cause giant cell syncytia in tissue culture; produce no demonstrable intracytoplasmic or intranuclear inclusion bodies; have not haemagglutinated test erythrocytes from a wide variety of species, and have not produced clinical disease after inoculation of 1 day old mice or hamsters. The previously defined simian foamy viruses were isolated from cynomolgus (Nos. 1 and 2) (refs. 8 and 9), rhesus (No. 3) (ref. 10), squirrel (No. 4) and Galagos monkeys (No. 5) (personal communication from P. Johnston). The first three have proved to be RNA viruses, whereas the fourth is inhibited by 5-iodo-deoxyuridine.

Pan 1 and Pan 2 fit these criteria well, and so we have assigned them positions as simian foamy virus 6 and 7, respectively, in conferences with Dr Paul Johnston of the Department of Microbiology, University of Louisville Medical Centre. Two strains of Pan virus 1 (that is, CV 1 and CV 18) and one of Pan virus 2 (that is, CV 11) were not neutralized by seven hyperimmune typing sera to the previously described simian foamy viruses: rabbit antiserum to types 1, 2 and 3, obtained from Dr J. A. Morris, of the Division of Biologics Standards, National Institutes of Health, and to types 1, 2, 3 and 4 from Dr Johnston. Our own antisera to Pan viruses 1 and 2, from rabbits hyperimmunized with three injections of HEK fluids at weekly intervals, have shown specific high titre neutralization to the homologous virus type without cross neutralization between the two types (Table 3). Typing sera from these two viruses are now being prepared in horses in the Simian Foamy Virus Typing Programme in Dr Johnston's laboratory. Cabasso¹⁰ has now identified an eighth agent fitting the criteria of this undoubtedly heterogeneous group.

Table 3. CROSS NEUTRALIZATION TEST OF PAN VIRUSES 1 AND 2

			utralizing ai	itibody titi	res †	
Virus*	Pan 1	Rabbit	Antisera	Pan 2	Rabbit	Antisera
	1	2	3	4	5	6
Pan 1	40	320	80	0	0	0
Pan 2	0	0	0	160	80	320

* Virus strains used in neutralization test and rabbit immunization were CV1 of Pan 1 and CV 11 of Pan 2. † Titres expressed as the reciprocal of the highest dilution of serum that neutralized 80 to 300 $TCID_{50}/$ inoculum in HEK cell cultures. Titres of less than 10 are indicated by 0.

Reoviruses from Chimpanzees

Pan viruses 3 and 4 in HEK cell culture fluids have haemagglutinated human group O erythrocytes to titres of 1:128. They produce a reovirus-like cytopathic effect in HEK in 3 to 4 days and infectivity titres of 105.3 to $10^{6.5}~TCID_{50}/0\cdot1$ ml. in 2 weeks. Specific typing sera against reoviruses 1, 2 and 3 have been used in haemagglutination inhibition tests with these agents. (Hyperimmunized rooster sera were obtained from the Communicable Disease Center, Atlanta, Georgia.)

Haemagglutination of each of two Pan 3 isolates (CV 2 and CV 4) was strongly inhibited by reovirus antiserum to titres of > 640 and 640, respectively. Both exhibited minimal crossing with reovirus type 1 (titres of 40 and 10, respectively). Haemagglutination of Pan virus 4 (CV 3) was inhibited by reovirus 1 antiserum at a dilution greater than 1:5,120 (crossing at a dilution of 1:10 with reovirus 2). It is noteworthy that Pan viruses 3 and 4 were isolated from different areas of the brain of the same chimpanzee (A 1; see Table 1). The sera of all the chimpanzees thus far tested have shown HAI antibody to both Pan 3 and Pan 4, with the exception of the sera from chimpanzee A 4 which failed to react with Pan 3

and showed minimal inhibition with Pan 4. The serum of a single kuru patient reacted with both. Considering the ubiquity of reoviruses, these last reactions are scarcely surprising.

Adenoviruses from Chimpanzees

Pan 5, 6 and 7 viruses, in HEK cell culture, originally were thought to be enterovirus like, but as cytopathic effect markedly increased, their adenovirus nature was indicated. This was confirmed by complement fixation, through the kindness of Dr Wallace Rowe (National Institutes of Health, Bethesda, Maryland), who has shown that these three viruses are not neutralized by antisera to human adenoviruses types 1 to 31, inclusive, yet they produce antigens in $H\tilde{E}K$ cell cultures which react to high titres with human adenovirus convalescent sera. As yet, they have not been identified as to type; they are not neutralized by antisera to the seventeen simian reoviruses, SV 1, 11, 15, 17, 20, 23, 25, 27, 30-34, 36-38. Screening neutralization tests, however, using many chimpanzee and hyperimmune rabbit sera indicate their dissimilarity, as illustrated by these three sera selected from Table 2.

Virus	Hyperimmune rabbit, Pan 5	Pre-bleed chimp, 35	Pre-bleed chimp, 33
Pan 5	+	+	+
6	0	0	+
7	0	+	+

The remaining virus strains shown in Table 1, as well as at least ten others from the two chimpanzees not included therein, are still under investigation. doubtedly, many will fall into the groups described above.

The significance of these results lies in the demonstration of a wide range of latent viruses, often two or more in a single animal, in tissues that are sterile by conventional techniques of virus isolation. These techniques have at times included inoculation of a 10 per cent suspension of various organs into different tissue cultures (HEK, BS-C-1, HEp_2 , green monkey kidney, and so on). Were monkey kidney, rather than HEK, cell cultures used as an indicator system for the explant viruses, many more agents might well have been isolated. internal milieu of an animal cannot be regarded as microbiologically sterile even in the case of such organs as the brain and spinal cord. The many viruses isolated from human adenoid tissue^{11,12} and the latency of herpes simplex virus are long familiar evidence in this direction. That many viruses are commonly latent in normal tissues has been known from the large number of simian viruses isolated from rhesus monkey kidney tissue cultures used in the cultivation of poliovirus for vaccine production¹³. The demonstration of viruses in germ free mice further supports this view¹⁴⁻¹⁶. Furthermore, a herpeslike virus has been demonstrated17 in, and a reovirus isolated18 from, long maintained explants of Burkitt lymphoma, and the maedi virus has been isolated from explants of sheep lung19.

The problem this profusion of chimpanzee virus strains poses in attempting to find the agent responsible for kuru is immense. So far, only one of the new viruses, the CV 11 strain of Pan 2, has been reinoculated into a chimpanzee intracerebrally after three serial cell culture passages at terminal dilution, with a final dilution factor of 10-13. Unfortunately, this animal was found dead 11 months after inoculation. Although explants were attempted, post-mortem bacterial invasion was so extensive that only a few grew. No viral agent has as yet been isolated from the few surviving explants. We are now trying to circumvent partially the problem of differentiating these numerous endosymbiotic viruses in the chimpanzee from a possible kuru agent by obtaining explants directly from human patients at lymph node biopsy or at early autopsy.

Although the few explants of tissue of kuru patients already in cultivation have not as yet yielded any agents. we have at present under study several unidentified agents from explants obtained from other human chronic neurological disorders.

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Stimulation of Biosynthesis of Glyceride

by

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The biosynthesis of glycerides from glycerolphosphate and palmitate, catalysed by mitochondrial and microsomal fractions, is greatly stimulated by the supernatant fraction (6,000,000 \times g-min). This is caused by the presence of a specific phosphatidate phosphohydrolase. unsaturated long-chain fatty acids and unspecific protein(s) in the supernatant fraction.

THE biosynthesis of triglycerides may proceed by two pathways-a monoglyceride pathway and a glycerolphosphate pathway—according to the glyceride glycerol precursor utilized (see Fig. 1). The individual enzymes catalysing these biosyntheses have been reported to be located in particulate subcellular structures1-16. Ten years

ago, however, Shapiro et al.17,18 observed that the biosynthesis of glycerides by the glycerolphosphate pathway catalysed by a mitochondrial fraction from rat liver was greatly stimulated by a supernatant fraction. observation has since been confirmed and extended in several laboratories; the stimulation of glyceride bio-

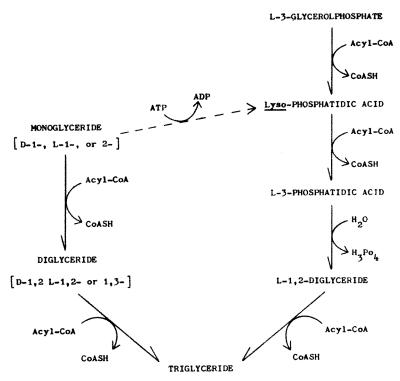


Fig. 1. Pathways in glyceride biosynthesis.

synthesis by the particle free supernatant (6,000,000g)has been observed with mitochondrial and microsomal fractions prepared from various tissues10,15,19-23. example of this supernatant stimulation is given in Table 1, which also shows that the active principles consist of a heat-stable and a heat-labile factor. The occurrence of two factors was discovered by Shapiro et al.18, who pointed out that the heat-stable factor could be partly extracted with ethanol-ether. Lately, detailed investigations of the heat-labile principle showed it to consist of more than one factor. Because these observa-tions are widely dispersed in the literature it seems appropriate to survey this particular aspect of glyceride biosynthesis. In this article we discuss the function and nature of the various factors present in the supernatant fraction in relation to the biosynthesis of glycerides by the glycerolphosphate pathway. The formation of glycerides by the monoglyceride pathway is not stimulated by the supernatant fraction.

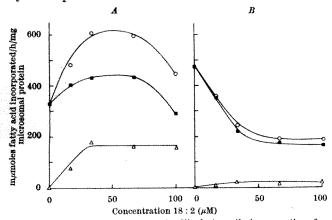


Fig. 2. Effect of increasing amounts of linoleate on the incorporation of palmitate into glycerides using a microsomal fraction from cat intestinal mucosa. The assay system was that described in Table 2 except that the concentration of linoleate was varied as indicated. The assay system contained 0.6 mg of microsomal protein and 2.4 mg of protein of an acetone-treated supernatant preparation. A, Fatty acids incorporated into glycerides: and B, fatty acids incorporated into phosphatidate.

\$\triangle \triangle \

The Heat-stable Factor

A clue to the nature and function of the heat-stable factor was obtained in investigations of the effect of unsaturated long-chain fatty acids on the formation of glycerides from glycerolphosphate and palmitate24. Palmitoleic, oleic, linoleic and linolenic acid were found to stimulate the incorporation of palmitate and were themselves incorporated. This is shown in Fig. 2 with sub-cellular preparations from the mucosa of cat small intestine. At optimum concentrations of linoleate, the molar ratio palmitate: linoleate present in the glyceride fraction—more than 80 per cent triglycerides—was 2.6, indicating that mixed acid triglycerides may have been formed. Fig. 2 also shows the amounts of labelled acids present in phosphatidate. These decreased with increasing concentrations of linoleate in the assay system and it is interesting that the molar ratio of palmitate: linoleate was > 10. This may indicate that intermediates containing linoleate were preferentially incorporated into glycerides. For example, phosphatidate phosphohydrolase may act preferentially on a phosphatidate containing palmitate and linoleate compared with one containing only palmitate.

The synergistic action of palmitate and unsaturated long-chain fatty acids was not observed when mixing palmitate with saturated long-chain fatty acids nor was

it seen when the formation of triglycerides by the mono-

glyceride pathway was studied24.

Thus we concluded that if the heat-stable factor of the supernatant fraction involves unsaturated long-chain fatty acids, these acids ought to occur in appropriate amounts in the supernatant fraction, and furthermore their removal from the supernatant fraction ought to reduce its stimulating activity. Both these assumptions were proved to be correct²⁴. The supernatant fraction was found to contain up to 25 mumoles of free fatty acids/mg of protein of which about 43 per cent was unsaturated C₁₈-acids. The removal of all lipids including free fatty acids from the supernatant fraction by treatment with acetone resulted in a decreased stimulating activity (Table 2). When small concentrations of linoleate were added to a supernatant treated with acetone, the stimulating activity was restored to the level obtained with the same but untreated supernatant preparation. Although unsaturated fatty acids are a heat-stable factor it cannot be excluded at this stage that an unspecific protein in fatty acid free supernatant preparations is also heat stable and thus contributes to the overall stimulation of glyceride biosynthesis. The effects of unsaturated fatty acids are small when compared with those of the whole supernatant fraction. Nevertheless, the increased rate of formation of triglycerides from a mixture of palmitate

STIMULATION OF GLYCRRIDE BIOSYNTHESIS BY SUPBRIATANT INS USING A MITOCHONDRIAL FRACTION FROM RAT LIVER

Addition of protein	mµmoles of palmitate incorporated into glycerides
None	11
Boiled supernatant fraction (11.8 mg)	$\begin{smallmatrix}13\\311\end{smallmatrix}$
Supernatant preparation (0.9 mg)	
Supernatant preparation (0.9 mg) + boiled supernatan fraction (11.8 mg)	1t 474

The assay system contained, in a final volume of 3 ml., 16·6 mmolar Sörensen phosphate buffer, pH 7·4, 6·6 mmolar ATP, 0·033 mmolar CoA, 20 mmolar D.1-3-glycerolphosphate, 6·6 mmolar potassium fluoride, 2·25 mmolar reduced glutathione, 0·8 mmolar palmitate and 3·6 mg of mitochondrial protein. Incubation lasted 40 min at 37° C. Supernatant preparations were obtained from rat liver. Boiled supernatant fraction was the supernatant fraction (6.000,000 × g-min) heated for 3 min at 100° C. Supernatant preparation was 0-45 per cent ammonium sulphate precipitate of the supernatant fraction (6.000,000 × g-min).

Table 2. REFECT OF LINOLEATE AND OF DEFATTED SUPERNATANT PREPARA-TIONS ON THE INCORPORATION OF PALMITATE INTO GLYCERIDES USING A MICROSOMAL FRACTION FROM THE MUCOSA OF CAT SMALL INTESTINE

Additions	mµmoles of palmit incorporated into glycerides
None	25
Supernatant preparation (1.2 mg)	111
Supernatant preparation (1.2 mg) + linoleate (67 µmolar)	144
Defatted supernatant preparation (1.2 mg)	84
Defatted supernatant preparation (1.2 mg) + linoleate (67 \(\mu\text{molar}\))	149

The assay system contained, in a final volume of 3 ml., 16.6 mmolar potassium phosphate buffer, pH 7.4, 6.6 mmolar ATP, 0.083 mmolar CoA, 3.3 mmolar D.L.3-glycerolphosphate, 13.3 mmolar potassium chloride, 2.7 mmolar reduced glutathione, 1.2 mmolar palmitate and 0.9 mg of microsomal protein. Incubation was carried out for 20 min at 37° C. All supernatant preparations were obtained from the mucosa of cat small intestine. Supernatant preparations were obtained from the mucosa of cat small intestine. Supernatant preparation was 0.50 per cent ammonium sulphate precipitate of the supernatant fraction (6,000,000g). Defatted supernatant preparation was the same supernatant preparation which had been treated with nine volumes of acetone at 0° C to remove all free fatty acids**.

Table 3. STIMULATION OF MITOCHONDRIAL GLYCERIDE BIOSYNTHESIS BY VARIOUS PROTEIN PREPARATIONS

Addition of protein	mμmoles of palmitate incorporated into glycerides
None	19
Defatted bovine serum albumin (25.0 mg)	53
Rat serum (21.5 mg)	45
Lipoprotein-rich fraction from rat serum (4.8 mg)	74
Supernatant preparation (3.9 mg)	260

Assay system was that described in Table 1. The mitochondrial fraction and the supernatant preparation (Table 1) were obtained from rat liver. Other additions were made as indicated. Each protein concentration noted was that giving maximum stimulation.

and linoleate compared with that from palmitate alone points to a fatty acid specificity of one (or more) of the enzyme(s) participating in the formation of triglycerides by the glycerolphosphate pathway, although previous investigations of the enzymes synthesizing phosphatidate using a mixture of stearoyl-CoA and lineloyl-CoA failed to show a definite fatty acid specificity with respect to saturated and unsaturated long-chain fatty acids²⁵. These enzymes were shown, however, to have a preference for long-chain fatty acids^{2,26}. Finally, as the concentrations of unsaturated fatty acids which same order as those found in the cell, this stimulation may have physiological implications. It has been shown that unsaturated fatty acids can stimulate the absorption of saturated acids by the small intestine²⁷.

The Heat-labile Factors

1. Nature and specificity. The heat-labile factors present in the supernatant fraction $(6,000,000 \times g\text{-min})$ are inactivated by heating for 3 min at 70° C and can be precipitated by ammonium sulphate with complete recovery and between three and six fold purification²⁰. When treated with papain, the supernatant fraction loses its activity and gel filtration on 'Sephadex G-200' shows that the minimum molecular weight of the heat-labile supernatant factor(s) is 200,000 (ref. 20). These results clearly indicate the protein nature of the active principles involved.

As glyceride biosynthesis is usually measured by incubating glycerolphosphate and a particulate subcellular fraction with either palmitoyl-CoA or palmitate, the known inhibitory effects of palmitoyl-CoA and free fatty acids on various enzyme systems should be considered. These inhibitory effects can be alleviated by adding protein and in particular albumin to the assay system. Consequently, the heat-labile principle in the supernatant fraction might exert its effect merely by acting similarly to albumin, and there are reports that albumin, various lipoprotein fractions or a boiled microsomal fraction will enhance the formation of glycerides^{8,20,28} or phospha-The stimulating activity of these proteins is low compared with that of the supernatant fraction 20 (Table 3). The stimulating effect of albumin (or lipoprotein) and of low concentrations of the supernatant fraction is, in some instances, additive, but at high concentrations of supernatant fraction the stimulating

effect of albumin or lipoproteins is strongly reduced or absent. The stimulation by lipoproteins decreased when they were defatted²⁸ and so it is possible that at least part of the stimulation caused by native lipoproteins results from the presence of unsaturated fatty acids in these proteins.

Thus the heat labile factors of the supernatant fraction are proteins and only a small portion of the overall stimulation of glyceride biosynthesis caused by the supernatant fraction can be ascribed to unspecific (non-enzyme)

2. The role of phosphatidate phosphohydrolase. Phosphatidate phosphohydrolase has been known for many years³⁰ as has its occurrence in particulate subcellular fractions^{4,6,11,16}. It was usually assumed that the phosphohydrolase located in mitochondrial and microsomal fractions was the enzyme involved in the biosynthesis of complex lipids. The observations described below, which were made independently in two laboratories^{23,31}, throw great doubt on this assumption and point to the involvement of a soluble phosphatidate phosphohydrolase in glyceride biosynthesis.

Analysis of the intermediates and products formed during glyceride biosynthesis in mitochondrial preparations showed that in the absence of the supernatant fraction, most of the label incorporated into total lipids was recovered in phosphatidate³¹ (Fig. 3). When the supernatant fraction was added, the amount of labelled palmitate found in glycerides increased sharply while that found in phosphatidate decreased. Fluoride is known to inhibit phosphatidate phosphohydrolase activity*,** and so it was omitted from the assay system (Fig. 3). The degree of the shift from phosphatidate to glycerides with respect to the amounts of labelled palmitate found in these two lipids at the end of the incubation depended on the amount of supernatant protein addeds. With some preparations, however, a slight increase in the amounts of palmitate in phosphatidate was observed at low concen-

The effect of the supernatant fraction was thus similar to that observed with unsaturated fatty acids except that the magnitude of stimulation of glyceride formation was far greater (compare Figs. 2 and 3). It was therefore essential to exclude the concomitant effect of unsaturated long chain fatty acids. This was done by using a supernatant preparation treated with acetone and free from fatty acids (Table 4). Heating the treated supernatant preparation completely abolished the stimulation of

trations of supernatant fraction.

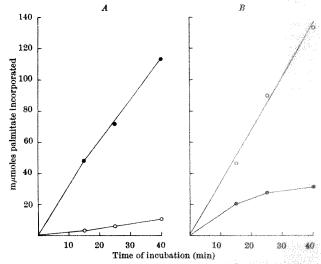


Fig. 3. The incorporation of palmitate into phosphatidate and giverides as a function of time using a mitochondrial preparation from rat liver. The assay system was that described in Table 1 except that potassium fluoride was omitted. A, 4.0 mg of mitochondrial protein; B, 4.0 mg of mitochondrial protein; B, 4.0 mg of mitochondrial protein +6.0 mg of protein of an ammonium sulphate precipitate of the supernatant fraction. • 1.00-palmitate incorporated into phosphatidate, and O, into giverids.

Table 4. EF EFFECT OF DEFATTED SUPERNATANT PREPARATIONS ON INCOR-OF PALMITATE INTO PHOSPHATIDATE AND GLYCHRIDES USING A MITOCHONDRIAL FRACTION FROM RAT LIVER

Addition of protein		les of palmit scorporated	ate
<u>-</u>		Glycerides	Total
None Defatted supernatant preparation (3.4 mg) Boiled defatted supernatant preparation	289 228	$\begin{array}{c} 10 \\ 147 \end{array}$	299 375
(3·4 mg) Defatted bovine serum albumin (3·1 mg)	330 329	13 11	343 340

Assay system was that described in Table 1. Supernatant preparations were obtained from rat liver. Defatted supernatant preparation was 0-45 per cent ammonium sulphate precipitate of the supernatant fraction (6,000,000 × g-min) treated with nine volumes of acetone at 0° C to remove all free fatty acids. Boiled defatted supernatant preparation was the same preparation heated for 3 min at 100° C.

glyceride formation, whereas the incorporation of palmitate into phosphatidate was increased to the same extent as that obtained with defatted albumin.

The results so far presented indicate that the supernatant fraction contained either phosphatidate phosphohydrolase activity or a protein which stimulated the mitochondrial phosphatidate phosphohydrolase. Table 5 shows that the first assumption is correct because heating the mitochondrial fraction did not decrease the formation of diglyceride. The substrate used in this experiment was labelled membrane-bound phosphatidate, which had been formed biosynthetically and freed from ATP and other cofactors by centrifugation through a sucrose density gradient³¹. It is interesting that there was very little hydrolysis of phosphatidate when the mitochondrial membrane fraction containing tightly bound phosphatidate was incubated in the absence of the supernatant fraction; this indicates that the phosphatidate phosphohydrolase present in the mitochondrial membrane preparation acted only slowly on the internal substrate.

Table 5. HYDROLYSIS OF MEMBRANE-BOUND PHOSPHATIDATE

Additions	 - Δ mµmoles of palmitate in phosphatidate 	+ Δ mμmoles of palmitate in glycerides
Membrane preparation + supernatant prepa-	6.8	< 2.0
ration Boiled membrane preparation + supernatant	47.8	83.4
preparation	63.0	58-4
Membrane preparation + boiled supernatant preparation	< 2.0	< 2.0

The preparation of the membrane-bound phosphatidate (membrane preparation) is indicated in the text. The membrane preparation (7.0 mg of protein) was incubated in a final volume of 2.0 ml. with 22 mmolar phosphate buffer, pH 7.4 and, where indicated, the supernatant preparation (9.9 mg of protein) for 10 min at 37° C. The supernatant preparation was 0-45 per cent ammonium sulphate precipitate of the supernatant fraction (6,000,000 × g-min). Boiled preparations were heated for 3 min at 100° C.

Phosphatidate phosphohydrolase is usually assayed with aqueous dispersions of phosphatidate and under these conditions the mitochendrial (or microsomal) phosphatidate phosphohydrolase is more active than the phosphatidate phosphohydrolase of the supernatant fraction (Table 6). The reverse is true, however, when membrane-bound phosphatidate is used as substrate. Of all the combinations recorded in Table 6, the supernatant fraction and membrane-bound phosphatidate gave the fastest reaction rate. Addition of freshly prepared mitochondrial fraction to membrane-bound phosphatidate did not bring about a hydrolysis of phosphatidate.

Although the results presented here refer to mitochondrial membranes, the same observations have been

Table 6. HYDROLYSIS OF MEMBRANE-BOUND PHOSPHATIDATE AND OF AQUEOUS DISPERSIONS OF PHOSPHATIDE

Source of enzyme	Physical state of substrate	mµmoles of phosphatidate hydrolysed/min/mg of protein
Mitochondrial fraction Mitochondrial fraction* Supernatant preparation Supernatant preparation	Aqueous dispersion Membrane-bound Aqueous dispersion Membrane bound	2·6 0·1 0·6
oupernment preparation		10-1

Membrane-bound phosphatidate was prepared as indicated in the test. Stable aqueous dispersions were prepared with phosphatidate obtained from egg phosphatidylcholine by enzyme hydrolysis. Each of the above combinations was assayed in slightly different conditions to obtain optimum reaction rates²¹. rates²¹.

* The mitochondrial enzyme was acting on the internal substrate.

made with microsomal fractions^{23,31}. Using either subcellular fraction, the phosphatidate phosphohydrolase activity of the supernatant fraction accounted for most of the stimulation of glyceride formation caused by the supernatant fraction.

If the phosphatidate phosphohydrolase of the supernatant fraction is involved in the biosynthesis of glycerides, it is surprising that of five enzymes participating in this biosynthesis, the phosphohydrolase is the only one found in the cell sap. Mitochondrial fractions prepared under mild conditions in 0.88 molar sucrose instead of the usual 0.3 molar sucrose still showed the same requirement for the supernatant fraction to form maximum amounts of glyceride. Furthermore, a supernatant fraction prepared in 0.88 molar sucrose had the same specific activity with respect to the hydrolysis of membrane-bound phosphatidate as a supernatant fraction prepared in 0.3 molar sucrose. Thus the primary subcellular localization of the enzyme hydrolysing the membrane-bound phosphatidate formed as an intermediate in the biosynthesis of complex lipids remains an open question. If, in the intact cell, this enzyme is bound to mitochondria and the membranes of the endoplasmic reticulum, its linkage is extremely weak.

 ${\bf 3.} \quad \textit{Further factors.} \quad \textbf{Although the presence of phosphati-}$ date phosphohydrolase activity, unsaturated long-chain fatty acids, and unspecific protein(s) in the supernatant fraction account for most of the stimulating activity, there is evidence suggesting that the supernatant fraction contains other stimulating factors.

Studies of the requirement for substrates and cofactors (glycerol phosphate, ATP, CoA and MgCl₂) showed that not even a small portion of the stimulation of glyceride formation by the supernatant fraction could be caused by the presence of any of the known cofactors in this frac-When assaying acyl-CoA synthetase or the synthesis of phosphatidate, however, small but significant stimulations were observed with supernatant preparations.

Using palmitate as substrate the mitochondrial acyl-CoA synthetase reaction was stimulated by 25-35 per cent on addition of 0.5 mg of protein of a supernatant preparation²⁰ (Table 7). For comparison, the data of Farstad, Bremer and Norum³³ are included which were obtained with a microsomal fraction from rat liver. While the exact mechanism of this stimulation is not fully understood, preliminary evidence suggests that the supernatant fraction contains an acyl-CoA synthetase kinase³⁴ the action of which might resemble that of the phosphorylase b kinase³⁵. The observations of Bar-Tana and Shapiro³⁶ may be relevant in this connexion. They found that the first part-reaction of a microsomal acyl-CoA

Table 7. STIMULATION OF ACYL-COA SYNTHETASE BY SUPERNATANT PREPARATIONS

Additions	mμmoles of palmitoyl CoA formed
Mitochondrial fraction	610
Mitochondria: fraction + supernatant preparation	820
Microsomal fraction	569
Microsomal fraction + supernatant preparation	880

All preparations were obtained from rat liver. Data referring to the mito-chondrial fraction are taken from Smith and Hübscher³⁰ and those referring to the microsomal fraction from Farstad, Bremer and Norum³².

Table 8. EFFECT OF SUPERNATANT PREPARATIONS ON INCORPORATION OF LABELLED GLYCEROLPHOSPHATE INTO PHOSPHATIDATE USING A MITOCHONDRIAL PREPARATION FROM RAT LIVER

Addition of protein	mµmoles of glycerol- phosphate incorporated into phosphatidate
None	62
Supernatant preparation (3.3 mg)	99
Boiled supernatant preparation (3.3 mg)	101

Assay system contained, in a final volume of 1.0 ml., 16.6 mmolar potassium phosphate buffer, pH 7.4, 3.3 mmolar ATP, 0.033 mmolar CoA, 8.6 mmolar L-3-glycerolphosphate (1 μ c./18.7 μ moles), 13.3 mmolar magnesium chloride, 50 mmolar potassium fluoride, 0.4 mmolar palmitate, 2.6 mmolar reduced glutathione and 1.0 mg of mitochondrial protein. Incubation was for 30 min. at 37° C. Supernatant preparation was 0.45 per cent ammonium sulphate precipitate of the supernatant fraction (6,000,000 × g-min). Boiled supernatant preparation was the same preparation heated for 3 min at 100° C.

synthetase required CoA and that the overall reaction rate of this enzyme was increased by preincubation in the presence of ATP and CoA. From this they decided that the active form of the acyl-CoA synthetase contains enzyme-bound CoA.

Finally, when assaying the biosynthesis of phosphatidate in the presence of high concentrations of potassium fluoride (50 mmolar) to inhibit all phosphatidate phosphohydrolase activity, we found a further small stimulation on adding a supernatant preparation (Table 8). stimulation was heat-stable, an observation already noted in the experiment quoted in Table 4. In that experiment an acetone-treated supernatant preparation was used, and so the heat stable stimulation quoted in Table 8 could not have been caused by unsaturated fatty acids. This observation agrees with those of Lands and Hart29, who reported an increased phosphatidate formation on addition of albumin or a boiled microsomal fraction. The mechanism of this stimulation is unknown but the participation of an acyl carrier protein similar to that studied with preparations from E. coli³⁷ or Cl. butyricum³⁸ cannot be excluded.

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Model for Origin of Monosaccharides

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Formaldehyde, alumina and two naturally occurring aluminosilicates, kaolinite and illite, were refluxed with an aqueous solution of formaldehyde. Formaldehyde was converted to monosaccharides. This provides the basis for a model for the primordial origin of monosaccharides.

In 1861 Butlerow demonstrated that formaldehyde could be converted into a mixture of monosaccharides when heated in aqueous alkaline solution. Others²⁻⁸ have studied extensively the base-induced condensations of formaldehyde. All these experiments involved strongly alkaline media using relatively high concentrations of formaldehyde.

The opinion that because formaldehyde is readily converted to sugars it can be regarded as a primordial precursor of sugars is strengthened by the observation that formaldehyde is one of the products formed when a mixture of methane, ammonia and water, the presumed primitive atmosphere of the Earth, is exposed to either an electric discharge or ionizing radiation 10,11. Further evidence in favour of formaldehyde as a possible intermediate in primitive sugar synthesis comes from the observation that several sugars, including ribose and deoxyribose, are formed by the action of ultraviolet light on a dilute solution of formaldehyde12.

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Some objections have been made to the general acceptance of formaldehyde as a precursor of sugars on the prebiotic Earth. Horowitz and Miller 13 objected to the high concentrations of formaldehyde used in some of the base-induced condensations of formaldehyde as unrealistic in primordial conditions. Abelson¹⁴ maintains that large bodies of water on the Earth were never highly alkaline and that the pH of the oceans has remained fairly constant. A serious difficulty arises because in the absence of an inhibitor, such as an alcohol, formaldehyde rapidly forms paraformaldehyde at moderate temperatures. If ammonia was abundant in the early atmosphere of the Earth, the highly stable compound, hexamethylenetetramine, would also be expected to be the predominant product of formaldehyde condensations.

For these reasons it was decided to examine the possible polymerization of formaldehyde by the action of boiling water in the presence of clay. In these conditions, paraformaldehyde would depolymerize, and there would be a minimum amount of dissolved ammonia present. Such a situation may reproduce more faithfully prebiological

conditions which could have occurred at hydrothermal springs. The microcrystalline aluminosilicate minerals are considered to have -0.66 equivalents of negative charge/unit cell¹⁵, and so they would probably be capable of inducing the condensation of formaldehyde to sugars.

The alumina, chromatography grade, was obtained from the J. T. Baker Chemical Company. The clay minerals were obtained from Ward's Natural Science Establishment and were classified as Kaolinite No. 17 from Lewiston, Montana, and Illite No. 35 from Fithian, Illinois. Kaolinites have the approximate empirical formula (OH)₈Si₄Al₄O₁₀ and illites have the formula

$${\rm (OH)_4} K_{1^-1\cdot 5} ({\rm Si_{6\cdot 5-7}Al_{1^-1\cdot 5}}) \ ({\rm Al_4Fe_4} Mg_4) {\rm O_{20}}$$

In addition, kaolinite is composed of a single silica tetrahedral sheet and a single alumina octahedral sheet, whereas illite has two silica tetrahedral sheets with a central alumina octahedral sheet¹⁵.

In preliminary qualitative experiments, 50 ml. of a 0.33 molar solution of aqueous formaldehyde was refluxed with a suspension of 10 g of either alumina or one of the two samples of aluminosilicate. After an arbitrary refluxing period of 5 h, the suspended material was removed by either filtration or centrifugation and the clear solution was evaporated at reduced pressure to a syrup. The mixture was separated by paper chromatography with butanol: ethanol: water (4:1:1 by volume) in one direction and butanol: pyridine: water (9:5:8 by volume) in the other. The sugars were located on the chromatogram by spraying with aniline hydrogen phthalate¹⁶. The groups of sugars were identified as hexoses, pentoses, tetroses and trioses by comparing their colour reaction with aniline hydrogen phthalate and the R_F values with those of standard monosaccharides. At this concentration of formaldehyde, hexoses were detected only by prolonged refluxing in an inert atmosphere.

In a control experiment, samples of the aluminosilicate minerals were subjected to extraction with boiling water for 5 h. No sugars were detected in the extracts. Siurries of alumina, kaolinite and illite in water exhibited a pH

of 7.4, 4.2 and 5.9, respectively.

A quantitative measure of the conversion of formaldehyde to carbohydrates was carried out by adding 0.1 mc. of ¹⁴C-formaldehyde to the 0.33 molar aqueous formaldehyde solution. Duplicate samples were chromatographed on thin-layer cellulose plates with butanol: pyridine: water (9:5:8) and butanol: ethyl acetate: water (7:1: 2). The radioactive areas were determined by autoradiography, after which the principal radioactive areas were removed from one of the plates. The activity of the individual areas was compared with the total amount of radioactive material applied to the plate. The areas were then identified by spraying the duplicate plate with aniline hydrogen phthalate. The alumina-induced condensation reaction yielded 22 per cent pentoses, 17 per cent tetroses and 38 per cent trioses plus glycolaldehyde. A similar experiment, substituting kaolinite for alumina, produced $3 \cdot \hat{0}$ per cent pentoses and $3 \cdot 9$ per cent tetroses and smaller units. Increasing the refluxing time to 24 h did not appreciably change the yields of monosaccharides (Table 1).

In an attempt to determine the lowest concentration of formaldehyde at which a detectable amount of monosaccharides would still be formed, the previously described condensation reactions were carried out at formaldehyde concentrations of 10^{-3} molar and 10^{-2} molar. A mixture

Table 1 Experiment 1 Alumina Experiment 2 Kaolinite Concentration of formaldehyde 0.33 M 10-8 M 0.33 M 10-4 M per cent Hexoses 3.3 Pentoses 3.0 Tetroses Trioses 3.9

of 0·2 ml. of 10⁻² molar formaldehyde (0·025 mc.), 1·8 ml. of water and 0·5 g of alumina was refluxed under an inert atmosphere for 5 h. The solution was concentrated and chromatographed with a mixture of monosaccharide standards. An autoradiograph was compared with the chromatogram after it had been sprayed with aniline hydrogen phthalate. Glycolaldehyde seemed to be formed but no other sugars were present. Most of the radioactive material remained near the origin. Prolonged refluxing resulted only in the disappearance of the glycolaldehyde.

In a second series of experiments, 0·1 mc. of ¹⁴C-form-aldehyde (0·79 ml. of 10^{-2} molar) was added to 10 ml. of 10^{-2} molar formaldehyde and 2 g of alumina. After refluxing for 5 h, the solution was concentrated and thin-layer chromatography was used again. The conversion to pentoses was 2·3 per cent and the combined conversion to tetroses and tioses was 2·4 per cent. It is surprising, in view of the results at higher concentrations, that there was a yield of 4·0 per cent hexoses. When kaolinite was substituted for alumina 3·3 per cent hexoses, 3·0 per cent pentoses and 4·4 per cent tetroses and trioses were produced. Refluxing for 24 h in an inert atmosphere resulted in a decreased yield of monosaccharides. As in previous experiments, most of the radioactive material remained near the origin.

Using the procedure of Mills¹⁷, ribose was electrophoretically separated from the pentose fraction produced from a 0·33 molar radioactive formaldehyde solution refluxed with alumina. In these conditions the total conversion of formaldehyde to ribose was 3·8 per cent. The results obtained with illite were similar to those found with kaolinite and no quantitative determinations were made.

Since the middle of the nineteenth century, it has been known that aqueous formaldehyde will undergo condensation to a mixture of sugars in the presence of alkali. It has been observed that for this reaction polyvalent and divalent bases serve as better catalysts than mono-Tetramethylammonium hydroxide is valent bases. completely devoid of catalytic activity¹⁸. It has also been observed that the initial rate of condensation is slow; that is, there appears to be what has been called by some investigators^{7,8} a "lag phase" followed by a much more rapid rate of reaction. This "lag phase" was found to be eliminated by the addition of small amounts of glyceraldehyde or dihydroxyacetone^{7,8,18}. These observations can be rationalized by recognizing that there are two distinct reactions involved in the condensation of formaldehyde to the sugar mixture.

It is obvious that the first condensation to occur is that of two molecules of formaldehyde to form glycolaldehyde. Further reactions of the glycolaldehyde with formaldehyde can then proceed through aldol-type condensations and enolizations to yield the sugar mixture. The first reaction, however, implies that the carbon atom of a molecule of formaldehyde must become a nucleophilic centre in order to attack the carbon atom of a second molecule of formaldehyde.

Formaldehyde exists primarily as its hydrate in aqueous solutions (ref. 19 and (1)). The two oxygen atoms would exert an inductive effect on the carbon atom, decreasing

its electron density and thereby stabilizing the anion which would result from the ionization of a proton

attached to carbon. Co-ordination of the oxygens with divalent cations (2) would greatly enhance this effect, which would explain in part the greater efficacy of divalent oxides as catalysts. It should be noted that the

microcrystalline clay minerals usually have alkaline earths, alkali metals and protons adsorbed as cations on their surfaces15. Franzen and Hauck20 have reported that alkaline earth cations form salts with aqueous formaldehyde, and although Staudinger et al.21 have shown that there is no definite composition to these materials, they are probably co-ordination complexes of the metals with water, hydroxide ions, and a variety of chain lengths of partially polymerized formaldehyde.

The polyvalent cations, which are Lewis acids, also co-ordinate with the carbonyl oxygen atom of nonhydrated formaldehyde, rendering the carbon atom much more subject to nucleophilic attack. It can further be expected that the polyvalent cations would also stabilize the enolate anions of the hydroxy aldehydes and ketones

In this work, it has been shown that a dilute (10-2 molar) solution of formaldehyde can be condensed to a mixture of sugars in conditions designed to simulate a primordial hydrothermal spring. An alkaline medium is unnecessary for this condensation to take place. The reaction takes place at mildly acid and neutral pH. The basic sites on the crystal lattice of the alumina and aluminosilicates probably serve to remove protons from the formaldehyde and various intermediates absorbed on the mineral surfaces, thereby providing the necessary reactant molecules.

A reasonable rationale for the production of hexoses in 10-2 molar solution of formaldehyde and not in a 0.33 molar solution is that in dilute solution an individual reaction intermediate will be absorbed for a longer time on the mineral surface. Conversely, in a more concentrated solution, the reaction intermediate is more quickly displaced from the mineral surface by the other solute molecules. It can be expected that the longer any given reactant remains on the active surface of the catalyst the greater are its chances of undergoing further reactions to form products of higher molecular weight.

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Synthesis of Sugars in Potentially Prebiotic Conditions

The formation of sugars from formaldehyde has been studied extensively1, but the reaction has usually been carried out in conditions too extreme to have existed on the primitive Earth. The claim that relatively pure ribose can be obtained by boiling formaldehyde with a suspension of calcium carbonate2 led us to investigate the catalytic activity of "carbonate-apatite". apatite, a very common phosphate mineral, is inactive; we argued that if carbon dioxide was abundant at any stage in the evolution of the ocean, carbonate incorporation into apatite must have been extensive.

A slurry of "carbonate-apatite" prepared freshly by the method of Hayek et al.3, was found to have a pH of 8.5. On boiling with 0.5 molar formaldehyde, it gave yields of up to 40 per cent of sugars. There was an initial induction period of several hours during which no sugars could be detected, then glycolaldehyde, trioses, tetroses, pentoses and hexoses appeared successively. Sugars were separated by chromatography in butanol-acetic acidwater (4:1:5, upper phase) and in acetic acid-pyridinewater (7:3:2) and estimated using the acid aniline Pentoses are present in maximal phthalate spray¹. amount after about 12 h and hexoses after about 24 h. When boiling was continued for longer periods the solution darkened and the sugars gradually disappeared; the hexoses persisted longest. We obtained similar results when we used calcium carbonate as a catalyst, but the whole sequence of reactions proceeded more slowly.

When this experiment was repeated using more dilute formaldehyde, we could still detect sugars from 0-01 melar solution but not from 0.001 molar. The minimum detectable yield in the latter experiment was 1-2 per cent. In these experiments, we used formaldehyde labelled with carbon-14 to obtain greater sensitivity in our analyses. The experiment was discontinued after 14 days. These results are similar to those reported by Gabel and Ponnamperuma4, who used a suspension of Al₂O₃ as catalyst.

We do not believe that the formose reaction as we and others have carried it out is a plausible model for the prebiotic accumulation of sugars. First, it requires concentrated formaldehyde solutions and, second, the sugars formed are decomposed quite quickly. If formaldehyde is the prebiotic precursor of ribose, some method of stabilizing the sugars is essential. The formation of ribosides of the natural bases-for example, adenine-is one possibility, but attempts to bring about this condensation have not been successful. Instead, rather unstable adducts were formed.

The formation of sugars in plausible conditions and their incorporation into nucleosides have not been achieved. Until the problem is solved or by-passed it remains a weakness in theories of abiotic nucleic-acid synthesis.

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Study of Microbial Evolution through Loss of Biosynthetic Functions: Establishment of "Defective" Mutants

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by

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On appropriate media, mutants which have lost a biosynthetic function may exhibit a selective advantage over parental strains. Is this how "parasitism" became established?

THE availability of complex media (such as higher organisms as hosts) was followed by the appearance of fastidious micro-organisms, defective in one or more biosynthetic functions. Lwoff¹, in his study of the establishment of parasitism, anticipated that, on adequate media, auxotrophic mutants could have a selective advantage over prototrophic parents because of energetic economy (fewer biosynthetic steps). Such an explanation would necessitate the assumption that the prototroph would continue to manufacture the particular metabolite, although it could simply take it from the medium as the auxotroph does. Since Lwoff's work1, feedback inhibition and gene repression mechanisms have been demonstrated which tend to suppress the biosynthetic steps of the prototroph if the end-product is available in the medium. it appeared debatable whether or not a prototroph is at a selective disadvantage compared with an auxotroph.

Here we present evidence which shows that, on adequate media, "defective" mutants, auxotrophs and others, indeed have a distinct selective advantage over the parental strain. The mechanisms of feedback inhibition and/or gene repression which would help the parental strain to economize are not universal and/or not complete enough to offset the advantage that the "defective" strain has in not being involved at all in this particular biosynthetic process. The results also indicate that the more biosynthetic steps dispensed with by the auxotroph (early block; final metabolite available) the more advantage it has. Furthermore, a de-repressed strain, producing more of the final metabolite, is also at a selective disadvantage with respect to the normal repressed one. The work has also been extended to the case in which the actual block is unknown (mutation to azide resistance).

The organism we used was Bacillus subtilis strain 168, its mutants and back-mutants. The purpose of using a back-mutant rather than some wild strain from the "collection" was to ascertain that the two strains studied differ by a single mutational step in the structural gene studied; this is, obviously, of primary importance. To study the selective advantage of one strain over another, the two were grown together in liquid minimal medium with appropriate supplements, and the population changes observed; it has been shown that the comparison of growth rates of the two strains cultivated separately may

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not lead to a reliable estimate of their selective values.

For a more precise determination of the selective behaviour, the strains were grown in a medium of constant

composition (continuous culture), in an improved version (unpublished work of Eichhorn, H. H., and Morimoto, H.) of the chemostat of Novick and Szilard⁴. At zero time, a sample (1 ml.) of a chemostat culture of one strain was added to a 20 ml. culture of the other strain in a second chemostat. In cases where approximately equal starting numbers of cells of both strains were desired, the contents of both chemostat tubes were mixed and 20 ml. introduced into a third chemostat. The chemostats were set at generation time 4 h (37°C; glucose limiting: 0.55 mmolar). At 24 h intervals (six generations) aliquots of 1 ml. were removed, diluted, spread on appropriate agar media and the proportions of the cells of each strain were determined

(Figs. 1 to 5).

Fig. 1 refers not to auxotrophs but to an azide-resistant mutant. When the spontaneous mutant 168/azi (resistant to 2 mmolar sodium azide on Penassay agar plates) was grown in the chemostat together with its azide-sensitive parent 168 in the absence of azide, it had a strong selective disadvantage: the proportion of 168/azi decreased 100fold in eighteen generations (Fig. 1). The decrease is at first exponential; the curves subsequently flatten out because of the appearance of additional 168/azi cells as a result of spontaneous mutations. The curves for two different ratios of 168/azi to 168 at zero time are parallel; this means that the azide-sensitive strain 168 has an intrinsic selective advantage and does not produce any inhibitor specific against 168/azi. Such inhibitors have been reported^{5,6} but seem to be rare cases. Another example of selection against a "drug" (mutagen)-resistant mutant has been reported recently. Such results may explain why a high proportion of azide-resistant mutants (and probably "drug" resistant mutants in general) does not usually occur in wild populations.

The nature of the genetic injury resulting in azide resistance is not known. More information is available in the case of auxotrophs. When the histidine requiring (his-) mutant 21, obtained by heat^{8,9}, was grown together with its histidine non-requiring spontaneous back-mutant (his+), in the presence of 0.32 mmolar L-histidine, it had a strong selective advantage over his+; the proportion of the latter decreased 4,000-fold in forty-eight generations (Fig. 2). Again, the curves are essentially parallel regard-

less of the initial ratio of the two strains, indicating that the auxotroph his- has an intrinsic selective advantage over its prototrophic parent his+ and is not producing some inhibitor specific (or more active) against his+. The concept that the selective advantage of auxotrophs over prototrophs is not the result of some specific inhibitors is also supported by the generality of the phenomenon.

An indole requiring auxotroph (ind-) in the presence of 0.25 mmolar L-tryptophan exhibits a strong selective advantage over its indole non-requiring back-mutant ind+ (Fig. 3, curves t): the proportion of the latter decreased more than 10^s-fold in fifty-four generations. The viability of chemostat cultures of ind- and ind+ strains before mixing was estimated by comparing the viable count on Penassay plates with the total count in a Petroff-Hauser counter. The average values were 97 per cent for ind- and 100 per cent for ind+. Thus the selective advantage of ind- was not due to its lower mortality.

The site of genetic injury in the his- mutant 21 is unknown; but the mutation in (ind-) is known to result in the absence of indole glycerol phosphate synthetase. In both cases, when the final metabolite (histidine or tryptophan) is present, the block may represent an omission of one or more synthetic steps that follow the blocked one; thus the auxotrophs have a definite selective advantage over the prototroph. Ind- strain, on tryptophan, did not have to (and could not) perform any steps towards the biosynthesis of tryptophan. It seemed of interest to study whether the advantage of this auxotroph over the prototroph is less pronounced when the two are grown on indole instead of tryptophan—that is, when the auxotroph still has to perform one step: conversion of indole to tryptophan. Curve i on Fig. 3 shows the result; the advantage of the auxotroph over the prototroph is not as pronounced as when the two strains are grown on tryptophan (curves t on Fig. 3).

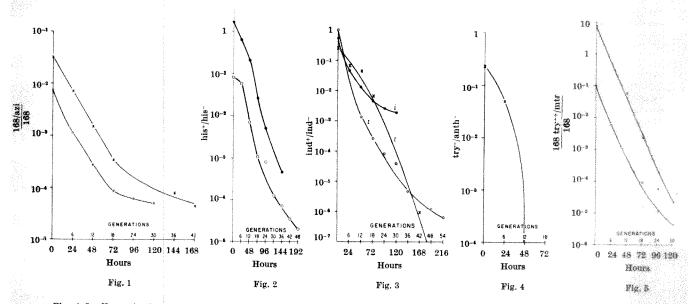
We then investigated whether an earlier block in the same pathway, such as the inability to synthesize anthranilate, has a selective advantage over a late block: in the presence of tryptophan the mutant blocked earlier should manufacture fewer intermediates than the mutant blocked further in the pathway. Moreover, if the point mutation in an early block were polar it would decrease the amount of enzymes made by the following cistrons

and should increase the selective advantage of the mutant-Anthranilate-requiring (anth-) strain SB194 (obtained from Dr E. Nester) was grown together with tryptophan-requiring (try-) strain T3 lacking tryptophan synthetase B (obtained from Dr C. Anagnastopoulos), in the presence of 0.25 mmolar L-tryptophan (Fig. 4). As expected, the anth- strain exhibited a strong selective advantage over try-: the latter disappeared in less than 48 h (twelve generations). In this case, the better growth of the anth-strain could be observed even before mixing, when the two strains were grown separately for 3 days in their chemostats: the try- mutant reached a cell density of

 4.2×10^7 /ml., whereas anth- reached 5.7×10^7 / ml. This predictable behaviour may serve as a tool in cases where one wants to predict which of the several existing pathways or bypasses is most economical and

will be used preferentially by the cell. The genes involved in the biosynthesis of tryptophan in E. coli are known to form an operon, with the operator situated in the anthranilate region 19,11; tryptophan itself (and its analogues such as 5-methyltryptophan) inhibits the synthesis of tryptophan, presumably by contributing to the repression of the operon. In B. subtilis the situation may be similar; the locus influenced by 5-methyltryptophan is also situated in the general location of the anthranilate region 12,13. These results indicate, however, that in the wild strain the repression of tryptophan synthesis by tryptophan may not be complete: some tryptophan synthetase is being made and some tryptophan synthesis may still go on, in contrast to the auxotroph in which this synthesis is completely blocked; the auxotroph can thus be at an advantage. For other pathways which are not controlled at all by a repressible operator, an auxotroph may have even more pronounced advantages. A similar situation may exist for those pathways which are controlled by feedback inhibition which in vivo is not complete.

It seemed of interest to study whether a "de-repressed" mutant is at selective disadvantage with respect to its wild parent. Presumably, in such mutants, the operator is not influenced by tryptophan and its analogues; such strains often contain high levels of tryptophan synthetase^{12,12} and excrete tryptophan¹³. One such "derepressed", spontaneous mutant, resistant to 5-methyl-



Figs. 1-5. Change in the ratio of cell numbers of *B. subtilis* strains indicated on the ordinate on growing them together in a chemestat in minimal broth for the number of hours or generations indicated on the abscissa. The two curves on Figs. 1, 2 and 5, and t on Fig. 3, refer to two different starting ratios. See text for strain designation. Total viable counts: Fig. 1, 2·3 to 2·6 × 10* fml.; Fig. 2, 8 × 10* to 4 × 10* fml.; Fig. 3, 6 × 10* to 1·1 × 10* fml.; Fig. 5, 5 to 6 × 10* fml. Mean viability over 90 per cent. Media supplements: Figs. 1 and 5, unsupplemented; Fig. 2, excess of L-histidine (0·32 mmolar); Fig. 3, excess of L-tryptophan (curves t) or of indole (curve t) (0·25 mmolar); Fig. 4, excess of L-tryptophan (0·25 mmolar).

tryptophan and excreting tryptophan (168 try++/mtr), was grown together with its wild parent 168 in unsupplemented basal medium. It can be seen (Fig. 5) that this "de-repressed" mutant is indeed at a distinct selective disadvantage: its proportion decreased 105-fold in twenty-six generations. Again, this result is independent of the initial proportion of the two strains, indicating an intrinsic selective disadvantage of the "de-repressed" strain rather than the action of a specific inhibitor manufactured by the wild parent. It seems most probable that the disadvantage of the "de-repressed" strain is its useless (uncontrolled) production of large amounts of tryptophan; it is likely that this is why a high proportion of "derepressed" mutants does not occur in wild populations.

These examples help to predict evolutionary trends by means of studies of competitive behaviour. Such studies can probably be extended to include substances other than simple metabolites. For example, the foregoing cases of gene inactivation (his-, ind-, try-, anth-) were results of point mutations. In such cases the economy is in the metabolite only: the structural gene, the messenger RNA, and in many cases (CRM+) the inactive enzyme itself, still have to be manufactured. If, however, such mutants were deletions resulting in the elimination of a DNA segment, and the shortening of the chromosome¹⁴, the additional advantages may include faster replication of the chromosome and saving on sugars, bases, aminoacids and ATP otherwise necessary to synthesize this portion of DNA, messenger RNA and protein. If this is true, a mutant with a deletion would be at a distinct selective advantage over a point mutant and would

supersede the latter. For example, study of tryptophan requiring wild strains of Streptococcus faecalis (ATCC 8042, 8043 and 9790) reveals that in these species the strains occurring in nature have lost one or more of their structural genes (for tryptophan biosynthesis) by deletions and not by point mutations (unpublished results of Zamenhof, S., and Heldenmuth, L. H.). Such mechanisms then could account for the complete disappearance of certain genes during the process of evolution.

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Erythrocyte Substitute for Perfusion of Brain

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In a simulated blood plasma 'FX-80', a liquid fluorocarbon with a high solubility for oxygen, maintains the electrical activity of a preparation of isolated rat brain. This is as effective as a suspension of erythrocytes in the same simulated blood plasma.

A SUBSTITUTE for whole blood must be able to transport oxygen, carbon dioxide and glucose (or other metabolic substrate). Its composition must permit exchange of water and ions with extracellular fluid while it maintains the steady state composition of the tissues it perfuses. The many substitutes for blood plasma which have been widely used are able to perform all these functions except the adequate transport of oxygen and carbon dioxide. There has hitherto been no useful substitute for the function of the haemoglobin of the erythrocyte. Haemoglobin is not unique in its ability to carry oxygen. Certain complexes of cobaltous salts are able to reversibly bind oxygen without being oxidized1,2, but attempts to make use of these substances as functional substitutes for haemoglobin have not been successful. The relatively high solubility of oxygen and carbon dioxide in some nonpolar liquids offers the possibility of transporting these gases in the physically dissolved, chemically unbound form. Such a simple mode of transport of oxygen may be better than transport as bound molecules, especially at low oxygen tensions.

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A liquid fluorocarbon designated as 'FX-80' (manufactured by the 3M Company, St Paul, Minnesota) has a high solubility for oxygen and carbon dioxide. 'FX-80' is composed predominantly of perfluorobutyltetrahydro-furan and its isomers. When saturated with oxygen, it contains 0.63 ml. of the dissolved gas/ml. (personal communication from J. W. Sargent). This capacity is greater than that of human erythrocytes which when fully saturated contain about 0.46 ml. of oxygen/ml. of cells3. The diffusion of oxygen through the fluorocarbon is quite rapid and it has been possible for mice to survive complete immersion for hours in 'FX-80' into which oxygen is bubbled4.

Gollan and Clark⁵ reported that isolated rat hearts continued to contract vigorously when perfused alternately with oxygenated FX-80° and with oxygenated diluted blood. Presumably, the perfusion with 'FX-80' alone could not be continuous because of its inability to transport substrate to the perfused tissue or to maintain ionic equilibrium. Water and polar substances such as glucose and salts are virtually insoluble in 'FX-80', and so this fluorocarbon cannot carry out the functions of whole blood in the perfusion of an organ, but it should be

able to carry out the functions of the erythrocytes in transporting oxygen and carbon dioxide on the basis of the high solubilities of these gases in the fluorocarbon.

The problem then is to convert the liquid fluorocarbon into particles which are approximately the size of erythrocytes and which are stable when suspended in blood plasma or a fluid resembling it. We have accomplished this by ultrasonic treatment of a mixture of FX-80' and a simulated blood plasma.

We have evaluated the ability of such a dispersion of 'FX-80' to transport oxygen and carbon dioxide and perform other functions of blood by using it to perfuse

an isolated rat brain preparation⁶.

The simulated blood plasma used was an 8 per cent solution of bovine serum albumin in Krebs-Ringer bicarbonate buffer solution prepared as previously described. To a 10 ml. portion of the simulated plasma in a Rosett cooling cell immersed in an ice bath was added 2 ml. of 'FX-80' and this mixture was insonated at 110 W power output for 20 sec with a Branson model W-185C cell disruptor fitted with a 0.5 in. tip. The dispersion so obtained has the appearance of homogenized milk. Microscopic examination of the dispersion showed that the 'FX-80' was dispersed quite uniformly into particles which were about 2-3µ in diameter. The dispersion was quite stable and showed only slight sedimentation after standing several hours. The dispersed material could be sedimented by centrifuging and the sedimented material was easily resuspended without further insonation. A sample of the dispersed material was washed with water by successive centrifugation to remove all soluble protein. The washed sedimented material was analysed by the Lowry method (Folin-Ciocalteau reagent) and found to contain about 5 per cent protein.

Insonation of 'FX-80' in water or buffer solution not

containing protein did not yield a stable dispersion.

The preparation and behaviour of our isolated perfused rat brain preparation have been described. The "blood" which has been used for perfusing this isolated brain is a suspension of washed dog erythrocytes in the same bovine albumin solution which was used to prepare the 'FX-80' dispersion. We have measured the electrical activity and some metabolic functions of the isolated brain when perfused with the 'FX-80' dispersion and compared the results with those obtained in identical experiments except that the "blood" was used for perfusion. In all experiments, closed circuit perfusion was performed at 25° C using 5 per cent carbon dioxide-95 per cent oxygen

and an initial glucose concentration in the perfusion fluid of 200 mg/100 ml. The perfusion fluid was always freshly prepared just before each experiment. In the perfusion fluid the volume of erythrocytes or of the dispersed 'FX-80' was approximately 20 per cent of the total volume. Bipolar electroencephalograms (EEG) were recorded from each side of the brain from four electrodes placed in direct contact with the bone in the frontal ann parietal regions on both sides. Samples of perfusiod fluid collected at intervals during the perfusion were analysed for glucose by a glucose oxidase method using the commercial reagent 'Glucostat' (Worthington Biochemical Co.); lactate was determined enzymatically with lactic dehydrogenase7. In some experiments, measurements of the pH, oxygen tension and carbon dioxide tension of perfusion fluid were made on samples drawn into capillary tubes from the arterial side (from the perfusion line just before the entrance to the brain) and from the venous side (from the superior sagittal sinus through a small hole in the bone and dura).

Table 1. DURATION OF MAINTENANCE OF EEG ACTIVITY OF AN ISOLATED, PERFUSED RAT BRAIN PREPARATION

Perfusion fluid	No, of experiments	Amplitude > 5 μ V (min)	Amplitude $<5 \mu V$ (min)
"Blood" ' FX -80' dispersion Simulated plasma	$^{10}_{10}_{3}$	46-180 (average 115) 44-245 (average 131) 0-4 (average 1)	10-135 (average 62) 23-181 (average 69) 0-2 (average 1)

We have shown that the spontaneous electrical activity of our perfused brain preparation is a very sensitive criterion of its functional and metabolic condition; cessation of perfusion for only 30 sec causes almost complete loss of electrical activity. In the present series of experiments, we have found that when the isolated brain was perfused with the bovine albumin solution alone, the spontaneous EEG never persisted more than 5 min When the perfusion was performed with either "blood" (washed dog erythrocytes in the same bovine albumin solution) or the 'FX-80' dispersion (also in the same albumin solution), the spontaneous EEG usually persisted for more than 2 h. In Fig. 1 are shown portions of the EEG recordings obtained in a representative experiment in which the 'FX-80' dispersion was used and similar recordings from an experiment using "blood". Table 1 lists the results of the series of perfusion experiments which provide a comparison of the ability of ${}^{\circ}FX$ -80' dispersion and artificial blood to maintain the EEG of the isolated rat brain. The duration of the EEG has been

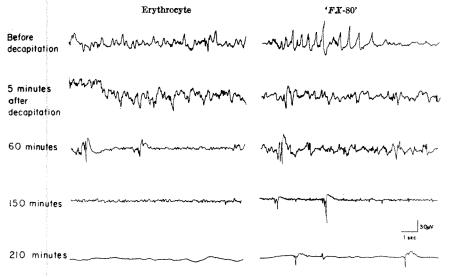


Fig. 1. The spontaneous EEG activity of an isolated rat brain preparation perfused with "blood" (recordings on the left) and another perfused with 'FX-80' dispersion (recordings on the right) at various times.

divided into a period when the amplitude of the activity is more than 5 μV and another when it is less. This was done because it is sometimes difficult to determine the exact time at which activity has ceased completely. Sometimes, after activity has been absent for a time, spontaneous seizure activity appears. It is therefore thought that a comparison of the periods of maintenance of activity greater than 5 µV is a more reliable one. The course of the changes in amplitude and frequency of the EEG with perfusion time was very similar for both the "blood" and 'FX-80' experiments. These results show that there is little difference between "blood" and 'FX-80' dispersion in ability to maintain the electrical activity of the brain and suggest that the dispersed 'FX-80' is adequately performing the gas transport functions which the erythrocyte ordinarily does.

Table 2. GLUCOSE CONSUMPTION AND LACTATE PRODUCTION OF ISOLATED RAT BRAIN PREPARATIONS PERFUSED WITH "BLOOD" OR 'FX-80' DISPERSION

Perfusion fluid	No. of ex- periments	Glucose consumption (mg/h)	Lactate production $(\mu \text{moles/h})$
"Blood" "FX-80" dispersion	5	4·0-6·8 (average 5·3)	12–30 (average 20)
	5	5·1-7·6 (average 6·7)	21–33 (average 26)

The changes with time in the glucose and lactate concentrations of the perfusion fluid for representative experiments with "blood" and 'FX-80' dispersion are shown in Fig. 2. The curves for glucose concentration are almost the same for both types of perfusion fluid. The lactate concentration curves are similar except that the lactate values are initially somewhat higher for the perfused "blood" samples and remain so. Even after exhaustive washing of erythrocytes some lactate is always present and the erythrocytes are producing small amounts of lactate continuously. From the analytical data, we have calculated the glucose consumption and the lactate production during the first hour of perfusion and these values are listed in Table 2. The differences between the values for the two perfusion fluids are not large but the values for the 'FX-80' experiments are significantly larger, both glucose consumption and lactate production being about 30 per cent greater.

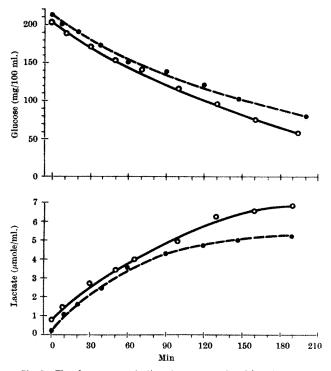


Fig. 2. The glucose concentrations (upper curves) and lactate concentrations (lower curves) of isolated rat brain preparations perfused with "blood" ($\bigcirc-\bigcirc$) or with 'FX-80' dispersion ($lue{lue{lue{---}}}$).

The differences in pH between the "arterial" perfusion fluid and the "venous" fluid were nearly all between 0.05 and 0.12 U ("arterial" always greater) and these differences showed no significant relation to perfusion time. The absolute values and the differences were very similar for the "blood" perfusion and 'FX-80' perfusion experiments.

The oxygen tension of the "arterial" fluid was between 400 and 500 mm of mercury in all experiments at all perfusion times. In the experiments with "blood", the difference in oxygen tension between the "arterial" and "venous" fluid was about 300 mm during the first hour of perfusion and decreased to 150-200 mm after 2 h of perfusion. In the experiments with 'FX-80' dispersion, these differences in oxygen tension were 200-250 mm during the first hour and they dropped to less than 100 mm after 2 h of perfusion. The smaller arteriovenous differences for 'FX-80' dispersion might be expected on the basis of the greater total amount of oxygen carried by 'FX-80' than by erythrocytes. These measurements were not of high precision, chiefly because of some difficulty in obtaining the samples of "venous" fluid. The results, however, demonstrate that there is a significant arteriovenous difference and that its order of magnitude is about the same with both "blood" and 'FX-80' dispersion. When substrate is absent from the perfusion fluid, the electrical activity of the perfused brain persists for only a relatively short time. In two experiments with 'FX-80' dispersion to which glucose was not added, the EEG disappeared in 20-30 min and the arteriovenous difference in oxygen tension dropped to almost zero in less than 1 h. This suggests that the arteriovenous differences we observed are indicative of maintenance of the metabolic and functional activity of the brain.

The values for CO₂ tension in the "arterial" fluid of all samples were in the range 32-40 mm and the "venous" samples were greater by 9-15 mm during the first hour. This difference decreased to 2-7 mm at the end of the second hour. There was practically no difference in these values whether "blood" or 'FX-80' dispersion was used as perfusion fluid.

Our results demonstrate that 'FX-80' in dispersed form is adequately carrying out the essential functions of the erythrocyte. The significant buffering capacity of the erythrocyte is, of course, lacking in 'FX-80'. The brain is such a sensitive organ with respect to oxygen supply that it is likely that 'FX-80' dispersions can be used with equal success for perfusion of other organs. Such dispersions may have considerable advantages over blood in organ perfusion experiments and for use in vitro. because haemolysis cannot occur and there is no haemoglobin or other pigment to interfere with spectrophotometric or other measurements. Furthermore, the ' $\dot{F}X$ -80' could be used in conditions of temperature and pH and in the presence of toxic agents which the erythrocyte cannot withstand.

We have done preliminary experiments on the intravenous infusion of 'FX-80' dispersions in intact animals. The animals have died and death seems to be caused by some unknown effect on the lungs.

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LETTERS TO THE EDITOR

ASTRONOMY

Continuum Radiation from Quasi-stellar Sources

It is known that the mechanism of synchrotron radiation from relativistic electrons in a magnetic field provides a valid explanation of the continuum electromagnetic radiation from extended cosmic sources, such as radio galaxies. This explanation, however, in respect of the nuclei of quasi-stellar sources meets serious difficulties. Assuming the quasi-stellar sources are at cosmological distances it is found that their internal radiation field is so intense that it causes overwhelming loss of fast electrons by inverse Compton scattering. Alternatively, a strong magnetic field reduces the lifetime of the fast electrons unacceptably.

I shall show that these difficulties are simply overcome if it is assumed that relativistic protons are responsible for the bulk of the synchrotron radiation, particularly at its maximum in the millimetre and infrared wavelengths, just beyond the limit usually set by self absorption.

For simplicity, I assume the relativistic protons to be mono-energetic with energy $W = \gamma m_0 c^2$, where m_0 is the rest mass and $\gamma \gg 1$, although no great difficulty arises in assuming a more plausible power-law energy spectrum.

Consider the frequency, v_* , below which self absorption occurs, which marks approximately the peak intensity of the radiation spectrum. Here the Rayleigh–Jeans surface intensity, v_*^2 $3W/c^2$, just matches the radiation emitted by all protons throughout the volume of the source. Thus

$$3\nu_{\bullet}^2\gamma m_0c^2/c^2 \simeq \frac{4\pi}{3} R^3n \ (4\pi R^2)^{-1} \frac{2e^4B^2\gamma^2}{3m_0^2c^3\nu_{\bullet}}$$
 (1)

where n is the particle density of protons per unit volume, R is the radius of the source, B is the magnetic field strength and e the proton charge. Here, a simple approximate formula for the synchrotron radiation emitted by one proton per unit frequency at v_* has been used.

Equating the radiated power to the flux received at distance D from the source gives

$$S_{r_*} = \frac{R^2}{D^2} \frac{3v_*^2 \gamma m_0 c^2}{c^2} \tag{2}$$

According to the theory of synchrotron radiation, the radiation from ultra-relativistic particles peaks at γ^2 times the gyro frequency of the rest mass, thus

$$v_* \simeq \gamma^2 eB/2\pi m_0 c \tag{3}$$

Eliminating \(\gamma \) from equations (2) and (3) yields

$$V_{\star}^{-5/2} S_{v_{\star}} \simeq \left(\frac{R}{D}\right)^2 m_0 (2\pi m_0 c/eB)^{1/2}$$
 (4)

To obtain another relation between R and B, assume equipartition between magnetic field energy and particle energy density, which is the most economical assumption possible, thus

$$nW = B^2/8\pi \tag{5}$$

From equations (1) and (5)

$$cv_*^3/R = \frac{4\pi^3}{27} \left(\frac{eB}{2\pi m_0 c}\right)^4$$
 (6)

is obtained.

From equations (4) and (6) R and B can be estimated independently, knowing y, S_{n} and D_{n} .

independently, knowing v_{\bullet} , $S_{r_{\bullet}}$ and D.

Take as an example the quasi-stellar source nucleus 3C 273B, which is assumed to be at a cosmological distance of 470 Mparsec. From observational data² take $S_{r_{\bullet}} = 2 \times 10^{-21}$ ergs cm⁻² c/s⁻¹ at $v_{\bullet} = 10^{12}$ c/s as approximately the self absorption limit. For these values equations (4) and (6) give $R = 2 \times 10^{15}$ cm and $B = 1.7 \times 10^{4}$ gauss. Then it follows that $n = 10^{7}$ cm⁻³ and $\gamma = 200$. In this field, the proton life time for radiating is about 1 yr. The total magnetic energy equals (by hypothesis) the total relativistic proton energy and is about 0.5×10^{54} ergs, which totals also about the rest energy of one solar mass. Curiously the values of B and γ are not so much greater than those which occur in solar flares².

In this model, based on proton radiation, I conclude that the quasi-stellar source nucleus is a small object with R about 1 light day, well within the limits imposed by observed short period fluctuations of intensity. The magnetic field is strong, yet the total magnetic energy is still very small in comparison with a radio galaxy.

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Case of the Vanished Correlation in Statistics of Quasi-stellar Objects

Longair and Scheuer recently reported the results of their exhaustive studies concerning the possible correlation between optical and radio intensities and the redshifts in a large sample of quasi-stellar objects. Plotting the observed visual optical magnitudes m, and the radio fluxes S_{178} and S_{1400} against the red shifts z, they found significant statistical correlations between these quan-The next step was to eliminate the effects of relativistic time dilation comprising: the fact that the radiation received was emitted with higher frequency; the diminished energy of each photon; the contraction of the observed frequency band relative to the band in which the radiation was emitted. All these effects of time dilation remain the same whether or not the redshift is considered to be the result of the expansion of the universe. After constructing the graphs of the corrected values m_b' , S_{178}' and S_{1400} the authors make the following statement: "The most striking result is that the correlation . has disappeared completely'

This disappearance, similar to that of the Cheshire cat, which left nothing but the grin, can easily be explained on the basis of the point of view which I recently proposed concerning the nature of the quasi-stellar objects. I indicated that the free path of a light ray propagating through the space of the universe populated by galaxies is comparable with the distance we can penetrate into space, using modern instrumentation. Thus if we assume that the original sources of white light are located at very large distances (thus corresponding to the very early evolutionary stages of the universe), their light must almost inevitably pass through at least one of the galaxies on its way to us. This will result in absorption lines similar to those caused by the interstellar gas in our Milky Way, so that the observed Doppler effect in the absorption lines will correspond not to the recession velocity of the source but rather to the recession velocity

of the intervening galaxy. It should be mentioned that, because of the continuous dispersion of the galaxies in the universe, a passage of that light ray through a galaxy is more likely to take place during the earlier stages of its journey rather than during the later ones. No correlation is to be expected between the intensity of light or radiowaves and the redshift of the absorption lines observed in quasi-stellar objects. This is exactly the conclusion at which Longair and Scheuer have arrived.

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Evidence of Continuum Emission from Jupiter at 18 Mc/s

In 1966 it was reported by Dulk and Clark¹ that almost continuous, bursty, radiation from Jupiter can be detected at low frequencies if an aerial receiver system of sufficient sensitivity is available. Relative to this, Stone, Alexander and Erickson² found a marked increase in emission probability at 26·3 Mc/s using the large array at Clark Lake Radio Observatory. Prolonged periods of activity were observed by Warwick³ on a number of occasions in 1960. Evidence is presented here of a related effect at 18 Mc/s observed on several occasions during the 1966–67 apparition of Jupiter.

The term "continuous" as used by Dulk and Clark¹ refers to prolonged periods of bursty radiation of the type already familiar to observers of Jupiter. The "continuum" radiation reported here is a relatively steady emission sometimes appearing as a background to the more usual bursty type of emission. According to Dulk (private

communication) true continuum was hardly ever observed during the course of his observations.

Our observations were made with an interferometer consisting of two identical broadside arrays, each of four whole wave dipoles, on an east-west baseline of sixteen wavelengths. The beam of each array was centred on the zenith and had half-power points at about ±13° in the north-south direction and about ±25° in the east-west direction. During the period of the observations, Jupiter was about 8° to the south of the zenith at the upper culmination and no other point sources of comparable intensity were within the response pattern of the aerials. The receiver was phase-switched with a variable timeconstant of either 0.1 or 1.0 sec and a bandwidth of 3.5 kc/s. This could, if necessary, be swept electronically over a frequency range of ± 0.1 Mc/s every 2 min; thus Jupiter could be distinguished from certain types of interference or from a distant station. Two different pen-recorders had to be used during the apparition. These had time-constants of about 0.25 sec and 0.5 sec as seen in Figs. 1 and 2, respectively. The overall response of the system was therefore effectively variable from about 0.25 to 1.0 sec. The event characteristics are given in Table 1. The corresponding periods of activity can be divided into two classes. In class 1, it can be seen that the conventional Jupiter bursts, for example, on November 7, December 9 and 10. 1966, seem to be superimposed on a fringe envelope and that the pen does not return to the zero-centred baseline either between each burst or throughout an entire fringea period of about 15 min. On December 16 and 17, 1966, and January 18, 1967, for example, in class 2, it can be seen that the pen returns to the baseline during a fringe as might be expected if the radiation consisted solely of sporadic outbursts. Both types of occurrence can be seen in different sections of the records, December 17, 1966, and February 12, 1967. In the record for December 10, 1966, the effect of shortening the receiver time-constant to 0.1 sec is shown. It can be seen that this did not alter the form of the fringes, and it did not cause the pen to

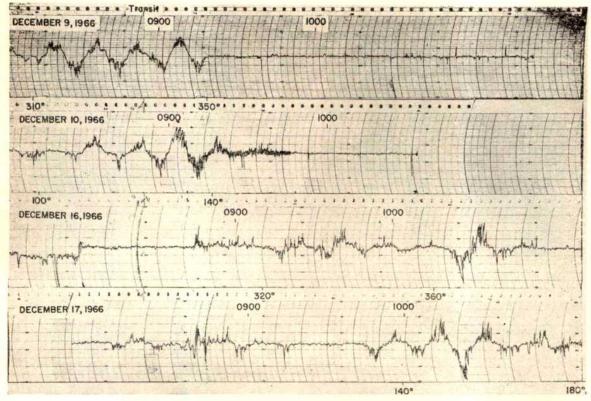


Fig. 1. Typical events recorded by the interferometer showing class 1 and class 2 types of activity. The receiver time constant is changed from 1.0 to 0.1 sec during the event of December 10, 1966.

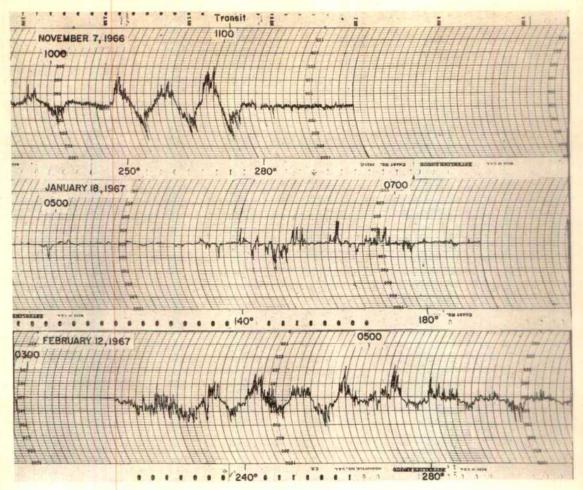


Fig. 2. Typical events recorded by the interferometer showing class 1 and class 2 types of activity.

Table 1. CHARACTERISTICS OF THE EVENTS

U.T. of e		f event λ_{iii}		п	Io longitude		Time con	stant (sec)		
Date	Begin	End	Begin	End	Begin	End	Receiver	Recorder	Type of activity	
November 7, 1966	0950	1010	232	244	225	228	1.0	0.5	Continuum	
	1020	1110	250	280	230	237	1.0	0.5	Continuum	
December 9, 1966	0805	0922	308	354	243	254	1.0	0.25	Continuum	
December 10, 1966	0753	0930	91	150	85	98	1.0*	0.25	Continuum	
December 16, 1966	0915	1100	325	28	238	252	1.0	0.25	Periods of both continuum and no continuum	
December 17, 1966	0840	0920	94	118	76	82	1-0	0-25	No continuum	
	0945	1105	134	182	85	97	1.0	0.25	Continuum	
January 18, 1967	0550	0715	133	185	87	99	0.1	0.5	No continuum	
February 12, 1967	0400	0610	233	284	122	134	0.1	0-5	Periods of both continuum and no continuum	

^{*} Changed to 0-1 from 0907 to 0948.

return to zero between the bursts of activity although the system noise increased somewhat.

It is suggested that on some occasions continuum emission must have been present in the Jupiter radiation to cause the class 1 type of activity. Absolute calibration is not available for these records, although the general adjustment procedure of the instrument implies that the intensity of the continuum radiation would be less than 10^{-21} W.m⁻²(c/s)⁻¹. A total of seventeen events out of the forty-three observed during the period November 5, 1966, to March 4, 1967, contained evidence of continuum emission, if this is arbitrarily defined as any event containing one complete fringe during which the pen did not return to the baseline. That this effect was not observed on every occasion presumably indicates that the continuum emission is either not a frequent occurrence at 18 Mc/s or, perhaps, that it is always present but usually of too low an

intensity to be recorded by the interferometer. Each array of the interferometer has been enlarged to ten whole-wave dipoles and made electronically steerable in the north-south direction for further observations during the next apparition.

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PLANETARY SCIENCE

Early History of the North Atlantic Ocean and its Margins

THE formation of the Atlantic Ocean in late geological history was conceived (as a scientific hypothesis) in the second half of the nineteenth century when sufficient became known of the stratigraphical framework of Earth history. Suess (1885-1904) surveyed the striking similarities which suggested continuity of structure and stratigraphy of the opposing margins of the Atlantic and Arctic oceans, and he postulated that foundering of part of a once more extensive Laurasian continent gave rise to the Atlantic Ocean. Against geophysical evidence this view survived until recently (for example, Beloussov). On the other hand. from speculation about the reasons for the distribution and shape of the continents and oceans, and especially about the origin and influence of the Moon, an Atlantic fission of Laurasia was successively postulated, notably by Owen in 1857, Snider in 1859, Fisher in 1881 and 1889 (possibly the first to suggest Atlantic separation by convection), Pickering in 1907, Taylor in 1910 (who first detailed a scheme for the opening of the Arctic and separation of Greenland from both Europe and America involving strike-slip between Greenland and Ellesmere Island) and Baker in 1911. Wegener (1912-1928), in spite of resort to a feeble mechanism for drift, by bringing palaeoclimatic and other evidence to bear, succeeded in gaining widespread attention and support. It needed only the addition of palaeomagnetic evidence to finally convince most scientists. Continental drift was so challenging a concept that most attention had been absorbed in the questions of its general validity and mechanism. Its exploitation to explain earlier events in Earth history was left to those who related the long history of orogenic activity to substantial continental movement. Alternatively, it has been suggested that continental drift may have been a process characteristic of the last 5 per cent of Earth history. But most "pre-drift" reconstructions even recently have been static in not being related stratigraphically.

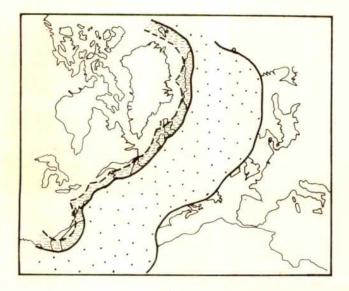
A recent application of the drift concept to earlier history has been made by J. T. Wilson. His question "Did the Atlantic close and then reopen?" assumed drift as a

phenomenon of general application and supplemented a specific hypothesis to test it (Fig. 1). Some aspects of this are examined here. The principal novel feature of Wilson's hypothesis does not seem to be supported by the evidence he gave, but it may well anticipate appropriate new data to support some part of it.

The part of Wilson's hypothesis which is not novel concerns the history of what is termed here the North Atlantic geosyncline (defining geosyncline by thickness of sediments and not by the depth of water in which they formed). For example, in 1928 W. van der Gracht² suggested (page 72) "It would seem as if in the older Paleozoic, before the Caledonian diastrophism, America might have moved westward faster than Eurasia, opening a Paleozoic Atlantic geosyncline, which was partly closed again during the Caledonian diastrophism. . . . In the Mesozoic period, and increasingly in the Tertiary revolutions, the drift of the American continent . . . tearing open the old rift and causing the present Atlantic." Van der Gracht's comprehensive hypothesis was well based on what was then known of the tectonic history of the Atlantic borderlands, but his thermal mechanism (adapted from that of Joly3) was shortly to be improved on by Holmes 4.5, who also used the concept of a Caledonian orogenic concentration of sial to generate a zone of upward and outward convection and so split it open along its length. For Wegener the Mid-Atlantic ridge had been the linear scar of continental fission analogous to the African rift valleys and Red Sea, but perhaps Molengraaf⁶ first saw its significance as a volcanic zone of extension, which refuted the westerly continental drift of Joly and van der Gracht and supported a

symmetrical spreading of the Atlantic.

It had long been known that the Atlantic orogenic structures arose from a complex of geosynclines, including Dana's type (Appalachian) "geosynclinal". Bailey and Holtedahl' considered the nature of the seas which covered the developing geosynclines and preceded the orogenic belt before closing and reopening. Caledonian geosynclines were then conceived essentially as thicknesses of early Palaeozoic rock*. It has now become clear, as the history of the crystalline parts of the orogens has been unravelled, that similar or much greater thicknesses of late Pre-Cambrian rocks are also involved in the Appalachians*-11, Scotland*12, Norway*13, East Greenland*14 and Spitsbergen*15. Thus similar conditions, probably marine, persisted in



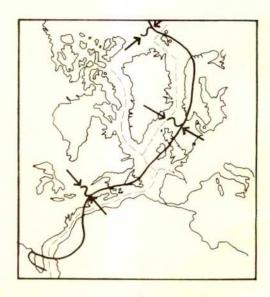


Fig. 1. Closing of proto-Atlantic Ocean in Palaeozoic time according to Wilson'. (a) Early Palaeozoic; coarse stipple is proto-Atlantic Ocean; dashed shading is island are geosyncline. (b) Mid- to late-Palaeozoic; the thick line marks the closure of the proto-Atlantic and the chief compressed geosyncline lies to the west of this; the dashed line marks the approximate line of later Atlantic opening.

these areas, possibly for some hundreds of millions of years before the Lower Palaeozoic orogeny¹⁶.

It may be still a matter for debate whether orogeny in general, or the Caledonian diastrophism in particular, involved significant and substantial compression with closing of the geosynclinal areas. This has long been assumed in general by most of those who advocated drift (for example, Argand, Termier, du Toit) and it has been argued (for example, recently; see refs. 17-19) that if convection caused continents to drift, the same kind of movements have also compressed zones of lithosphere. In one of the first hypotheses giving a detailed sequence of continental movements through Phanerozoic time in the North Atlantic-Arctic region 20, I made such an assumption and later illustrated it by a sequence of palaeogeological maps²¹ (Fig. 2). The first of these shows an area of Pre-Cambrian and Lower Palaeozoic sedimentation but gives no indication of the depth of water (which may well have varied considerably in place and time). There is, however, some evidence of shallow water facies and occasionally of desiccation and there is no reason to suppose that any of the facies are abyssal. The aqueous environment is usually assumed to have been marine, judging from the Palaeozoic faunas, Pre-Cambrian glauconite, occasional halite pseudomorphs and possibly from the widespread dolomites and stromatolitic limestones. But Wilson¹ supplements this idea by inserting his wide and deep proto-Atlantic ocean to the east of the geosyncline which he depicts as a relatively narrow system of island arcs (drawn as one involving little orogenic compression).

The closure of this area (marine geosynclinal + ocean basin) was then suggested by Wilson to have been gradually accomplished through Upper Ordovician to Carboni ferous time (Fig. 1b). The closure had been analysed into component movements allowing a distinction to be made between the northern and southern parts of the North Atlantic region^{20,21} (Fig. 2a-d). Phases of disturbance. reflected in discordances and facies in the Carboniferous record, are evident throughout the region, but evidence of substantial late Palaeozoic compression is limited to the Armorican-Appalachian belt (Suess' Altaids), whereas increasingly northwards Permian conditions seem to have been progressively more quiescent. On the other hand, Mid-Palaeozoic (Caledonian, Taconian and Acadian) structures were formed with considerable intensity along the whole site of the subsequent North Atlantic fission.

The explanation of the contrasting history of northern and southern parts of this region may be found in the existence of a mobile (palaeo-) Tethys. Three relatively stable blocks (North America and Greenland, Spitsbergen and Northern Europe and Africa) moved somewhat independently. In mid-Palaeozoic time the whole North Atlantic geosyncline, from Alabama to Spitsbergen, was compressed—not necessarily simultaneously in all areas

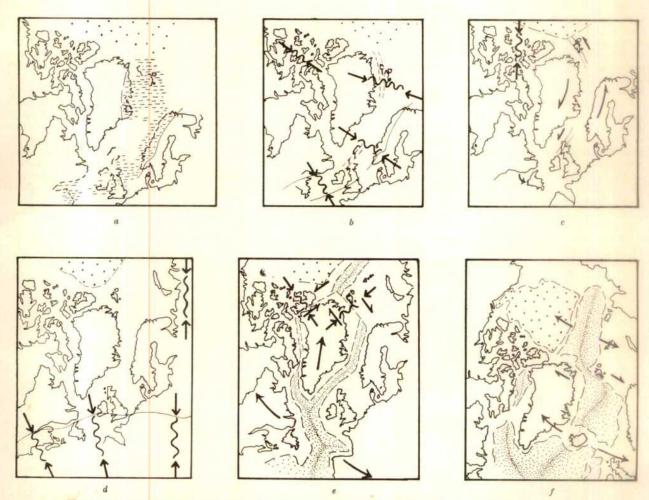


Fig. 2. Sequence of movements in North Atlantic-Arctic region during Phanerozoic time, schematized from Harland^{28,31}. (a) Early Palacozoic; the shading shows the extent of the North Atlantic geosyncline in Palacozoic time (other Palacozoic geosynclines are not shown). (b) Silurian; directions of "Caledonian" compression are shown diagrammatically by arrows and folded lines. (c) Late Devonian showing the new positions after sinistral strike-slip movements along the Atlantic orogen. (d) Late Palacozoic; directions of "Hercynian-Appalachian" compression. (e) Early Tertiary Atlantic fission and northward movement of Greenland is shown by finely stippled new ocean and compression to the north-east and north-west of Greenland. (f) Recent; spreading of new Atlantic and Arctic Ocean basins continues and the maps also show the continuing ancient Arctic basin by coarse stipple.

(Fig. 2b). This closed and to a large extent eliminated the seas between Europe and America. In late Palaeozoic time Africa moved differentially with respect to the newly welded Laurasia with a net movement towards Greenland and along a direction parallel to its axis (Fig. 2d). Seen from North Europe the closing direction was thus turned between early Silurian and late Carboniferous time through a large angle (60°-90°) and initiated both the graben faulting characteristic of Old Red Sandstone times and the postulated sinistral late mid-Palaeozoic strike-slip faulting of Scotland^{22,23} and Spitsbergen²⁰. My own reconstruction of this phase^{20,21} fits, but does not depend on, the report of analogous movements described by Wilson²⁴ as the Cabot Fault, which has been seriously questioned²⁵ (Fig. 2c). A prediction of this hypothesis, however, is that sinistral strike-slip faulting can be expected to extend somewhere in the Appalachian region or towards north-west Africa. Such a fundamental fault system might well provide a suitable zone for later fission 20.

The subsequent opening of the Atlantic has similarly been analysed into distinct elements. An independent northward movement of Greenland against Spitsbergen and Ellesmere Island resulted first in compression and then, respectively, in dextral and sinistral strike-slip which accompanied the opening of the North Atlantic and Arctic ocean basins^{20,21,26,27}. In so far as Africa moved independently with respect to Meso-Europe, this led to differential opening of the northern and southern parts of the Atlantic basins. The final stage in this oceanic evolution is the familiar situation accommodating the mid-oceanic rifts and ridges6,28-30. My account of later continental movements has been greatly simplified, partly by omitting the qualification, in each case, that continents might be associated with equally stable oceanic extensions; for it is assumed that the differential movements in the crust and upper mantle depend on activity in the deeper mantle.

The distinctive feature of Wilson's hypothesis1 is the postulate of a proto-Atlantic ocean basin in Lower Palaeozoic time alongside the North Atlantic geosyncline. Wilson showed the chief part of the geosyncline as a thin zone bounding eastern Laurentia. It was separated from Nordaustlandet in Spitsbergen, Fennoscandia, southern British Isles, eastern Newfoundland and maritime provinces, and some of North West Africa, by the postulated ocean basin of width similar to that of the present Atlantic, and with parallel sinuous sides. This is a daring reconstruction.

The almost exact parallelism of the two margins of the supposed proto-Atlantic as drawn by Wilson (analogous to those of the present Atlantic) suggests that it was formed by fission (? along a still earlier orogen) as van der Gracht suggested2. This may not have been intended, but if the reconstruction is true it suggests a prior question: Did an eo-Atlantic geosyncline close (? Grenville) before the proto-Atlantic opened again to close (Caledonian) before re-opening as the Atlantic? Alternatively, the evidence of late Pre-Cambrian diastrophism (for example, Grenville, Carolinidian) can be explained as an earlier stage in the closing of a North Atlantic geosyncline that was initiated still earlier.

Much of the evidence on which Wilson based his separation of the two sides of a proto-Atlantic depends on a familiar recourse to faunal provinces. But these are probably too uncertain to be used as a basis for such a construction. I have been thanked in general for private information on Spitsbergen¹ and I must disclaim communicating any evidence from Spitsbergen which would support such a separation of Nordaustlandet and Vestspitsbergen as Wilson proposes.

The Hecla Hoek (part of the North Atlantic geosyncline) is not (as Wilson attributes to me) a Lower Palaeozoic eugeosyncline. The chief volcanic record of the geosyncline (comprising about 12 km in Ny Friesland) terminates about 7 km beneath the Lower Cambrian and Ordovician carbonate strata, so that in latest Pre-Cambrian and Lower Palaeozoic time conditions were relatively stable, only exposing sediments occasionally above sea level as evidenced by desiccation cracks. Whereas previously²¹, I had supported Orvin32 and Sandford33 in regarding Nordaustlandet as the margin of the geosyncline, questioned it in 196034 and our current view16, pending publication of a Norsk Polarinstitutt investigation of Nordaustlandet, is to regard the succession on both sides of Hinlopenstretet as broadly similar. Even if the earlier view is correct it relates only to the earlier part of the Pre-Cambrian succession. Indeed, it is perhaps easier to correlate Pre-Cambrian Hecla Hoek rocks in detail across the strait than across any other unexposed area. Wilson's hypothesis of a proto-Atlantic ocean basin as it concerns Spitsbergen in early Palaeozoic time seems to be as surely wrong as anything in nature can be.

There may, however, be some truth in Wilson's hypothetical proto-Atlantic. It is unlikely that there would be very thick sediments throughout the North Atlantic geosynclinal region (Fig. 2a). Geosynclines and ocean basins may have been interspersed and evidence for these may be sought in the sedimentary record. But abyssal facies are difficult to identify after metamorphism. In particular, it is not known on what kind of basement the geosyncline rested in most places. In Spitsbergen no certain evidence of a pre-Hecla Hoek basement has yet been published. Late Pre-Cambrian thermal events³⁵ are difficult to interpret but could relate either to a metamorphic episode within some geanticlines or to a marginal (?) Carolinidian diastrophism brought into proximity with Ny Friesland by sinistral strike-slip faulting in late Caledonian time¹⁶. The early, largely basic, eugeosynclinal phase could have initiated geosynclines on a marginal ocean floor. If so it was a late Pre-Cambrian ocean, possibly even pre-Grenville. But we do not yet know whether it existed, nor do we know on which margins of such a hypothetical ocean the geosynclines could have developed. The possibility of such a proto-Atlantic ocean may be tested by investigating the distribution of eugeosynclinal facies (or otherwise) at the base of late Pre-Cambrian geosynclinal successions throughout the North Atlantic. The (proto) Arctic basin shown in Fig. 2a-f could well be an extension of such a proto-Atlantic ocean and the geosynclinal area in Fig. 2a would need supplementing and widening by a trough or by basins of non-geosynclinal ocean floor.

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Variations in Two New Zealand Glaciers during the Past 800 Years

A METHOD for dating surfaces which uses measurements of the maximum diameters of the lichen thalli which encrust

rocks has been developed and fully described1.

We have used this procedure, with some slight modifications, to study the moraines of the Mueller and Tasman glaciers in the Mount Cook region, Canterbury, New Zealand. The termini of these glaciers are at the same altitude (about 2,500 ft.) and are only 4 miles apart so that conditions for lichen growth are similar at both places. Two deviations were made from Beschel's method: first, in the case of oval thalli the longest axis rather than the shortest was measured; second, defined sampling areas were not used because of the irregularity of the terrain. The results obtained allowed us to construct comparable chronologies for advances of the two glaciers covering a period of about 400 yr. On older surfaces at the Tasman glacier the record extends back a further 400 yr.

A glacial advance is defined for our purposes as an increase in ice volume substantial enough to form distinct, ridged lateral moraines and terminal push moraines. Complex series of these moraine ridges are present near the glaciers at Mount Cook, the oldest existing laterals and latero-terminals being most distant from the present position of the ice. The morphology, relative spatial positions, relative heights and vegetation cover of the ridges demonstrate their chronological position relative to one another and show that parts of them have been free from ice since they were formed. It is on these surfaces that lichen measurements were made. In many cases only short fragments remain of what were obviously once extensive moraine loops. Subsequent destruction of much of the length of these moraines has been chiefly through the agency of vigorous melt-water streams or by overriding by later ice advances. In some instances, there is evidence for forward movement of the glacier snouts during glacial advances, but in other cases there is no such evidence, either because the terminal moraines proper have been destroyed by subsequent events or because forward movement did not occur. Changes in height of the glacier surfaces are clearly recorded by the series of lateral and latero-terminal ridges.

We identified surfaces of known ages at the Mueller glacier, formed about AD 1930, 1913 and 1890, from historic records^{2,3} and a surface formed between AD 1730 and 1750 from the study of Lawrence and Lawrence* in which tree growth rings were used. Calibration growth curves for two species of lichen present on these surfaces, Rhizocarpon tinei (Tornab.) Run. and R. candidum Dodge (both identified by Mr D. Galloway, Department of Biochemistry,

University of Otago), are given in Fig. 1.

The age of only one surface—AD 1890—is known from historic records at the Tasman, but a curve drawn through the plot of maximum diameter of R. tinei on this surface, against surface age, closely agrees with that from the Mueller. Similarly, a curve for R. candidum plotted from a surface dated at about AD 1740 (by means of the R. tinei

Table 1. MAXIMUM AGES RECORDED BY TWO LICHEN SPECIES ON MUELLER AND TASMAN MORAINES (YEARS AD)

	Mueller	Tas	man
R. tinei	R. candidum	R. tinei	R. candidum
1930	*	*	manus 🛊
1890	•	1890	
1835-50	1840-50	1850	1850
1805	1810	1810-20	Section 8
1770-90	1795	*	
1720-50	1710-40	173050	1730
1650	1655)	1690	1670
_	1580 \$	1670**	1580-1680
1550	1540	7010	3,000,000
100011	101011	£	
	•	1630**	1430-60
	•	1260-1390	1360-708
	,	1470**	1150(
	1350++		

Thalli of this species absent.

† Surface not identified.

† Surface not identified, possibly obliterated by subsequent advance.

§ Two advances not clearly distinguished by the morphology of the moraine, but earlier lichen date on distal side of the moraine and later dates

on its crest.

|| Possible minimal age.
|| No dates available. At least five advance moraines overgrown by scrub and forest.
|• Minimal age. Older date taken as nearest approximation to true age.
| + Minimal age. Probably older than surfaces of similar date at Tasman.
| A few undated, older moraine fragments lie outside the limits of those recorded here.

curve) parallels the curve obtained for this species at the Mueller. The ages of undated surfaces at both the glaciers were extrapolated from these growth curves. shows the ages of distinct advance moraine ridges as determined from the largest lichen diameters, taking the dates at their face value to the nearest 5 yr back to AD 1600, and to the nearest 10 yr from AD 1600 back to AD 1150. In some cases, ranges of dates are given which, back to AD 1580, represent approximate time ranges from beginning to end of the glacial advances. On older moraines these ranges simply represent the scatter of dates. Because the age estimates are likely to be minimal, the oldest date on each surface may be taken as the nearest approximation to true age on surfaces older than about 400 yr.

The estimates of the dates of glacial advances determined in this way match those for historically recorded advances of glaciers in the European Alps and glaciers in the Caucasus Mountains from the late sixteenth century onwards, and seem to show that most of these glacial events were synchronous in the northern and southern hemispheres. Matthes noted that the Grindelwald glaciers advanced in 1595-1620, 1719, 1743, 1770-79, 1814-22, 1838-50, the Vernagtferner in 1599-1601, 1678-81, 1771, 1820, 1848 and advances of the glaciers of the European Alps occurred generally in the 1850s, 1875-1890s, 1910-13. 1916-20 and 1926. In the Caucasus, advances occurred in 1850-60, 1877-87, 1907-14, 1927-33.

As far as we are aware, the Mount Cook moraines seem to provide a more complete record of glacier variations

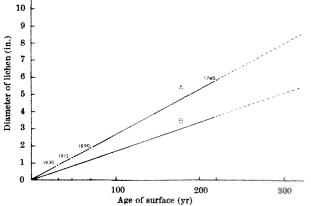


Fig. 1. Growth curves for Rhizocarpon tinei (A) and R. candidum (B) on surfaces of known age at the Mueller glacier.

during the past millenium than any which have so far been studied elsewhere in the world. The relative heights and positions of latero-terminal moraines indicate that the advances were of varying magnitude. In particular, the advances in the seventeenth and eighteenth centuries were massive and both Mueller and Tasman glaciers apparently pushed out beyond older terminal moraines at those times, but did not destroy parts of the earlier lateral moraines. On some occasions, however, the two glaciers behaved differently. The advance of the Mueller in the mid-eighteenth century was greater than most earlier and all later advances, but the advance of the Tasman in 1890 overrode most of the length of the mid-eighteenth century moraine and was the greatest advance since the thirteenth century. Ice levels remained high at the Mueller and Tasman glaciers between 1890 and about 1916, but so far there is no evidence for distinct advances during this period. Since about 1916 some hundreds of vertical feet of ice have been lost from the surface of both glaciers by down-wasting, but there has been little terminal retreat.

The Beschel method¹ has recently been criticized by Jochimsen⁶ on theoretical grounds related to the physiology and ecology of lichen and as a result of his observations in Austria. In our opinion this criticism does not apply to our results for the following reasons: (a) it is possible to draw a straight line through measurements of the greatest diameter of two lichen species on surfaces of known age; and (b) comparable results were obtained for two lichen species on the moraines of two glaciers.

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Givetian Unner Stringgeenhalen

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Devonian Conodonts from Kashmir

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THE increasing use of conodonts in studies of Palaeozoic stratigraphy in Europe and North America has recently received added impetus by the discovery of faunas in other continents where broadly similar faunas have been described from strata of similar ages. So far, however, there has been only one published record of conodonts from the Indian subcontinent¹. A well preserved fauna of some 1,200 individual conodonts has recently been extracted from a sample of siliceous limestone, collected from a locality near Lutherwan, Kashmir (33° 6′ 90″ N., 75° 35′ The insoluble residue also yielded many fragments of bony material and numerous fish teeth. The strata yielding conodonts lie just below the Muth Quartzite which has been assigned a Middle Devonian age on the basis of fossils collected from a locality 1 mile north of Naubug (33° 44′ 30″ N., 75° 23′ 30″ E.), Anantnag Distt, Kashmir.

The area was first mapped by Middlemiss², who assigned

Manticoceras

Table 1. CONODONT SPECIES FROM THE DEVONIAN OF KASHMIR AND THEIR RANGES IN THE DEVONIAN OF GERMANY

	Upper Stringocephalen Stufe			Manticoceras Stufe			
	Oderhäuser Kalk	Sparganophyllumkalk	varca-Subzone	ordinata-dubia-Subzone	đubia-rotundiloba- Subzone	asymmetrica-martenbergensis- Subzone	martenbergensis-triangularis- Subzone
olygnathus cristata Hinde							
olygnathus ordinata Bryant							
olygnathus pennata Hinde	 	<u> </u>				1	
olygnathus webbi Stauffer	 	-	 				
olygnathus linguiformis Hinde	 	_				╢	
olygnathus rugosa Huddle				<u> </u>		1	
olygnathus cf. P. dengleri Ziegler					 	-	
olygnathus ef. P. varca Stauffer			 	 		1	
ncyrodella rugosa Branson and Mehl					 	₩	-
ncyrodella rotundiloba (Bryant)						₩	
ryantodus cf. B. paeckelmanni Bischoff and Ziegler			-	-			
criodus spp.	 	 	 	 	 	╂	+
onchodina sp.	 	 	 	-		 	+
richonodella sp.	-	+	 		 	 	-
ryantodus spp.	-	+	 	 	 	#	+
zarkodina spp.	-	+	 	 		 	
'indeodella sp.	-	-		 		 	
anderodus sp.							
igonodina sp.	 	+	 	 	<u> </u>	<u> </u>	
	LL			***************************************			

Ranges from Bischoff and Zieglers. Full line shows common occurrence; broken line indicates rare occurrence. For further details, see Zieglers.

an Upper Silurian age to the beds, and Gupta³ later remapped the area and named the strata the Naubug Beds. The beds consist of blue grey to rusty calcareous sandy shales and siliceous limestones, and have yielded a large fauna consisting of brachiopods, trilobites, corals, crinoids, cystoids and nautiloids. This has been described by Gupta³, who interprets it as of Upper Silurian-Lower Devonian age.

The Naubug Beds are succeeded, apparently conformably, by the Muth Quartzite. Devonian osteolepid and dipterid fish remains have been collected from dark calcareous shales situated at the base of the Muth Quartzite south-west of the Margan Pass⁴, and these suggest a Lower or Middle Devonian age for the base of the Muth Quartzite.

Gupta³ has collected a rich fauna of brachiopods, corals (including Calceola sandalina), trilobites, pelecypods and gastropods from the middle portion of the Muth Quartzites, and these suggest a Middle Devonian age for the fossil-

iferous portion of the quartzites.

The conodont fauna is dominated by polygnathid specimens, although other individuals are also abundant. The following species are present: Polygnathus cristata Hinde; P. ordinata Bryant; P. pennata Hinde; P. webbi Stauffer; P. linguiformis Hinde; P. rugosa Huddle; Polygnathus cf. P. dengleri Bischoff and Ziegler; Polygnathus cf. P. varca Stauffer; Ancyrodella rugosa Branson and Mehl; A. rotundiloba (Bryant); Bryantolus cf. B. paeckelmanni Bischoff and Ziegler; Icriodus spp.; Lonchodina sp.; Trichonodella sp.; Bryantodus spp.; Ozarkodina spp.; Hindeodella sp.; Panderodus sp.; Ligonodina sp.
All these individuals are in a comparable state of

preservation, most being fragmentary, although detailed preservation of the fragments is good. The specimens of Panderodus are lighter in colour and may be reworked. Numbers of polygnathid specimens show morphological trends in which they approach palmatolepids and ancyrodellids. There are two specimens in which individual conodonts are used together by material that resembles

"bony material" in colour and texture.

Table 1 shows the ranges of these various species, as recorded by Bischoff and Ziegler⁵ for Germany. This fauna clearly lies near the Middle-Upper Devonian boundary, and it probably falls within the dubia rotundiloba-Subzone, as originally defined. The precise zonal position in Germany is still not fully established, however, although Ziegler has erected a new hermanni-cristata-Zone between the varca and the asymmetrica-Subzones. He regards this latter subzone as lowest Upper Devonian, but there is a gap between the vertical ranges of the two diagnostic cephalopod genera, Maenioceras and Pharciceras.

The importance of the present fauna lies partly in the fact that the nearest known faunas of comparable age are those of Europe, the Spanish Sahara7 and Australia6, and partly in the remarkable overall similarity of the faunal assemblage to those of other areas. The correlation which we propose on the basis of conodonts agrees well with that proposed independently on the basis of other in-

vertebrate fossils3.

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PHYSICS

Electron-beam Dosimetry using the Radiation-induced Fluorescence of Polyethylene

WE have recently examined various effects of high-speed electron radiation on the properties of polyethylene. Among other effects, we observed that the intensity of fluorescence (excited by mercury-lamp illumination of a polyethylene sample previously electron-beam irradiated) increased linearly with radiation dosage in the range from 0 to 40 Mrads. The fluorescence is persistent, reproducible and easily measured, and thus may be useful for dosimetry applications.

Irradiation of polyethylene has been studied from many viewpoints, but relatively little dosimetry work has Charlesby¹ has suggested that polyethylene might be used as a dosimeter by using infrared spectroscopy to follow changes in unsaturation caused by irradia-The fluorescence, thermoluminescence and phosphorescence of polyethylene have all been investigated**.

but not from the dosimetry standpoint.

In our experiments, a variety of polyethylene samples with density and molecular weight variations of different form (that is, bulk or sheet), and with and without antioxidant, were examined. For standardization purposes TR 201 'Marlex' in 1/16 inch sheets was selected for our studies. Irradiation was carried out with a High Voltage Engineering 1.0 MeV Insulating Core Transformer Electron Accelerator. Dosages ranged from 0 to 160 Mrads, in 10 Mrad steps. All irradiations were performed in air at room temperature, at 10-0 Mrads per pass

The fluorescence spectra were obtained with a Cary 15 spectrophotometer and 1512 fluorescence attachment. A Hanovia 735 A 0070 source was used in conjunction with a filter of aqueous $NiSO_4.6H_2O$ (500 g/l.) and a Corning 5840 glass filter. The path length of the nickel sulphate solution was 1 cm; the principal excitation wavelengths were in the areas of 3120 Å and 3330 Å, with lesser amounts at 3650 Å and 2537 Å. The wavelength of the polyethylene fluorescence emission spectrum selected for measurements was 4470 Å; the spectrum appeared as a broad band from 6000 Å to about 3600 Å (Fig. 1).

The results of our measurements are shown in Figs. 2 and 3, and may be summarized as follows. Fluorescence intensity at the 4470 Å wavelength varied linearly with

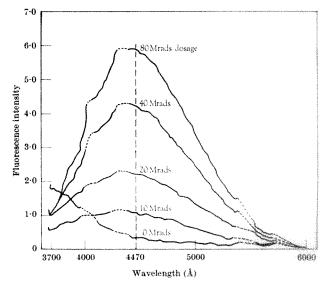


Fig. 1. Fluorescence spectra of TR 201 'Marlex' 1/16 in, polyethyiene sheet irradiated with different dosages. The scattered mercury lines of the exciting illumination are indicated as breaks in the fluorescence spectra.

dosage up to 40 Mrads and then fell off. Anti-oxidants did not affect linearity but seemed to have some effect on intensity. The anti-oxidant itself showed no fluorescence before or after irradiation. The fluorescence persists for periods at least as long as three years after irradiation. The effect of heat is to quench the fluorescence.

It seems from the results that the polyethylene fluorescence may in fact be useful as a dosimetry system for electron-beam irradiation in the appropriate dosage

We have not, at this point, made an extensive investigation of the fluorescence mechanism. It has, however, been shown by various authors5-7 that several forms of unsaturation are present in irradiated polyethylene. It is

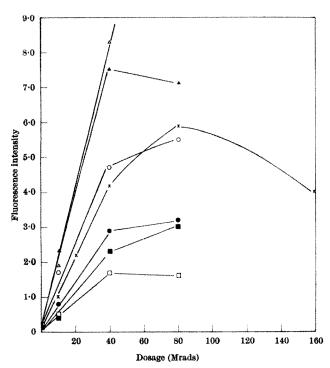
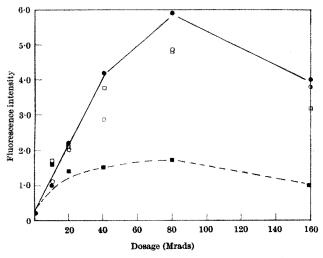


Fig. 2. A plot of fluorescence intensity against dosage for a variety of polyethylene samples. \triangle , Allied 1220 (bulk), high molecular weight, no anti-oxidant; \triangle , Allied 1230 (bulk), high molecular weight, with anti-oxidant; \bigcirc , 'Marlex' TR 201 (sheet), irradiated in 1966; \times , 'Marlex' TR 201 (sheet), irradiated in 1964; \rightarrow 'Marlex' TR 201 (bulk), with anti-oxidant; \rightarrow 'Marlex' 704 (bulk), no anti-oxidant; \rightarrow 'Marlex' 604 (bulk), with anti-oxidant.



Effect of heat on fluorescence intensity of irradiated TR 201 1/16 in specis. • Room temperature 25°C continuous; 'Marlex' 1/16 in. sheets. ● Room temperature 25° C continuous; O, heated at 50° C for 2 h; □, heated at 100° C for 2 h; ■, heated at 150° C for 2 h.

possible that the carbonyl group, which absorbs ultraviolet radiation in the 2700 Å-3400 Å region, is involved, because our principal excitation wavelengths range from 3120 Å-3330 Å. We are proceeding with our investigation of this aspect.

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Stability of Dilute Viscoelastic Flows

STEMMING from Toms'1 original observation, several investigators² have observed the phenomenon that dilute concentrations of linear polymers of high molecular weight in water and other solvents will act to reduce greatly the hydraulic friction. Although the viscosity in solution of the effective polymers is higher than that of the base liquid, it has been shown that the frictional resistance is reduced even when the data are correlated on a solvent-based Reynolds number³.

This phenomenon is usually attributed to a modification of the turbulent flow structure4, and a pronounced thickening of the laminar sublayer of the turbulent flow has been observed from measurements of velocity profiles. Although the use of pitot tubes for velocity profile measurements may be subject to normal stress errors, the causal relationship between sublayer and thickening and friction reduction seems to be proved. Such a conclusion suggests that the polymers act to stabilize the flow in the buffer region. Furthermore, if stabilization is enhanced in the presence of turbulence, it would be expected that the polymers would also delay the point of instability and In earlier, unreported extend the transition regime. experiments by one of us (W. B. G.), this delay of transition was observed. It was subsequently found that the flow remained laminar even in the presence of severe roughness elements7.

In the literature, two types of pipe friction factor data are found. In one case, the flow goes into normal transition⁸, following the Newtonian turbulent flow characteristic with a subsequent and increasing reduction of friction at higher Reynolds number (or higher strain rates). By using higher polymeric concentrations or more effective polymers, there are instead one or two breaks in the friction factor characteristic which indicate increasing flow resistance with respect to laminar Newtonian flow. Ram and Tamir¹⁰ assume that the first break indicates transition into turbulence and identify this region as structured turbulence. It is also possible to assume that the higher strain rates have induced a change in rheological fluid properties rather than a change in flow state, such as suggested by Peterlin¹¹ for a very high molecular weight polymer in a very viscous solvent. The purpose of this study was to clarify this point by measuring the friction factor characteristics in association with observations of flow stability.

Various concentrations (0, 0.5, 1, 2, 5, 10, 30, 300 p.p.m.) of polyethylene oxide ('Polyox' coagulant, Union Carbide

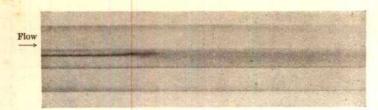


Fig. 1. Dye burst in water. $R_d = 2,280$, and tube diameter = 0.058 in.

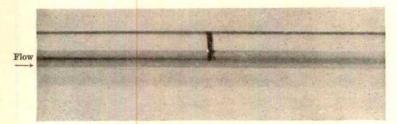


Fig. 2. Dye burst in aqueous solution of 0.5 p.p.m. of polyethylene oxide. $R_d = 4,440$, and tube diameter = 0.058 in

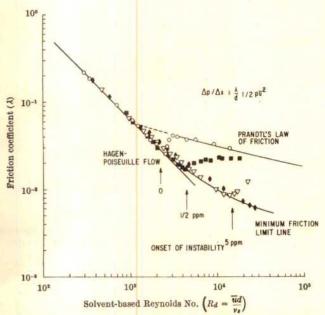


Fig. 3. Flow resistance of aqueous solutions of polyethylene oxide in a 0-055 in. diameter capillary. O. Tap water; .05 p.p.m. 'Polyox' coagulant; .05 p.p.m. 'Polyox' coagulant; .05 p.p.m. 'Polyox' coagulant.

Co.) were tested in capillary tubes of 0.055 and 0.101 in. diameter. Several static pressure taps were used to avoid entry length effects. Flow stability was observed by means of a dye filament in a 0.058 in. diameter glass tube with a 6 to 1 inlet contraction nozzle; and also by observation of the stability of the exit jet¹² of both the glass and metal capillary tubes.

Flow instability is characterized by turbulent diffusion of the dye filament, as shown with water in Fig. 1, and also erratic behaviour of the exit jet as each turbulent burst develops and passes through the tube. The onset of instability with water occurred at a diametral Reynolds number of

$$R_d = \frac{\bar{u}d}{v} = 2,240$$

approximately 6 in. downstream of the inlet. The same behaviour was found to occur with 0.5 p.p.m. of 'Polyox' coagulant in water, except that instability was delayed until $R_d = 4,400$ and occurred farther downstream at about 24 in. from the inlet. Close inspection revealed that transition was composed of turbulent bursts separated by

laminar regimes, just as is observed with Newtonian flows¹³. Figs, 1 and 2 show the ends of these turbulent bursts, at camera positions of 10 and 34 in., respectively.

With higher polymer concentrations, the stability increased monotonically. A critical Reynolds number of 14,840 was obtained at a concentration of 5 p.p.m., and at 30 p.p.m. the flow remained stable up to the limits of the test facility $(R_4 = 26,000)$.

These results are shown in Fig. 3, superimposed on the friction factor-Reynolds number data of representative concentrations (0, 0.5, 5, 30 p.p.m.) in the 0.055 in diameter tube, correlated using a solvent-based Reynolds number. The data points are seen to depart from the laminar characteristic much earlier than the loss of flow stability. Thus this fluid system, which is pseudoplastic or shear thinning at sufficiently high concentrations, exhibits shear thickening at high strain rates; and a dominant factor in the ability of linear polymers of high molecular weight to reduce

friction is caused by the enhancement of flow stability, both in delaying transition and in stabilizing the buffer region of turbulent flow.

The envelope of these data prescribes a minimum friction limit line where the friction factor may be described in the form

$$\frac{1}{\lambda} = 8.70 \log (R_d \sqrt{\lambda}) - 16.1$$

or, approximated in the simpler form of

$$\lambda = 1/\sqrt{R_d}$$

Furthermore, it is found that good correlation is obtained from this shear thickening, laminar flow regime by using a solvent based Reynolds number, as shown in Fig. 4. Here data for 30 and 300 p.p.m. of 'Polyox' coagulant in tap water are shown for two tube diameters, 0.055 and 0.101 in.

These observations of the delay in transition taken together with thickened laminar sublayers and suppressed influence of roughness elements suggest that the macromolecules enhance flow stability in a very general manner;

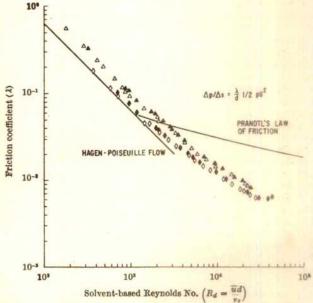


Fig. 4. Flow resistance of aqueous solutions of 'Polyox' coagulant. △, 300 p.p.m., tube diameter of 0.055 in.; ♠, 300 p.p.m., tube diameter of 0.101 in.; ♦, 30 p.p.m., tube diameter of 0.055 in.; ♠, 30 p.p.m., tube diameter of 0.101 in.

not only through a more stable velocity profile, but also through elastic properties in the Orr-Sommerfeld equations.

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THE SOLID STATE

Irradiation Creep in Several Metals and Alloys at 100° C

Enhanced stress relaxation under neutron irradiation is well known and has been observed in more than a dozen non-fissile materials^{1,2} (ref. 1 gives a review of earlier work), and recently steady state irradiation creep has been observed in nickel³. This communication briefly describes the results of an experiment designed to compare the irradiation creep of several metals and alloys at about 100° C. So far, no published data have shown the effect of differing metallurgical condition on the irradiation creep behaviour of a given material and the effects of such a variation, in two alloys, were also investigated.

The specimens were made from molybdenum, nickel, titanium, Nimonic alloys PE16 and 80A and an AISI type 316 stainless steel, in the form of helical springs of 0.5 in. diameter and wound from wire of 0.04 in. diameter; each spring had twenty-five active turns. These dimensions are such that a deflexion of 0.05 in. corresponds to a surface shear strain of 10-4. The chemical compositions of the metals and alloys are given in Table 1. Twelve springs were used; they were hung in two strings of six springs each, under tensile load, in a D₂O-flooded thimble in a hollow fuel element in the Dounreay Materials Testing Reactor. The temperature of the springs was determined by y-heating in the various components of the apparatus and boiling of the surrounding D₂O. At each of six successive reactor shutdowns the springs were hoisted into a portable lead cell, which was positioned above the hollow fuel element, and photographed through lead glass windows in the cell walls. Measurements of these photographs, showing reference surfaces above and below each spring and an adjacent scale, permitted the spring extensions to be calculated to ± 0.3 mm. The final calculated neutron dose, achieved after about 2,000 h irradiation, was 3×10^{20} n.v.t. (> 1 MeV) at the centre line of the apparatus. The neutron flux drops by less than 10 per cent over the region occupied by the springs and the centre line dose is used, in Table 2, for every spring.

Examination of plots of extension versus dose showed linear creep for nickel, Nimonic 80A, Nimonic PE16 in the fully heat treated (FHT) condition and titanium. The titanium plots passed through the origin, but the lines for the other three materials made positive intercepts with the strain axes suggesting the existence of a transient in the period between zero dose and the first measurement at 6×10^{19} n.v.t. (> 1 MeV). The stainless steel specimens all showed entirely transient forms; by 6×10^{19} n.v.t. (> 1 MeV) the strain in the annealed specimen had apparently saturated, but the cold-worked (CW) specimens did not saturate until between 12 and 18×10^{19} n.v.t. (> 1 MeV). The Nimonic PE16 specimen in the solution treated $(S\Gamma)$ condition also saturated before the first measurement. Table 2 summarizes the observed transient deflexions, steady state deflexion rates and total creep strains. For control tests, one spring of each material was exposed at 98° C in water, for the same length of time the springs were in-pile and their extension measured with a cathetometer. In no instance was a deflexion exceeding 0.5 mm observed.

Table 1. COMPOSITION OF METALS AND ALLOYS (WEIGHT PER CENT)

Element	Nimonic 80A	PE16	Ti	Mo	Ni	316
C	0.06	0.06			< 0.2	0.036
Ši	0.80	0.36		< 0.008	< 0.1	0.640
Mn	0.09	0.09		. 0 000	< 0.35	1.87
s	0.00	~~~			< 0.02	0.010
$\check{\mathbf{P}}$					~ O O2	0.006
Ĉr	19.3	16.75				16.7
Ni	Balance	42.9			99-4	13.7
Sn	Dantellee	T2 0			00 4	< 0.01
Τï	2.25	1.25	Balance			< 0.015
Mo	220	1 20	Datatice	Balance		2.29
Nb				Datance		< 0.05
Co	0.66	0.11				< 0.025
v	0.00	0.11				0.03
'n						
B N ₂		*****				0-0004
iV g						0.039
Aŝ		73 . 3	0.00	0.000		< 0.005
Fe	0.74	Balance	0.09	0.008	< 0.5	
\mathbf{H}_{2}			0.003			
Cu	0.14	0.03	Arrange .	waren.	< 0.25	manus.
Mg	0.01	******	******	*****		
Αľ	1.11	1.13		-		

The composition given for the nickel is not an analysis but a commercial specification.

Table 2. Eo is the initial elastic deflexion, E_t the transient deflex-ION, ÉS THE STEADY STATE DEFLEXION RATE AND & THE TOTAL STRAIN

		Stress			Es/Eo	
		(108	Eo		(per 10	²⁸ €1
Material	Condition	dynes/	(cm)	E_t/E_0	n/cm²	$(\times 10^4)$
		cm²)			> 1 MeV	7)`
Nickel	2 h at 700° C	4.5	0.90	0.79	0.43	15.6
	,, ,,	3.4	0.69	0.71	0.39	9.95
Nimonic PE16	1 hat 1,080° C+16 h					
	at 700° C (FHT)	5.6	1.05	0.19	0.06	3.54
	1 h at 1,080° C, air					
	cooled (ST)	5.7	1.06	0.15		1.34
Nimonic 80A	1 h at 1,080° C+16 h		2			
***************************************	at 700° C	5.0	0.89	0.22	0.26	7.8
Titanium	30 min at 300° C	4.48	1.18		0.40	11.1
	00 11111 40 000	3.40	0.91	Manager .	0.26	5.48
Molybdenum	1 h' at 1.095° C.	0.10	0 0 4		0 20	- 1
morj buoman	cooled in vacuo	6.15	0.61	*	*	*
		5.12	0.54	*	*	*
316 stainless	Cold worked (20%)	6.25	1.11	0.61	-	5.35
steel	CO14 WOLLEG (20 /8)	3.82	0.70	0.61		3.42
50001	20 min at 1,050° C	002	0.0	001		0 12
	(CW), water					
	quenched (annealed)	3.95	0.66	0.20	-	0.96
* 0 - 4 4	quenemon (universe)	0.00	0.00	0 20		0.00
* See text,						

In magnitude the pure metals creep the most, the higher stressed nickel spring having extended slightly more than two elastic deflexions. This agrees with more than two elastic deflexions. previous work1, where greater rates of stress relaxation were observed in pure metals compared with alloys. The small creep strain and early saturation observed in the annealed 316 stainless steel also agrees with earlier stress relaxation work on type 18/8 stainless steel¹. Comparison of the cold-worked and annealed stainless steel specimens shows that the former show more irradiation creep although they are stronger in thermal creep at temperatures below which recovery occurs. This inversion is also seen in Nimonic PE16, where the solution treated spring creeps less than the normally stronger, fully heat treated spring. The behaviour of the Nimonic 80A is similar to that observed by Taylor and Jeffs2; a transient followed by steady state creep. The heat treatment given by Taylor and Jeffs to their specimens is different from that used in this experiment and a more quantitative comparison does not seem possible. The stress exponent of creep is evidently close to 1 for nickel (both transient and steady state) and cold-worked stainless steel (transient)

and to 2.6 for titanium (steady state).

Unexpectedly, it was found that the titanium springs twisted, apparently linearly with time, in the horizontal plane. After the final exposure in-pile the lower and higher stressed springs had twisted by about 100° and 130°, respectively, the horizontal angular rotation of the wire ends tending to uncoil the springs. The possibility of ratcheting of the springs on the screwed connectors to The possibility of which they were spot welded when the apparatus was assembled was excluded by comparing the positions of the wire ends on photographs taken after assembly and after the final exposure in-pile. Twisting of helical springs is expected for geometrical reasons4 but is usually very small. Titanium springs when tested in the laboratory twisted slightly $(\sim 10^{\circ})$ when either stressed to give, on loading, the same total deflexion as the irradiated specimens or allowed to creep to the same extent at a higher stress and temperature than experienced by the in-pile springs. Titanium displays irradiation growth when bombarded by fission fragments and might be expected to grow under neutron irradiation also. The wire texture was determined and it was found that there was a pronounced preferred orientation with the basal plane of the h.c.p. cell parallel to the wire axis. To obtain the observed sense of twisting a contraction along the wire would be required which is incompatible with the preferred orientation and the elongation in the [1120] direction observed by Buckley⁶. Hydride platelets may precipitate in the titanium, but would give twisting of opposite sense to that observed if the platelets are deposited on the {10I0} planes which are known to be the primary hydride habit planes6.

Meaningful strains cannot be quoted for the molybdenum springs which were found to be disappearing rapidly presumably by radiolytic oxidation. The wire diameters were measured after the final exposure and were 0.027 in. and 0.026 in. for the higher and lower stressed springs, respectively. From elasticity theory the corresponding deflexions ought to have been 2.1 cm and 2.2 cm, the actual deflexions were 4.2 cm and 4.8 cm. It therefore seems likely that creep has occurred, but because the stresses on the springs have more than doubled it is probable that a large proportion of this would be thermal creep. Rapid corrosion of molybdenum under irradiation has previously been reported.

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Formation of Zinc Ferrite at Low Temperatures

It has been considered that phase changes such as that of cubic (or tetragonal) to trigonal iron (III) oxide¹ (often called γ and α ferric oxides, respectively) involve diffusionless, topotactic or epitactic processes^{2,3}. The evidence for this view has been derived from observations on selected single crystals-namely, the comparatively large ones suitable for study by X ray diffraction, or the very small ones satisfactory for electron diffraction. Recent work on powders of calcium sulphate4, cubic iron (III) oxide8 and aluminium oxide, however, indicates that phase changes in these materials do not take place until the temperature is high enough for a considerable degree of mobility to exist in the system. In each case the transformations were associated with marked reductions in surface area, visible sintering and improvements in the crystallinity of the systems. These phenomena indicate a substantial degree of movement as would be the case if the materials crystallized by the diffusion-fed growth of nuclei. Nevertheless, the transformations in question occur well below the Tammann temperatures, being first detected at about a third of the melting point expressed in degrees absolutethat is at temperatures where surface diffusion alone might be expected to be in operation?.

It was thought that striking evidence as to the extent of mobility in iron (III) oxide at temperatures at which the rate of transformation of the cubic to the trigonal form becomes appreciable, could be gained by establishing evidence of reaction with a second compound. Such a reaction could take place only by diffusion, and this communication gives some details of the reaction of iron (III) oxide with zinc oxide at temperatures in the neighbourhood of

400° C.

Reaction between zinc oxide and the trigonal and cubic forms of iron (III) oxide was brought about as follows. Zinc oxide and the appropriate hydrous iron (III) oxide or hydroxide were mixed for 3 h in a mechanical mortar in the correct proportions to give zine territe (zinc iron (III) oxide—spinel type, ZnFe₂O₄). Samples (2 g) of the mixture were then placed in a reaction vessel attached to an all-glass apparatus already described, and heated for 6 h at 270° C in vacuo. Water, which is largely removed by this treatment4,5, is highly active in the system and its effects were minimized and standardized by dehydrating and outgassing under a constant set of conditions. It was shown that no ferrite was formed during this treatment. When the sample had been dehydrated the temperature was raised to a selected value for a further 6 h.

Zinc oxide (composition: zinc oxide, 99.5 per cent; water, 0.39 per cent; organic oxides, 0.13 per cent; sodium. 0.005 per cent; magnesium oxide, 0.0001 per cent) of particle size 0·1-1µ, and a surface area of about 10 m²/g was used. Unless indicated to the contrary, the mixtures were made up from precipitated hydrous iron (III) oxide (composition: ferric oxide, 78-3 per cent; water, 19-9 per cent; ferrous oxide, 0.05 per cent; nitrate, 0.001 per cent; chlorine, 0.010 per cent; ammonia, 0.04 per cent) very similar to that used by Molony and Ridge' when the trigonal iron (III) oxide was required. For the cubic oxide (the material was highly disordered and so was not regarded as tetragonal1) precipitated iron (III) oxide hydroxide crystallographically similar to the mineral lepidocrocite was used. In this case, the material was taken from the same batch used by Ridge, Molony and Boell*. Measurements by nitrogen adsorption gave values of about 150 and 110 $\rm m^2/g$ for the surface areas of the two oxides, respectively.

The products of heating the mixtures of oxides were analysed for zinc ferrite by X-ray diffractometry, the integrated intensities of the diffraction peak for the 2.99 Å spacing being used to determine the amount of ferrite. The values used are the means of two independent

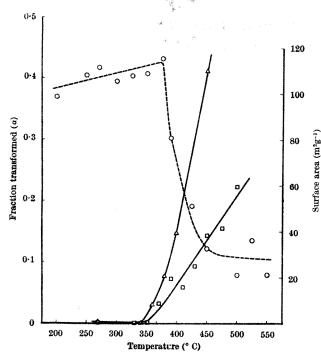


Fig. 1. Fraction (a) of iron (III) oxide combined with zinc oxide in 6 h in vacuo plotted against temperature.

Cubic iron (III) oxide;

C, the surface areas are for cubic iron (III) oxide;

oxide alone as determined by Ridge, Molony and Boeil*.

determinations and I per cent of ferrite could easily be detected in artificial mixtures. It was shown that the small neighbouring diffraction peak in the case of the cubic oxide did not interfere.

Fig. 1 shows temperature plotted against the fraction (a) of the reactants converted into ferrite for systems containing iron (III) oxide in the cubic and trigonal forms. It is seen that reaction can be detected at 360° C and a equals about 0.5 at 450° C for trigonal oxide. The surface areas obtained by Ridge, Molony and Boell⁵ for the cubic oxide are also shown, and it is seen that reaction coincides with the sharp reduction in the surface. These facts indicate that there was sufficient mobility in the cubic oxide studied previously to account for the phase change on the basis of the growth of diffusion-fed nuclei.

The formation of zinc ferrite has been extensively studied using what was probably relatively unreactive iron oxide (say fired at 600° C) or haematite in systems open to the atmosphere 7,10-12. No reaction was detected until the temperature had been raised to about 650° C. We believe that the difference between these findings and those reported earlier results from differences in the conditions of the materials and experiments. The comparatively high surface areas of the oxides allow intimate contact. Furthermore, the high degree of cleanliness of the surfaces could allow higher rates of surface diffusion. A possible example of the effects of surface cleanliness is provided by the fact that a mixture of zinc oxide and powdered haematite gave a yield of about 2 per cent zinc ferrite when heated for six hours at 450° C in vacuo after having been outgassed at 270° for 6 h. When the same mixture was heated at 450° C in a crucible in a furnace or when air was admitted to the reaction vessel after outgassing at 270° C, no reaction took place. Apart from enhanced diffusion over clean surfaces the only other possible explanation for this behaviour is that the higher content of vacancies in materials heated in vacuo increases the rate of body diffusion. This, however, does not seem likely at 450° C, at which temperature the loss of oxygen was less than 0.0007 per cent, as determined by collecting the evolved gases and analysing them.

When a mixture of zinc oxide and the highly reactive iron (III) oxide described here was heated at 450° C in the air, zinc ferrite was formed.

For reaction to occur between the two oxides, they must both be capable of diffusion. The melting point of zinc oxide is 1,800° C indicating a Tammann temperature of about 760° C (400° C for surface diffusion). Bearing in mind the approximate nature of the derivation of these temperatures, the reaction of zinc oxide at about 360° C is probably in accord with the concept of the Tammann temperature and the analogous temperature for surface diffusion. Nevertheless, it seems a little difficult to believe that surface diffusion alone was responsible for the formation of zinc ferrite at 450° C in view of the rapidity of the reaction-namely, a yield of about 50 per cent in a period of 6 h (see Fig. 1).

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CHEMISTRY

Nuclear Magnetic Resonance as a Method for Continuously Monitoring Rehydration

Previous work¹ has established nuclear magnetic resonance (NMR) as a method for determining moisture content of foodstuffs. The principles have been further employed² in obtaining rehydration and dehydration curves for batches of samples pretreated for different times. We have extended the use of NMR to include continuous monitoring of rehydration processes.

The uptake of water by several dried materials (for example, macaroni, peas, lentils, collagen fibres) has been followed with an NMR process control instrument (PCI). The experimental method is simple: a known volume of water is added to a weighed dry material in a 100 ml. Nessler tube in the instrument probe. The sample requires no control in packing. The relative rate of uptake of water shown in Fig. 1 is obtained by observing the increase with time in the NMR signal area as recorded directly by the PCI. Calibration of the instrument and a knowledge of the initial water content of the dry sample are required for determination of the absolute water uptake.

The method is based on the fact that pure water has proton magnetic resonance relaxation times of a few seconds3. When water is close to, or bonded with, an immersed solid the relaxation times shorten4. Conditions can be chosen such that in a water-solid mixture the protons of the pure (free) water are RF saturated5 to give a low signal, whereas those of the water associated with the solid are virtually unsaturated and give a much higher signal. If this is achieved, the signal per unit mass from the associated water will be much greater than that from the free water. If we consider the case of rehydration

of a solid sample, the signal will increase as the amount of free water decreases and associated water correspondingly increases. Thus by selecting suitable instrumental conditions, it is theoretically possible to follow rehydration processes. It should be noted that the process control instrument used in this work, manufactured by Newport Instruments, Newport Pagnell, Buckinghamshire, has a gate which defines the width of signal accepted. If the relaxation times shorten greatly, the signal width may exceed the gate width and the peak area observed may decrease. This, however, is not at present relevant.

It is possible to derive an equation predicting the shape of the signal increase curve. Let f_t = fraction of water associated with the solid sample at time t; F = signal/gof the free water; B = signal/g of the water associated with the sample; W = total weight of water present; $S_t =$ observed NMR signal area at time t; and Z = zero point

error of the instrument.

Then, assuming that Z, B, W and F are constant (for any experiment),

$$S_t = (1 - f_t) WF + f_t WB + Z$$

= $f_t W (B + F) + WF + Z$

This equation shows that the time scale of the rehydration of the sample can be represented as in Fig. 1 by the NMR signal curve. By weighing the rehydrated sample and knowing the dry weight and water content of the sample initially, the NMR signal can be related to weight change and Fig. 1 altered to a weight against time curve. The curve obtained for rehydration of macaroni supports these ideas. A weight increase against time curve was obtained independently for the macaroni by weighing samples of wet macaroni after different uptake times. When points on this weight curve are plotted against the NMR signal from the process control instrument, at corresponding times, a straight line results. This suggests that the curves correspond exactly in shape and the

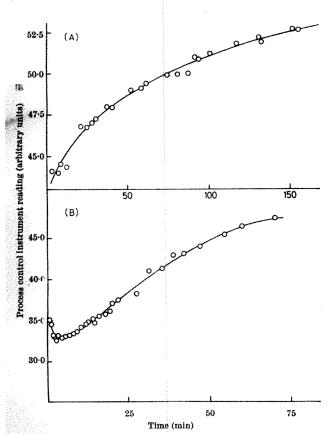


Fig. 1. A, Rehydration curve for macaroni; B, rehydration curve for

method is valid. Both the weighing and NMR rehydration experiments were carried out at 22° C.

Usip only the PCI signal curve and the dimensions of the sample, it is possible to calculate diffusion coefficients for water into dry materials. Ref. 6 gives equations for planar, spherical, cylindrical and tubular samples relating the fractional uptake of water at any time to the sample dimensions and diffusion coefficient. If we assume that the water uptake by macaroni is diffusion controlled and apply the equation in ref. 6 to the PCI curve, we obtain a value at 22° C for the diffusion coefficient of water into macaroni of 1.6 to 2.3 × 10-7 cm² sec-1 (this compares with the value obtained by Fish of 2.4×10^{-3} cm² sec⁻¹ for potato starch gel at 25° C).

The initial dip observed in the rehydration of dried peas in Fig. 1 is interesting. The origin of the peas and the experimental temperature affect the size of the dip, which increases at lower temperatures and disappears at about 25° C, above which temperature it cannot be observed. The dip is thought to be caused by leaching out of pea sugars, for if the peas are repeatedly soaked and washed and then dehydrated, the rehydration curve shows no initial dip. The dip also depends on the RF level used,

and is unobservable at low RF levels.

It may be mentioned that dehydration rates can be similarly observed. Warm air is blown through a moist sample while it is in the instrument probe, and the reduction in NMR signal as water is removed is recorded.

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Optical Nitrogen-15 Analysis of Small Nitrogen Samples using a Noble Gas to sustain the Discharge

In view of the dominant part which protein plays in nature, the isotope nitrogen-15 is valuable as a tracer for nitrogen, especially in agriculture, biology and medicine.

Nitrogen-15 is usually analysed by mass spectrometry, but it is now well established 1-4 that the abundance of nitrogen-15 in biological samples can be routinely analysed by optical emission spectroscopy.

The sensitivity of this tracer method is ultimately determined by the smallest amount of excess nitrogen-15 (above natural nitrogen-15) which can be accurately measured, which is at present about 10-100 ng of excess nitrogen-15 by the mass spectrometric methods and by the optical method. This sensitivity is, however, not sufficient in many tracer investigations. For example, an amino-acid spot on a paper chromatogram may contain only 0-1 µg of nitrogen with 0-1 ng of excess nitrogen-15. In this particular case, an increase in sensitivity of measurement of at least a hundred-fold would be necessary.

We have found that a considerable increase in the sensitivity of the optical method can be obtained by using a noble gas as a sustainer in the electrodeless discharge

tube containing the nitrogen sample.

We used pure nitrogen and noble gases from the Linde Company, München, in a standard discharge tube made of rasotherm ('Pyrex') glass by the Karl Kummer Company, Thüringen, a high frequency generator constructed in our laboratory⁷ and a Hilger and Watts quartz spectrograph, model E 742, modified in our laboratory⁸. Our vacuum line was essentially the same as that described elsewhere with the addition of the nitrogen and noble gas containers, and appropriate dosing volumes.

The discharge tube was evacuated by baking and sputtering with krypton or argon before the nitrogen or the mixture of nitrogen and krypton or argon was added. The various pressures, and thereby the mixing ratios, were measured with an 'Autovac' gauge, type 3294 B, from LKB, Stockholm. The tube was air-cooled during

discharge.

Using the standard discharge tube, it has recently been found that 5 µg is the smallest amount of nitrogen that can be analysed by the optical method. This we have found to be reproducible with pure nitrogen. Furthermore, using krypton or argon at a pressure of 1 torr as a sustainer, quantities of natural nitrogen in the range 0.5-5 µg were analysed for nitrogen-15 abundance to an accuracy of ±5 per cent. This constitutes a reduction of the lower limit by an order of magnitude.

Using a quartz capillary, 2 mm bore and 3 in. long, the smallest quantity of nitrogen which could be analysed was reduced to about 0.1 µg. This quantity of nitrogen contained about 0.5 ng of nitrogen-15, which approaches the amount mentioned in the amino-acid example given

A detailed investigation of the use of adding noble gases as sustainers (and possibly, internal standards) in optical nitrogen-15 analysis will be made in the near future.

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Chemisorption of Propane on Platinum Surfaces in the Presence of Pre-adsorbed Water

The chemisorption of saturated hydrocarbons on platinum surfaces has been studied by several authors¹⁻³, whose results imply that chemisorption of hydrocarbons is accompanied by extensive dissociation of the molecule, at least at temperatures above 80° C (ref. 3). It is assumed that some C-C and much C-H is dissociated at temperatures of about 100° C.

For catalytic processes in the presence of water, in which the chemisorption of saturated hydrocarbons is

involved, it is interesting to know how the chemisorption is affected by adsorbed water. In order to study this question, we have investigated the chemisorption of propane on platinum in the presence of pre-adsorbed water.

In the experiments, Raney platinum was used as adsorbent. Its surface was cleaned by an oxidationreduction treatment with gaseous oxygen and hydrogen

and aged as described elsewhere2.

The adsorption experiments were carried out with an electro-magnetic vacuum microbalance (Sartorius-Werke, Göttingen); the experimental temperature was 100° C. Water vapour at various pressures was admitted for various periods of time, and the increase in weight caused by water adsorption was recorded. The apparatus was evacuated (10^{-6} mm mercury) in order to remove gaseous and weakly adsorbed water, leaving a certain quantity of water adsorbed on the surface. Subsequent admission of propane (1 mm mercury) led to a further increase in weight, which was reduced on evacuation. The remaining increase in weight of the sample will be referred to here as "net weight increase on chemisorption of propane". Because this net weight increase might include, besides propane chemisorption, a contribution resulting from water desorption, we carried out independent determinations of the amount of propane chemisorbed by oxidizing the chemisorbate by admission of oxygen (1 mm mercury) at 100° C. This led to the complete oxidation of the surface species to give carbon dioxide, which desorbed. Mass spectrometry of the product gas allowed us to determine the carbon content of the chemisorbate, and thus the number of chemisorbed propane molecules.

The experimental results are plotted in Fig. 1 and Fig. 2which show, respectively, the amount of chemisorbed propane and the net weight change on propane chemisorption as a function of the amount of water pre-adsorbed on the catalyst surface. Fig. 2 also includes a plot of the difference of the amount of chemisorbed propane and the net weight change, which represents the amount of water displaced

from the surface on chemisorption of propane.

The results may be summarized as follows. (a) The quantity of chemisorbed propane is independent of the degree to which the surface is covered with pre-adsorbed water, which ranged between 0 and 1.9×10^{15} molecules of water/cm² in this investigation. (b) Compared with the number of platinum surface atoms-

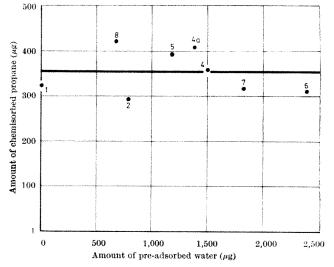


Fig. 1. Amount of chemisorbed propane as a function of coverage of the surface with pre-adsorbed water. The numbers at the dots denote the experiment number; the solid line represents the average value of the experimental data. Temperature, 100° C; weight of catalyst sample, 0.800 g; BET specific surface area of catalyst, $5.2~\mathrm{m}^2/\mathrm{g}.$

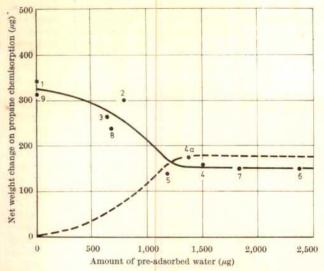


Fig. 2. , Net weight change on propane chemisorption as a function of surface coverage with pre-adsorbed water. The numbers at the dots denote the experiment number. - - - , Amount of pre-adsorbed water displaced on chemisorption of propane (difference between amount of chemisorbed propane—355 µg, solid line of Fig. 1—and net weight change). Temperature, 100° C; weight of catalyst sample, 0.800 g; BET specific surface area, 5.2 m²/g.

cm2, which is the mean of the values for the three most important crystal faces (100), (110) and (111)—the number of chemisorbed propane molecules is small- 1.2×10^{14} /cm². Thus one propane molecule is chemisorbed for every ten platinum surface atoms. (c) At low surface water concentrations, the amount of water displaced from the catalyst surface on chemisorption of propane increases with increasing coverage of the surface with pre-adsorbed water. The displaced quantity is constant above a water coverage of 1·1×1015 water molecules/cm2 or about one water molecule for each platinum surface atom. (d) The maximum amount of water displaced on chemisorption of propane is 205 µg per sample. This corresponds to about 1.6 × 1014 molecules of water/cm2. Compared with the propane value of 1.2×10^{14} , this leads, approximately, to a one-to-one correlation between chemisorbed propane and displaced water molecules.

A qualitative picture of the chemisorption of propane at 100° C in the presence of adsorbed water may be built up from the results. If the platinum surface is completely covered with adsorbed water, chemisorption of one propane molecule requires the displacement of one water molecule. In view of the large difference in the cross sectional areas of propane (about 35 Å2, estimated to be the mean value of cross sectional areas of n-butane and ethane4) and water (10.6 Å2 (ref. 4)) in physisorption, this seems to be caused by valency reasons rather than steric hindrance. At lower water coverages, the average number of displaced water molecules is naturally smaller.

At water coverages above a certain limiting value which corresponds to about one molecule of water for each platinum surface atom, the additionally adsorbed water is in a state in which it does not interfere with subsequently adsorbed propane.

The observations that the chemisorbed amount of propane is not changed by coverage of the surface with pre-adsorbed water and that only one water molecule is displaced by one propane molecule do not allow the conclusion that extensive dissociation does not follow the first propane chemisorption step. It may well be that instead of a simple dissociation occurring in the water-free case, we are dealing, in the case of water coverage, with a reaction of the chemisorbate with adsorbed water under simultaneous dissociation.

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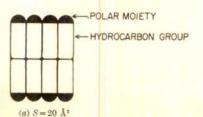
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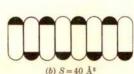
Optically Positive, Isotropic and Negative Lamellar Liquid Crystalline Solutions

The subject of the structure of liquid crystalline solutions of amphiphiles is of importance both with regard to the theory and technology of these substances and in relation to the liquid crystalline character of certain biological units, for example, cell membranes.

The aqueous lamellar liquid crystalline solution phase (neat phase, G) formed by many amphiphiles usually constitutes a positive uniaxial crystal. In this phase the amphiphile molecules form bimolecular leaflets with the hydrocarbon chains in contact (Fig. 1). The polar groups are associated with the water layers interposed between successive leaflets2.

In this model, the hydrocarbon chains, although commonly represented diagrammatically as lying normal to the planes of the lamellae, Fig. 1a, are believed to be in quasiliquid state and subject to thermal motion which, apart from the restriction imposed by their attachment to the polar groups in the lamellae, is similar to that in a liquid hydrocarbon of corresponding molecular weight. Except in certain circumstances considered here, the terminal attachment of the hydrocarbon chains to the polar groups will, however, lead to a predominating direction of carbon-carbon bonds across rather than along the lamellae -that is along rather than at right angles to the optic The predominating direction of carbon-carbon bonds along the optic axis provides the principal source





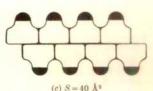


Fig. 1. Schematic representation of possible hypothetical territorial envelopes of amphiphile molecules in thermal motion in lamellae in which the effective area, S, per polar group is (a) 20 Å², (b) and (c) 40 Å²; in reality the envelopes will overlap.

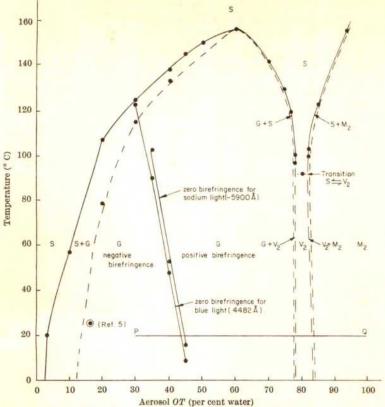


Fig. 2. Phase diagram for the Aerosol OT/water system. The boundaries indicated by broken lines are tentative. S is the mobile isotropic phase; G is the neat phase; M_z is the inverse middle phase; and V_z is the inverse viscous isotropic phase.

of the positive birefringence shown by the majority of G phases3.

It was noted by one of us (P. A. W.)4 that the stability of liquid crystalline G phases, over the wide ranges of concentration experimentally observed, indicates that changes in the effective area for each polar group must occur with changes in concentration although the order of the changes involved, as indicated by X-ray diffraction measurements⁵, was small. Definite, experimental

evidence of such changes, however, has been provided by more recent X-ray measurements. Thus with the potassium soaps of the n-fatty acids at 86°C, within the concentration limits of stability of the G phase, the effective area, S, for each polar group has been found to vary from 30.2 Å2 to 41.4 Å2.

In view of the quasi-liquid structure of the hydrocarbon regions of the G phase, it might be expected that if the directions of all the carbon-carbon bonds at any instant are resolved and summed as components normal to and parallel to the lamellae, then the ratio, X, of components normal to the lamellae against components parallel to the lamellae will tend to decrease with increase in the effective area, S, per polar group. Furthermore, if possible sources of birefringence other than preferred orientation of the carbon-carbon bonds are neglected, the positive birefringence of the G phase will decrease with decreasing values of X, becoming zero when X is about 0.5, and subsequently negative. X, however, is not necessarily a single-valued function of S, but at a given value of S, may vary with temperature. Thus if hypothetical values of S are taken as 20 Å^2 (close packing) and 40 Å2, possible hypothetical conformations of the territories occupied by amphiphile molecules in thermal motion within the bimolecular leaflets of the G phase may be

represented diagrammatically as in Fig. 1. Conformations (a) and $(b)^7$, in spite of the different values of S, would show The birefringence positive birefringence. of the conformation (c), in which the territories are statistically flattened with respect to the plane of the polar groups, would be less positive than that of (a) or (b). Forms intermediate between (b) and (c) and of consequently intermediate birefringence may

also be envisaged.

With amphiphiles containing a single n-alkane chain, the observed sign of birefringence of the lamellar phase is, apparently, always positive. From the experimental values of S found within the limits of stability of the lamellar phase produced with the fatty acid soaps⁶, this result is, on the basis of Fig. 1, not surprising. For branched-chain amphiphiles, however, molecular configurations within the G phase at which X could pass from > 0.5 at higher concentrations to <0.5 at lower concentrations, with increase in the effective area for each polar group2,6, would seem more

For example, with Aerosol OT (sodium sulpho-di(2-ethylhexyl) succinic ester) the two C₈ branches could be envisaged as forming a compact more acute-angled V (positive birefringence) at higher concentra-

tions which gradually opened to a more recumbent obtuse-angled V (negative birefringence) as the concentra-

tion was diminished and S increased.

In agreement with these considerations, we have recently observed a concentration-dependent change in the sign of birefringence with the aqueous G phase formed by this amphiphile and also by the corresponding potassium

With Aerosol MA (sodium sulpho-di(1,3-dimethylbutyl)

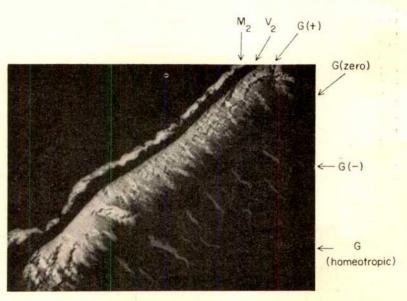


Fig. 3. Phase succession and change in birefringence produced by peripheral evaporation of the optically negative aqueous neat phase, G, formed by Aerosol OT (30 per cent weight). (\times 24.)

succinic ester), on the other hand, although the positive birefringence of the G phase at higher concentrations diminishes markedly with dilution, the phase breaks down at a concentration of about 37 per cent weight at room temperature, giving the isotropic micellar phase, before an actual change in sign of its birefringence has taken place.

Fig. 2 gives a tentative phase diagram which we have recently constructed on the basis of optical properties, nuclear magnetic resonance spectra and viscosity for a carefully purified sample of Aerosol OT. Fig. 3 illustrates the effect, as observed with a polarizing microscope, using white light, of allowing a sample of a negatively birefringent G solution of Aerosol OT (30 per cent weight) to undergo peripheral evaporation between slide and cover slip. In this way, an increasing concentration from the centre towards the edge of the sample is produced. Within the G phase itself, positive (outer, more concentrated) and negative (inner, less concentrated) zones may be distinguished which are separated by a narrow band not showing birefringence. The sign of the birefringence of the two zones was determined with a Red I compensator3. The textural features which extend across the two zones, in the one zone correspond to the fast direction, in the other correspond to the slow and in the intermediate band show zero birefringence (Fig. 3). The additional zones shown in Fig. 3 correspond to the phase succession $G \rightarrow V_2 \rightarrow M_2$ (ref. 8), indicated in Fig. 2 along the line PQ. The zone of null birefringence within the G phase in Fig. 3 corresponds in composition to the point of intersection of PQ and the line of zero birefringence for white light in Fig. 2.

It will be seen from Fig. 2 that for G solutions with birefringence close to zero the sign of the birefringence is sensitive to temperature. A rise of temperature produces an effect similar to that of an increase in concentration. A similar correspondence in the effects of increase in temperature or concentration is indicated by slight slopes of the $G-V_2-M_2$ phase boundaries in Fig. 2. results suggest a reduction in the effective area for each polar group with rise of temperature. This contrasts with the conclusions derived from X-ray diffraction measurements with the alkali-metal soaps, where an increase in area with rising temperature is found. The polar moiety of Aerosol OT (including the non-ionic ester carbonyl groups), however, differs considerably from the polar group of the soaps, and the interactions of the two types of group with the aqueous regions of the G phase may be affected differently by changes in temperature.

When a sample of the G phase about 5 mm in diameter, having any composition that gives negative birefringence (Fig. 2), is held between crossed polaroids and subjected to a gradual change in temperature which changes the sign of birefringence, a succession of interference colours can be observed with white light. The colours observed with white light at the points of zero birefringence as determined with sodium light or blue light are not black, but indigo blue and brownish yellow, respectively. These observations indicate that the Aerosol OT G phase, in the region of minimum birefringence, shows appreciable dispersion of the birefringence, so that the compositions corresponding to zero birefringence in Fig. 2 are, as shown, dependent on the wavelength of the light used (compare ref. 3, p. 338). J. Rogers

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Effect of Hydrogen Atom and Electron Scavengers on the Radiolysis of Ammonia Gas

Ammonia gas is decomposed by the action of ionizing radiations, hydrogen and nitrogen being the only products formed in significant amounts¹⁻³. Although some information about the nature of the primary processes in the radiolysis is available, chiefly as a result of mass-spectrometric studies4-8, the relative contributions of the different primary processes to the overall decomposition are not known. We report here an investigation aimed at determining the primary yield of hydrogen atoms in the radiolysis of ammonia and the contribution of ion neutralization to this yield.

It has been shown previously that the principal primary processes which are likely to play a part in the radiolysis of ammonia result ultimately in the formation of the radicals H and NH2 and it can be assumed that it is the reactions of these species which give rise to the products hydrogen and nitrogen. Thus the chief positive ions, formed by the processes (1) and (2)

$$NH_3 \land \land \rightarrow NH_3^+ + e^-$$
 (1)

$$NH_3 \land \land \rightarrow NH_2^+ + H + e^-$$
 (2)

react with ammonia by the fast reactions (3) and (4)

$$NH_2^+ + NH_3 \rightarrow NH_2 + NH_3^+ \tag{3}$$

$$NH_3^+ + NH_3 \rightarrow NH_4^+ + NH_2$$
 (4)

The ammonium ion persists (probably in the clustered form NH₄+nNH₃ (ref. 9)) until it is neutralized. Because negative ions are formed only to a negligible extent in pure ammonia7, the reaction of ammonium ions with electrons is the main neutralization process. Two possible reactions have been suggested³

$$NH_4^+ + e^- \rightarrow NH_3 + H$$
 (5)

$$NH_4^+ + e^- \rightarrow NH_2 + H_2$$
 (6)

Studies of the photolysis of ammonia10 indicate that the chief consequence of electronic excitation in the radiolysis will be N-H bond scission

$$NH_3 \wedge \rightarrow NH_2 + H$$
 (7)

The elimination of molecular hydrogen may be expected to occur to a minor extent10

$$NH_3 \land \land \rightarrow NH + H_2$$
 (8)

In order to measure the yield of hydrogen atoms and hydrogen from reactions (1) to (8), we used propane as scavenger. This reacts efficiently with free radicals but not with the other important intermediates, ammonium ions and electrons. The gases were irradiated with cobalt-60 γ-rays in sealed 'Pyrex' vessels. The dose rate, measured by the nitrous oxide dosimeter, assuming $G(N_2)=10$ molecules/100 eV (ref. 11), was 2×10^{15} eV/ml./min in ammonia at 760 torr, 20° C. The gaseous products, collected over traps at 77° K, were measured volumetrically and analysed mass spectrometrically.

Fig. 1 shows the dependence on temperature of the yields of hydrogen and nitrogen from pure ammonia, ammonia mixed with propane (1.5 mole per cent) and sulphur hexafluoride (0.3 mole per cent). In agreement with previous observations, the yields from pure ammonia are markedly dependent on temperature, and the ratio of the yields, hydrogen/nitrogen, is very close to the stoichiometric ratio, 3:1, which implies that the yield of hydrazine is negligible. In the presence of propane, the formation of nitrogen is greatly decreased and the hydrogen yield, $G(H_2) = 8.0 \pm 0.3$, is independent of temperature.

The marked depression of the nitrogen yield by propane can be accounted for in terms of reaction (9)

$$NH_2 + C_3H_8 \rightarrow NH_3 + C_3H_7 \tag{9}$$

which removes the NH2 radicals which in pure ammonia react to give nitrogen.

Hydrogen atoms react with propane

$$H + C_3H_8 \rightarrow H_2 + C_3H_7 \tag{10}$$

The yield of hydrogen is independent of the concentration of propane, from 1.5 to 6 mole per cent, showing that, at the concentrations used, reaction (10) competes favourably with any alternative reactions of hydrogen atoms. The yield of hydrogen $G(H_2) = 8.0 \pm 0.3$ is therefore a measure of the total yield of hydrogen atoms plus the molecular hydrogen formed directly—for example, by reactions (6) and (8). With propylene or benzene present (1.5 mole per cent), the yield of hydrogen fell to $G(H_2) < 0.8$. These additives remove free radicals by addition reactions so that the hydrogen observed is only that formed by molecular processes. The effect of propylene and benzene therefore suggests that the molecular yield is only a small fraction of the total primary yield.

Addition of sulphur hexafluoride to mixtures of ammonia and propane decreases the hydrogen yield by an amount $\Delta G(\hat{H}_2) = 3.9 \pm 0.3$ (Fig. 1). Sulphur hexafluoride captures electrons efficiently12, and it can be assumed that, in the presence of this additive, neutralization of the ammonium ion will involve reaction with the negative ion, SF₆-. The depression of the yield of hydrogen by sulphur hexafluoride is close to the yield of ammonium ions, $G(NH_4^+)$ = 3.8 (calculated from W = 26.5 (ref. 13)) and is therefore consistent with a mechanism in which neutralization of an ammonium ion by an electron results in the net formation of one hydrogen atom whereas neutralization by an SF₆- ion does not give any hydrogen atoms, for example,

$$NH_4^+ + SF_6^- \rightarrow NH_3 + HF + SF_5$$
 (11)

The effect of SF₆ in this system is similar to that previously observed in water containing scavenger concentrations of propane14. The residual yield of hydrogen from mixtures of ammonia with propane and sulphur hexafluoride

is presumably caused by reactions (2), (7) and (8). The observation that total yield of hydrogen from ammonia-propane mixtures, and the depression of this yield by sulphur hexafluoride, are independent of temperature, at least over the range 20°-200° C, strongly suggests that the primary reactions leading to hydrogen atoms in this system are not influenced by temperature. This finding is in conflict with an earlier suggestion¹⁵ that the extent of clustering of the ammonium ion, varying with temperature, might influence the ion-neutralization process and hence the extent of the primary decomposition.

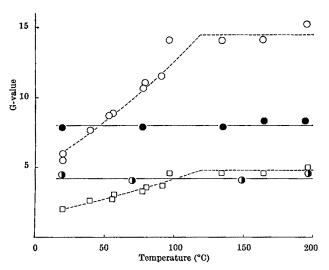


Fig. 1. Dependence on temperature of the yields from the radiolysis of ammonia in the absence and presence of scavengers. O, Hydrogen; D, nitrogen from pure ammonia; , hydrogen from ammonia + propane (1.5 mole per cent); , hydrogen from ammonia + propane (1.5 mole per cent) + sulphur hexafluoride (0.3 mole per cent). Total pressure, 1 atmosphere.

In the radiolysis of pure ammonia, the inter-reactions of the free radicals H and NH2 can result either in the formation of hydrogen and nitrogen or in the re-formation of ammonia. Without knowing the actual mechanism of the reactions involved, it is impossible to draw any firm conclusions from the total product yields about the extent of primary decomposition in pure ammonia. The results with propane as scavenger indicate, however, that the primary decomposition is independent of temperature. It is suggested therefore that the effect of temperature on the net decomposition of pure ammonia is the result of the competing reactions, which involve H and NH2 and, possibly, other intermediates formed from these radicals. This competition favours the re-formation of ammonia at low temperatures and the formation of hydrogen and nitrogen at high temperatures. The observed dependence on temperature of the yields would be consistent with an activation energy difference between the competing processes of about 2 kcal/mole (Fig. 1).

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BIOLOGY

Molecular Theory of Sweet Taste

THE molecular feature common to the many different sweet tasting compounds has been sought for many years¹. For the sugars, it was proposed²⁻⁵ that the sweet unit is the glycol group, and that intensity of sweetness varied inversely with the degree to which glycol OH groups appear to be intramolecularly hydrogen bonded. It is now apparent that vicinal OH groups in the glycol unit need to be approximately gauche, or in a staggered conformation. Vicinal OH groups which are in the anti conformation apparently are too far apart to cause sweet taste. Glycol OH groups which are eclipsed probably participate in an intramolecular hydrogen bond which competitively inhibits interaction of glycol with the These steric features of sweet and nonreceptor site. sweet sugar glycol units are shown in perspective in Fig. 1. Glycol conformational parameters, and the gross conformation of pyranose and furanose rings, have been used to explain the varying sweetness of the sugars²⁻⁵.

A corollary to the thesis that sugar sweetness is inhibited by glycol intramolecular hydrogen bonds is that the primary mechanism for the sweet taste response is intermolecular hydrogen bonding between the glycol unit and the taste bud receptor site. The sweet unit of the sugars, that is the glycol unit, can be viewed as a bifunctional entity with an AH and a B component.

It is in this sense that we recognize a molecular feature common to the many compounds which taste sweet. In an AH, B system, A and B are electronegative atoms separated by a distance of greater than $2\cdot 5$ Å, but less than 4 Å. H is a hydrogen atom attached to one of the electronegative atoms by a covalent bond. Thus sweet tasting compounds must have a slightly acidic proton within a specific distance of an electronegative orbital. Usually A and B are either oxygen or nitrogen, but in certain cases one of them may be a carbon or chlorine atom or a centre of unsaturation. AH is a proton donor and B is a proton acceptor. Examples of sweet compounds and their AH, B system are shown in Fig. 2.

As an example of the sugars, β -D-fructopyranose is used. Probably all vicinal OH groups constitute sweet glycol units. The anomeric OH group, however, possesses the most acidic hydrogen in the molecule, and constitutes an AH moiety. In close proximity, and having free rotation, is the methylene OH group which could serve as the B moiety of the AH, B system.

Saccharine is the better known of the so-called "synthetic" sweetening agents. The AH moiety is the NH group. Two possible B moieties exist, the carbonyl oxygen atom and the sulphoxide oxygen atom. The latter is favoured, for pseudo-saccharine (enol-saccharine) does not taste sweet, and an AH, B system using the imide and sulphoxide groups also accounts for the sweetness of the cyclamates.

Chloroform is an example of a small molecule which is quite sweet. Because of the electron withdrawing power of the chlorine substituents, the carbon atom of the molecule is electronegative and the hydrogen atom is slightly acidic, and this constitutes the AH moiety. The electronegative chlorine atoms comprise the B unit to complete the bifunctional system.

Unsaturated alcohols, with the structure shown in Fig. 2, possess an OH group which is α - to a double bond, and consequently this OH group is slightly acidic. The unsaturated centre is an excellent proton acceptor site.

Alanine, whether D or L, is sweet¹. The $\rm NH_3+$ group in solution is an obvious AH moiety, and the COO-group would serve as the proton acceptor site. Other amino-acids have varying degrees of sweetness depending upon their size and the stereochemistry of the α -carbon atom.

An example of a nitro-benzene compound that tastes sweet is 1-propoxy-2-amino-4-nitrobenzene. The sweet-

Fig. 1. Sweet and non-sweet sugar glycol units.

NOT SWEET

Fig. 2. Compounds of various chemical classes and their common (AH, B) unit.

2-AMINO-4-NITROBENZENES

ALANINE

ness of the nitrobenzenes is greatly affected by substitution on the benzene ring. Thus it is apparent that the electron density at a specific point in the molecule will determine whether or not that molecule tastes sweet. As pointed out by Deutsch and Hansch⁶, electron donating groups para to the nitro group, or an electron attracting group meta to the nitro group, are related to sweetness. Our contention is that the ortho hydrogen is the proton of the AH, B system and the influence of other substituents on the nitrobenzene ring alter sweetness by changing the acidity (or H bonding power) of the hydrogen ortho to the nitro group. The importance of electron distribution in the aromatic ring is further emphasized by the significance of the Hammett σ value in the Deutsch and Hansch equation⁶.

Table 1. INTERATOMIC DISTANCES OF AH, B SYSTEMS IN REPRESENTATIVE

	177 t D			
Compound	AH	В	A-B distance (Å)	orbital distance (Å)
β -D-Fructopyranose	—он	-0-	3-5	2-4
Saccharine	NH	→0	2.5	3.0
Chloroform	-CH	Cl	1.9	2.8-3.2
1-Octene-3-ol (unsaturated alcohol) Alanine	—он — n н‡	H H C=C- C00-	3·0 3·0	2-4 2-4
1-Propoxy-2-amino- 4-nitrobenzene	ArH	→ 0	2.7	-

The A-B distance of sweet compounds does not appear especially important with respect to sweetness, as shown in Table 1. The AH proton-B orbital distance, however, is more significant. This is an average value of about 3 Å for all sweet compounds examined.

It follows that the receptor site is also a bifunctional unit similar in nature to the AH-B system of a sweet compound itself. Then the interaction between the receptor site and the sweet unit is a "concerted interaction" involving two simultaneous hydrogen bonds

Receptor
$$A - H \dots B$$
 Sweet unit

The interaction is neither a proton transfer nor an electrostatic interaction, but probably involves London dispersion, the principal element of hydrogen bonds7. It was proposed that the site could be the protein peptide bond3, but it could also be a protein amide group of glutamine or asparagine. Dastoli and Prices showed that a protein from bovine taste buds does indeed respond to sweet tasting compounds.

It would now seem that the complementary AH, B system needed to elicit sweet taste is a unit common to sweet tasting compounds which satisfies, regardless of chemical class, the spacial and hydrogen bonding require-

ments imposed by the receptor site.

Compounds with mixed tastes, but which are exceedingly sweet, such as saccharine and the cyclamates, also possess a hydrophobic bonding system which may account for the rapid "impact" time of chemicals when they elicit sweet taste. The hydrophobic bonding, how-ever, may also impart the intrinsic "bitter" afternote. Properties such as a hydrophobic nature may influence the intensity of sweetness, but the AH, B system described here is prerequisite for a compound to be sweet.

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Hereditary Defect in Iron Absorption in Mice

Sex linked anaemia, which is hypochromic and microcytic1, was first observed in the male descendants of an irradiated mouse, and was shown to be an X linked recessive trait2. The gene symbol has been designated sla and its locus lies close to Tabby (Ta) and Brindled (Mo^{br}) in linkage group XX^2 .

The mechanism of the anaemia has been investigated in this laboratory using descendants of the original mixed strain, partially inbred by brother-sister matings, and on hybrids of the original mixed strain and the standard C57Bl/6 Jax strain, of the second to fourth backeross generations. In anaemic mice (hemizygous males and homozygous females), there is characteristic marked reduction in mean corpuscular haemoglobin concentration and mean cell volume. The anaemia is most severe in young animals, in which the haemoglobin concentrations range from 5 to 8 g/100 ml., and it regresses as the animals age3,4.

Siderocytes and sideroblasts are absent and histochemical studies of the iron stores reveal severe depletion in anaemic mice, particularly in the spleen4,5. Depletion of tissue iron stores is confirmed by direct chemical estimations of the total iron content of mouse carcasses

Table 1. TOTAL BODY IRON CONTENT Total body iron Genotype P content (μg/100 g)* Normal † 5.49 ± 0.27 (+/-)4.550 < 0.001 Anaemic ‡ (sla/ - and sla/sla) 3.72 ± 0.27

(Table 1). The anaemia responds to parenteral iron dextran in doses containing 0.5 and 5 mg of elemental iron^{3,4}. Serum iron concentrations and total serum iron binding capacity were determined and the results are given in Table 2; anaemic mice have a low serum iron concentration and increased iron binding capacity as is characteristic of iron deficiency in man?. Also consistent with iron deficiency is the presence of increased concentrations of free erythrocyte protoporphyrin8,8 as measured by the method of Grinstein and Wintrobe¹⁰ (Table 3).

Table 2. SERUM IRON AND TOTAL IRON BINDING CAPACITY (TIBO) Genotype Serum iron* (µg/100 ml.) TIBC (µg/100 ml.) Normal 254 ± 8.3 (27) 420 ± 13·1 (8) (+/-)Anaemic (sla/-) $142 \pm 26 \cdot 2 (25)$ 612 ± 30·3 (9) 5·509 < 0·001 4.192 < 0.001

Means ± 1 S.E., numbers in each group are given in parentheses.

Table 3.	FREE ERYTHROCYTE PROTOPORPHYRIN .
Genotype	Free erythrocyte protoporphyrin (µg/100 ml. of red cells)*
Normal $(+/-)$	49·7 ± 7·32 (11)
(+/-) Anaemic $(sla/-)$	$272 \cdot 1 \pm 57 \cdot 2$ (8)
t P	4·502 < 0·001

* Means ± 1 S.E., numbers in each group are given in parentheses.

Investigations with radioactive iron further support a hypothesis of iron deficiency in mice with X linked Tracer doses given intravenously are cleared from the plasma more rapidly than normal. Utilization of this tracer, as determined by its reappearance in the peripheral blood, is also more rapid than normal, and more complete (Table 4).

e 4. CLEARANCE OF INTRAVENOUSLY ADMINISTERED TRACER DOSES OF IRON-59 AND ITS REAPPEARANCE IN THE PERIPHERAL BLOOD

Genotype	*Clearance half time of iron-59 from plasma		e in peripheral ministration †
	(min)	1 day	5 days
Normal	46·1 ± 2·55	29·0 ± 1·43	58·6 ± 3·23
(+/-) Anaemic	23.4 ± 4.66	72.2 ± 6.27	(16) 76·1 ± 4·70
(sla/-)	(8)	(9)	(14)
t	4.653	7.387	3.510
\boldsymbol{P}	< 0.001	< 0.001	< 0.005

* Means \pm 1 S.E., numbers in each group are given in parenthesis. † Percentage of administered dose of radioactive iron.

The iron content of the diet used (0.019 per cent by weight) is adequate to maintain normal haemoglobin concentrations and normal body stores in genetically normal littermates of anaemic mice4,5. Thus the possibility of malabsorption of iron as an explanation for X linked mouse anaemia has been investigated. The gross anatomical appearance of the small intestinal mucosa is normal in anaemic mice, but there is clear histochemical evidence of excessive iron deposition in the duodenal and upper jejunal mucosal epithelium, in contrast to the absence of stainable iron stores in the spleen and other organs of anaemic mice. The excess iron laid down in the mucosal epithelial cells is presumably shed as the epithelium is exfoliated. The retention of tracer doses of radioactive iron as ferrous sulphate, 5 days after oral administration by stomach tube, has been measured by whole body counting of normal and anaemic mice in a 'Tobor' (Nuclear-Chicago) large volume gamma scintil-

^{*} Means ± 1 S.E. † Twenty-eight normal male (+/-) mice. ‡ Eighteen anaemic male (sla/-) and seven anaemic female (sla/sla) mice.

Table 5. RETENTION OF TRACER DOSES OF IRON-59 5 DAYS AFTER ORAL

ADMINISTRATION								
Dose (µg)	Genotype	Retention of iron-59 (per cent)*	P					
0.1	Normal $(+/-)$	22.8 ± 4.63 (10)	< 0.02					
	Anaemic (sla/ –)	$9.6 \pm 2.20 (10)$						
1	Normal (+/-)	15.4 ± 2.36 (26)	< 0.005					
	Anaemic $(sla/-)$	5.7 ± 0.67 (10)						
10	Normal (+/-)	9·7 ± 2·35 (11)	< 0.025					
	Angemic (sla/-)	5.0 ± 0.55 (15)						

^{*} Means \pm 1 S.E., numbers in each group are given in parentheses.

lation counter. Three doses of elemental iron have been tested-0·1 μg, I μg and 10 μg. With each dose the anaemic animals retain significantly less of the labelled iron than do the normal mice (Table 5).

Thus it seems that the anaemia of sla mice is caused by iron deficiency resulting from a defect in a genetically controlled step in the transfer of iron from the mucosal epithelial cells of the small bowel to the plasma compartment. The precise nature of this step and its relationship to the process of iron absorption as a whole remain to be elucidated.

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Stimulation of Yolk Deposition in an Ichneumonid Parasitoid by feeding Synthetic Juvenile Hormone

Some ichneumonid parasitoids need to feed on their hosts for egg maturation. Leius¹ found significant differences in fecundity of the parasitoid Scambus buolianae (Htg.) when it was fed various species of lepidopterous hosts, and he has attributed these to variations in the chemical composition of the haemolymph of the different hosts. Exeristes comstockii (Cresson) is another example of an ichneumonid in which full maturation of ovaries occurs only when fed on a host or a synthetic diet2. development in a number of insects is regulated by endocrine activity of the corpus allatum³, and compounds like farnesol can mimic the activity of the corpus allatum hormone in promoting yolk deposition when applied topically to decapitated *Rhodnius*⁴ and allatectomized *Periplaneta americana*⁵. The following experiments were

conducted to determine (1) whether feeding E. comstockii sugar solution containing farnesol or its derivative farnesyl methyl ether, as well as topical application of the latter, would promote maturation of the ovaries, and (2) whether farnesyl methyl ether added to a synthetic diet would increase egg deposition.

Separate groups of from ten to twenty newly emerged and mated female E. comstockii were fed a 10 per cent solution of sucrose only, 0.1 per cent farnesyl methyl ether in 10 per cent solution of sucrose, 0.3 per cent farnesol (Calbiochem) in 10 per cent solution of sucrose and a chemical diet². All diets contained 0·1 per cent of 'Tween 80' as emulsifier. After 9 days, females from each group were dissected and the number of eggs more than 1.2 mm long in the ovaries was counted. These numbers were transformed to $\sqrt{x+0.5}$ to correct for heterogeneous variance and were then compared by t tests. The mean number of eggs in the ovaries of females fed farnesyl methyl ether in sucrose was 18.3 ± 0.3 , whereas that of females fed chemical diet was 7.6 ± 0.6 . This difference was significant (P < 0.01). In the females fed sucrose only and those fed 0.3 per cent farnesol in 10 per cent of sucrose the mean numbers of eggs in the ovaries were 2.2 ± 0.4 and 2.8 ± 0.4 , respectively. These means are significantly lower than those of females fed farnesyl methyl ether and chemical diets, but are not different from each other.

To compare the effect of topical application of farnesyl methyl ether with that of feeding, we tested three groups of eleven mated females treated as follows: one had pure farnesyl methyl ether applied directly to the abdomen with a small brush and was then fed 10 per cent sucrose solution; a second was fed a solution of 0.1 per cent farnesyl methyl ether in 10 per cent sucrose solution; and a third was fed sucrose solution only. Ovarian development was assessed after 9 days. The mean number of eggs in the ovaries of those fed farnesyl methyl ether in sucrose was significantly higher than that of those treated with farnesyl methyl ether externally $(12.7 \pm 0.9$ against 8.3 ± 1.1 ; P < 0.01), and both treated groups had significantly more eggs in ovaries than the sugar-fed control (2.8 ± 0.8) . The external treatment was applied once only at the start of the experiment, whereas treatment in the food was continuous; this might account for the greater ovarian development observed in the feeding treatment.

To determine whether females fed 0.1 per cent farnesyl methyl ether in sucrose would oviposit a greater number of eggs than those fed sucrose alone and, similarly, if females fed 0.1 per cent farnesyl methyl ether in synthetic diet would oviposit more than females fed diet alone, four groups of fifteen newly emerged and mated females were caged individually and each group fed one of the foregoing diets. Coddled Galleria mellonella L. larvae were offered daily in wooden blocks, so that the parasitoid could not feed on them, but could oviposit on them2. Egg deposition was recorded for 2 weeks.

Females fed farnesyl methyl ether in 10 per cent sucrose solution oviposited more eggs than females fed sucrose alone $(3.7 \pm 0.7 \text{ against } 0.7 \pm 0.3; P = 0.02)$. The mean number of eggs from each female fed synthetic diet and farnesyl methyl ether, and fed synthetic diet alone, was 16.2 ± 4.3 and 21.4 ± 4.4 , respectively. These means do not differ significantly from each other, but are significantly higher than the means for females fed sucrose alone and for those fed sucrose and farnesyl methyl ether.

It was previously believed that adult ichneumonid parasitoids feed on host tissues because they bring very low nutritional reserves into the adult stage for maturation of the ovaries. The results reported here show that this view is not completely correct. E. comstockii evidently carries enough nutritional reserves for full ovarian maturation without recourse to protein feeding. These reserves can be utilized for yolk deposition if the insect is treated with farnesyl methyl ether, which mimics the activity of the corpus allatum. This observation is similar to that of Johansson⁶. He found that implantation of an active corpus allatum induced egg development in starved females of the milkweed bug, Oncopeltus fasciatus. It is interesting to note that the chemical is especially effective for E. comstockii when presented in the food.

Although increased egg deposition occurred in females fed with farnesyl methyl ether as compared with females fed sucrose alone, only about a quarter of the eggs developed in the ovaries was actually deposited. This suggests that although the chemical stimulates yolk deposition, it is unable to stimulate those factors that regulate oviposition. Addition of protein in the diet stimulates both yolk deposition and oviposition. Thus an additional mechanism controlling oviposition is present and it is undoubtedly linked with nutritional factors.

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Artificial Feeding Technique for Rhipicephalus appendiculatus and the Transmission of Theileria parva from the Salivary Secretion

Nuttall and Hindle 1 were the first to suggest that Theileriaparva, which is the causal organism of East Coast fever in cattle, develops to the infective stage in its common tick vector, Rhipicephalus appendiculatus, only after the ingestion of blood by the tick. This finding was supported by Cowdry and Ham² and, more recently, by Martin et al.³, who found the greatest numbers of parasites in salivary glands after 4 days of feeding although they did observe parasites in the glands after as little as 24 h. Infectivity therefore seems to be dependent on the feeding process.

During an attempt to study this phenomenon and to isolate the infective stages of T. parva which are secreted, we have found that adult R. appendiculatus partially fed previously on rabbits or cattle will absorb blood from capillary tubes placed over the hypostomes or capitula and that after 2 h the residual blood pool may be infective when injected into cattle.

Nymphal R. appendiculatus derived from the experimental stock were fed on cattle which were either uninfected or carrying patent infections of the Muguga strain of T. parva (> 10 piroplasms/1,000 red blood cells⁴). After moulting, equal numbers of males and females were

placed in calico bags on the ears of rabbits or cattle4. They were examined after 24 h and again after 48 h, and as they attached the ticks were marked with cellulose paint. They were considered as having fed for one day if they had attached during the previous 24 h and those ticks which did not attach within 48 h were discarded. After the initial feeding period (usually 4 days) the ticks were detached from the skin of the host and restrained, ventral surface uppermost, on wads of 'Plasticine'. A glass capillary tube (mean diameter 1·17 mm), slightly rounded at the tip and containing about 0.05 ml. of heparinized or defibrinated blood, serum or plasma, was placed loosely over the mouthparts of the ticks, and was supported at about 30° above the horizontal by a further wad of 'Plasticine'. In early experiments, only the hypostome was enclosed, but when the entire capitulum was inserted into the capillary tube, palpal movements and feeding occurred equally well. A temperature of approximately 27° C (24°-29° C) was maintained by a bench light placed overhead.

The capillary tubes, which were manufactured to a standard specification, were calibrated so that the volume of fluid absorbed by the ticks could be assessed by measuring the length of the column in the tube Forty tubes were calibrated in this way and a mean value of $1.08 \text{ mm}^3 \equiv 1 \text{ mm column length } (S.D. 0.036) \text{ was}$ obtained. A correction for the loss caused by evaporation under the same conditions was obtained from tubes sealed at one end and filled with blood or serum. volumes of fluid consumed in 24 h by two batches of adult ticks fed by this method on different substrates are given in Table 1. Each value is the mean for ten ticks. The greatest volume was consumed when serum or plasma was offered. Female ticks initially fed on cattle did not feed as well as those from rabbits. This may be the result of different mating behaviour on the two hosts, which in these experiments may also be related to the age of the ticks. Among those which had fed on the rabbit, certain individuals fed particularly well which may have been the result of early mating, for Gregson⁵ has shown that the rate of engorgement in Dermacentor andersoni is related to mating. Most of the females removed from the host at this stage, however, do not seem to engorge completely. Nevertheless, some individuals which have been fed for periods up to 7 days on capillary tubes have proceeded to lay viable eggs. To maintain feeding during these extended periods it was necessary to replace the tube of blood at intervals because it became occluded by the hypostomal cement. When fed on blood, both sexes excreted from the anus spherical droplets which rapidly hardened and were hollow. The droplets of excreta when smeared on a microscope slide were seen to contain numbers of undamaged red blood cells. When blood infected with T. parva was used, some piroplasms within the erythrocytes were seen to pass intact through the tick.

To demonstrate the infectivity of the residual feeding pool, batches of twenty to twenty-six adult ticks infected with T. parva were withdrawn from rabbits after 4 days of feeding. They were then fed on tubes of uninfected heparinized or defibrinated bovine blood or serum for 2 h. The residual fluid in the capillary tubes was pooled, made up to 1 ml. with uninfected bovine blood or serum,

Table 1. YOLUME OF BLOOD SUBSTRATES CONSUMED IN 24 h BY ADULT R. appendiculatus

			Mean volume and range consumed (mm³/tick) Substrate						
Initial feed	Batch No.	Age at attachment	Sex	Serum	Plasma	Defibrinated blood	Heparinized blood		
4 days on rabbit	971 (infected)	2 weeks	Male Female	$ \begin{array}{r} 5.8 \\ (0.0-20.5) \\ 10.3 \\ (1.2-41.4) \end{array} $	6·3 (2·2~18·1) 8·6 (0~51·7)	1·0 (0·0-3·1) 4·2 (0·0-13·0)	$^{1\cdot 1}_{(0\cdot 0-2\cdot 9)}\ ^{4\cdot 3}_{(0\cdot 5-19\cdot 8)}$		
5 days on bovine	207 (uninfected)	15 weeks	Male Female	13·2 (1·3-35·4) 4·1 (0·0-14·0)	8·4 (0·3–19·2) 3·7 (0·0–10·7)	5·3 (1·3-16·1) 1·8 (0·0-6·0)	$ \begin{array}{c} 3.7 \\ (0.0-10.0) \\ 2.8 \\ (0.0-7.1) \end{array} $		

and injected intradermally into the base of the right ear of a susceptible steer.

So far, eleven animals have been injected with the residual feeding pool in this way. Three out of five animals receiving heparinized blood developed fever, and hyperplasia of the lymph nodes in which schizonts of T. parva were found; one out of four animals receiving defibrinated blood also developed a similar infection, while neither of the two which received serum pools reacted. All four infected animals developed piroplasms typical of T. parva in the blood and two of them died.

Chabaud⁶ used a similar artificial feeding technique for some species of Ixodidae (including R. sanguineus), and Gregson 7-9 used the method to study salivation and feeding in D. andersoni. Both these authors found it necessary to spread the palps, whereas this does not seem to be so for the feeding of R. appendiculatus. We shall use this technique to obtain further quantitative data on the effects of sex, temperature and substrate on feeding activity.

The infectivity of the residual pool is of particular It indicates that salivation occurs during capillary feeding for as short a period as 2 h, and offers a method of obtaining the infective stages of T. parva. Further experiments are in progress to increase the efficiency of the method and to study the properties of the infective stages so obtained.

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MICROBIOLOGY

Mycobacteriophages isolated from Human Sources

THE isolation of mycobacteriophages from stool and resection specimens of patients with tuberculosis or sarcoidosis was first described in 1961 (ref. 1). Further results obtained during the past 5 yr in isolations of mycobacteriophages are reproduced in Table 1. These figures show that healthy persons rarely harbour mycobacteriophages in their intestinal tract, whereas stool and resection specimens of patients with tuberculosis or sarcoidosis frequently contain these mycobacteriophages.

Table 1. ISOLATION OF MYCOBACTERIOPHAGES FROM STOOL AND RESECTION SPECIMENS

Specimen Tuberculosis Sarcoidosis Healthy persons Stool 76/155 (49 per cent) 73/190 (38.4 per cent) Biopsy or 41/130 (31.5 per cent) 48/86 (55.8 per cent) resection 2/121 (1.6 per cent)

This communication deals with attempts to demonstrate mycobacteriophages in serum samples collected from normal persons and from patients with pulmonary tuberculosis or sarcoidosis. The diagnosis of tuberculosis was always confirmed by the isolation of M. tuberculosis. The diagnosis of sarcoidosis was established on clinical grounds, X-ray evidence, the histopathological examination of biopsies of lymph nodes, lungs or bronchial mucosa, and/ or Kveim tests.

Bacteriophages were isolated by successive passages of the serum in the RVB 6 medium described by Redmond and Ward², and from which the albumin V fraction had been omitted. The media were inoculated either with strain $H_{37}Rv$ or ATCC 607 before the addition of the patient's serum, which was added to a final concentration of 10 per cent. When a patient was on antituberculosis drug therapy or had received therapy shortly before the blood was taken, his serum was added to a culture of strain R 100 which is resistant to 100 $\mu g/ml$. of streptomycin and to 10 µg/ml. of isoniazid. After each passage in enriched medium, the culture filtrates were essayed 'spotting' on mycobacterial indicator strains3. Plaques were isolated and passaged once in liquid medium. Filtrates, adjusted to contain 1 routine test dilution in particles lytic for the strain on which the bacteriophage had been propagated, were spotted on other indicator strains to establish the lytic spectrum of each bacteriophage close to the time of its isolation.

No mycobacteriophages were isolated from serum samples of fifty-one healthy persons or from fifty-two with tuberculosis. Ninety-nine sera were examined from patients with pulmonary sarcoidosis. Of these, twentyfour had been collected in Montreal hospitals; thirty four in the Epidemiology Division of the Department of National Health and Welfare, Ottawa, and forty-one in the Sanatorium Hochenschwand in Germany. Eighteen of the cultures of sera collected in the Montreal area (75 per cent), fifteen of the sera from the Ottawa area (44 per cent) and twenty of the sera from Germany (49 per cent) contained particles lytic for mycobacteria.

The host range of the mycobacteriophages isolated from the sera of patients with sarcoidosis was limited in two phages to the strain ATCC 607. All other bacteriophages had a wide host range including virulent tubercle bacilli, atypical and saprophytic mycobacteria.

The frequent isolations of mycobacteriophages from patients with sarcoidosis and the previously reported absence of phage neutralizing antibodies in the sera of these patients suggest a state of "anergy" in these persons which parallels their non-reactivity towards tuberculin and PPD after natural exposure to tubercle bacilli or after BCG vaccination5.

Tuberculin reactions are sometimes depressed during viral infections. In recent experiments, it has been observed that BCG-vaccinated guinea-pigs which react strongly to intracutaneous tuberculin show a depression of the tuberculin reaction after the intraperitoneal inocula-This depression tion of mycobacteriophage lysates. exceeds the 5 week period during which mycobacteriophage particles can be isolated from their liver, spleen or the circulating blood.

This observation suggests that the "anergy" of patients with sarcoidosis towards tuberculin may result from the viraemia; or both phenomena (the suppression of this delayed type of allergy and the absence of phage-neutralizing antibodies which results in the long survival of the phage particles) may stem from the same (unifactorial or multifactorial) immunological defect.

There have been many studies of serum antibody concentrations in patients with sarcoidosis after immunization with typhoid-paratyphoid vaccines or other bacterial No defect in the production of circulating antigens. antibodies to these antigens could be detected. Kallings and Loefgren, however, reported the absence of phage neutralizing antibodies to coli T2 bacteriophages in the sera of 22 per cent of patients examined, while the sera of healthy persons contained appreciable titres of these "natural" antibodies. antibodies.

The mycobacteriophages which were isolated from the sarcoid tissue or from the sera of patients with sarcoidosis could have originated from the lysogenic mycobacteria which were demonstrated in the tissue biopsies of these patients⁸ but which were never demonstrated in their sera. These bacteria, or phage-infected mammalian cells, may be the reservoir for phage particles that are released into the circulation.

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Degradation of Salicylate by Aspergillus niger

SALICYLATE continues to be a leading cause of accidental poisoning in the United States; in 1964 there were more than 16,000 cases of accidental aspirin ingestion among all age groups, accounting for 25.8 per cent of all accidental poisonings reported1. Existing methods for the treatment of salicylate poisoning are aimed at alkalinization of the urine and extrarenal removal of the drug^{2,3}. The natural elimination of salicylate in man is a slow process. The average half-life of a single 1 g dose of aspirin is reported to be 6 h (ref. 4), and high doses have given half-life values up to 19 h (ref. 5). Administration of salicylate labelled with carbon-14 to man's has given the following metabolites (here reported as average mean values): free salicylate 61 per cent; salicylic phenolic glucuronide 22 per cent; salicyluric acid 8 per cent; salicylic acyl glucuronide 5 per cent; and gentisic acid 1 per cent. The conjugates of salicylic acid are less diffusible across cell membranes and thus have given higher clearances7. Furthermore, the excretion of salicyluric acid after administration of acetylsalicylate or salicylate is limited by the ability to conjugate salicylate with glycine rather than the excretion capacity of the kidneys8.

Metabolic manipulation aimed at converting free salicylate to conjugated forms may be useful as a third therapeutic approach to the treatment of salicylate toxicity. Theoretically, this could be accomplished by the use of specific enzymes isolated from organisms and capable of degrading salicylate. Although enormous obstacles might still have to be overcome before any such enzymes could be used in vivo, the potential value of such an enzyme justifies its investigation. This communication presents the results of preliminary studies using fungi to degrade the salicylate molecule.

Table 1. Growth of funci in still and shake culture on a salioylate medium

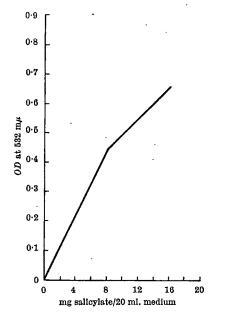
Organism	Still culture (days)				Shake culture (days)			
	1	2	3		1	2	3	
Trichoderma species (I-193)		_	+ 8			+ 6	+ ,	
Rhizopus nigricans (314 +)	-	_	_			-	-	
Dactylium dendroides (I-108)	-		_		_	_	_	
Cladosporium species (I-75)	_	_		•	_	_	_	
Cladosporium cladosporiodes (I-83)	_	_			_	- ·	_	
Saccharomyces cerevisiae (1)	_	_				_	_	
Aspergillus niger (10)	-	+8	+ m		-	+ m	+h	
Uninoculated control	-	_	_		_	_		

All cultures used were obtained from the Ohio State University stock culture collection. The stock culture number is in parentheses. The symbols $+_8$ (slight), $+_m$ (moderate) and $+_h$ (heavy) refer to visual observation of the growth of the fungus in culture.

All studies were carried out in synthetic liquid media with the following composition: 1 g of NH₄NO₃; 1.0 g of KH₂PO₄; 0.5 g of MgSO₄·7H₂O; 0.13 g of CaCl₂; 0.1 g of NaCl; and 0.01 g of Fe₂(SO₄)₃·nH₂O made up to 1 l. Sodium salicylate (NaC, H,O), the only source of earbon, was added in a concentration of 1 mg/ml. and dispensed in 20 ml. samples in 125 Erlenmeyer flasks. The final pH was 4.5. Flasks were inoculated with 0.2 ml. of water suspension of spores from a slant culture of the organism (10 ml. of sterile distilled water/PDA agar slant). Incubation was at 20°C in still culture or as shake cultures on an Eberbach reciprocating shaker (100 oscillations/min). Salicylate degradation was measured according to a slight modification of the methods of Trinder⁹ and DeMarco and Marcus¹⁰ utilizing a Bausch and Lomb 'Spectronic 20'.

Table 1 shows representative data about the growth of seven organisms on still and shake cultures. Two organisms which grew in a medium containing salicylate as the sole carbon source were Aspergillus niger and a species of Trichoderma. Aspergillus niger was selected for further tests because of its rapid growth in shake culture and because it has been intensively studied and approved by the US Federal Drug Administration.

Fig. 1 presents standard curve data obtained from the addition of 5 ml. of a solution of 0.05 per cent FeCl₃ made up in 0·1 normal HCl to 5 ml. of a 1:10 dilution of the salicylate medium. Readings were taken at 532 mu 5 min after formation of the ferric salicylate complex, against a suitable reagent blank. The system obeyed Beer's law at the lower concentrations of salicylate used (1-8 mg).



. Standard curve obtained from addition of ferric chloride solution to the sodium salicylate medium (details in text).

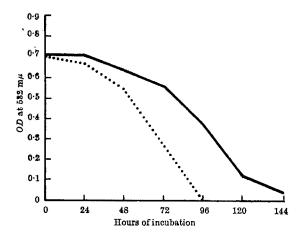


Fig. 2. Salicylate degradation by Aspergillus niger in still (----) and shake (.....) culture.

As shown in Fig. 2, the degradation of salicylate by Aspergillus niger occurred in both shake and still cultures. There appeared to be an initial lag phase of approximately 24 h followed by an exponential utilization of the salicylate. In one series of flasks, salicylate had disappeared as early as 76 h after inoculation in shake culture. We are now trying to isolate the enzyme or enzymes from Aspergillus niger which are responsible for this rapid degradation of salicylate, for use in investigations in mammalian systems.

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CYTOLOGY

Variation in the Human Chromosome Number

INCREASED aneuploidy has been described in cultured human peripheral blood cells, beginning in the sixth decade of life for females, and in the seventh decade of life for males1. This increased aneuploidy has been attributed largely to the presence of cells containing forty-five chromosomes, with an XO sex chromosome constitution.

During cytogenetic studies of atomic bomb survivors of Hiroshima and Nagasaki2,3 we have been able to evaluate the hypothesis of selective loss of the sex chromosomes with increasing age. We have now counted and examined the chromosomes of more than 18,000 cells from 329 patients, aged between 20 and 88 yr. Our results indicate that variation in the chromosome number occurs within a very narrow range throughout the adult years. The presence of cells with forty-five chromosomes is largely responsible for the existing deviation from the modal number, and chromosome loss seems to be neither completely random nor entirely related to chromosome

The population studied was selected from the Adult Health Study sample, a group of nearly 20,000 people who are examined every 2 yr in the Hiroshima and Nagasaki clinics of the Atomic Bomb Casualty Commission. Characteristics of this sample have been given elsewhere4. For cytogenetic study, index patients were selected from those who are estimated to have received 200 rads or more of mixed gamma and neutron radiation at the time of the atomic bombings. A control was selected for each index patient, matched by age, sex and city, from those who were over 3,000 m from the atomic bomb hypocentres. The controls are estimated to have been exposed to less than 1 rad. Any person with a history of malignant disease was excluded from the study, as were those with a history of radiation therapy, radioisotope exposure or those who had evidence of a viral exanthem on physical examination. Those patients with a history of diagnostic X-irradiation, exclusive of chest X-rays, in the preceding 12 months were also excluded. In all cases, peripheral leucocytes were cultured according to the method of Moorhead et al.5, and were collected between 66 and 72 h of culture.

In 20-49 yr old patients the chromosomes were counted only in the first thirty cells examined, although whenever possible a total of 100 cells was examined for the presence of structural aberrations. In patients more than 50 yr old, an attempt was made to both count and analyse 100 cells each. For these older patients, whenever cells with missing or additional chromosomes were encountered an attempt was made to classify such chromosomes by group. Cells were selected under low power (x100), according to (a) their apparent integrity and (b) the quality of chromosome spreading. Once considered to be suitable, a metaphase was included in the study, whether or not structural alterations were present. In determining the chromosome count of a cell, the number of centric chromosomes alone was used.

The blood samples were processed, and the slides were read, without technician or observer knowledge of the age, sex or exposure status of the patients.

There was no systematic difference in the distribution of chromosome number between cells of the heavily exposed and those of the controls, and so the data for these two groups have been combined. As shown in Table 1, 135 males and 194 females were studied cytogenetically. In the 74 males and 114 females who were between 20 and 49 yr old, from 94.4 to 97.8 per cent of cells counted contained forty-six chromosomes. Most aneuploid cells were hypomodal, with 45-chromosome cells predominating.

Among the 141 persons between 50 and 88 yr old, sixty-one were males and eighty were females. Fifty-three of the 141 were more than 70 yr old. As seen in Table 1, the percentage of cells with forty-six chromosomes ranged, in this older group, from a low of 94·1 in males older than 70 yr, to a high of 96.9 in females in the seventh decade of life. The lack of a consistent trend with age is shown graphically in Fig. 1. There was little difference between the sexes in terms of aneuploidy in old age; and there was, further, no pronounced tendency for males or females to show increased aneuploidy late in life as compared with the earlier years.

Fig. 1 also shows the distribution of the percentage of cells with forty-five chromosomes by sex and age of the patients at the time of examination. While a total of 422 cells with forty-five chromosomes was found, 100 of

Table 1. RELATIONSHIP OF CHROMOSOME NUMBER TO AGE AND SEX

Age at	No. of	Average	No. of	Chro		ne No. stribut		ntage
examina- tion	persons examined	age	cells examined	< 45	45	46		Poly- ploid
20-29 30-39 40-49 50-59 60-69 > 70 Total	12 44 18 18 20 23	24·7 35·9 44·2 56·0 65·0 72·8 49·3	Male 353 1,286 540 1,499 1,728 1,952 7,358	1.7 1.6 0.6 1.2 0.3 0.7	2·0 2·1 0·9 2·2 2·1 4·0 2·6	96·0 95·5 97·8 95·7 96·5 94·1 95·6	0·3 0·4 0·7 0·5 0·8 0·7	0·1 0·3 0·1 0·4 0·3 0·5 0·3
20-29 30-39 40-49 50-59 60-69 > 70 Total	12 62 40 24 26 30 194	25·2 35·0 42·8 54·9 64·2 74·8 48·6	Female 360 1,907 1,196 2,237 2,502 2,545 10,747	2·5 1·1 0·7 0·4 0·3 0·9 0·7	2·5 1·7 1·6 2·8 1·8 2·6 2·2	94·4 96·3 96·2 95·8 96·9 95·2 96·0	0·3 0·7 0·4 0·7 0·8 1·0 0·7	0·2 0·2 0·4 0·3 0·2 0·4 0·3

* Polyploidy here also includes endoreduplication, and the percentage of polyploid cells is based, where possible, on 100 cell chromosome counts.

Table 2. DISTRIBUTION, BY GROUP, OF MISSING CHROMOSOMES IN FORTY-FIVE

		0.	DYPOTEODORY	CIMINO		
Chromo	-	Male			Female	
	Observed	Expected*	Expected †	Observed	Expected*	Expected †
A	5	15.0	6-8	3	19.4	9.0
В	3	10.0	5.8	10	13.0	7.7
C	22	37.5	27.7	48	51.8	39-0
D	8	15.0	15.7	28	19.4	20.9
Œ	21	15.0	18-2	20	19-4	$24 \cdot 2$
F	13	10.0	15.2	8	13.0	20.2
G	43	12.5	25-7	32	13-0	27.9
Total	115	115.0	115.1	149	149.0	148.9

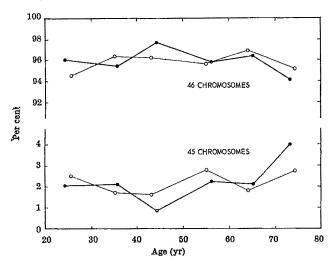
The X chromosomes were here grouped with the Cs; the Y chromosomes with the Gs.

* Expected values assuming random loss.

† Expected values assuming probability of loss to be inversely proportional to size.

these were from patients younger than 50 yr and no attempt has been made to identify the missing chromosome. This was done only in the remaining 322 forty-five chromosome cells seen in the 141 patients who were more than 50 yr old. In 264 instances, the missing chromosome could be identified by group.

The distribution of these cells is shown, separately by sex, in Table 2, together with expected numbers based on two different sets of assumptions regarding loss. First, if loss were entirely random, then the probability of loss from each group would be determined completely by the number of chromosomes in the group. It is apparent that our observations do not conform to the pattern expected with random loss, there being generally a lack of cells missing the larger chromosomes and a surfeit of those missing the smaller chromosomes. The pattern suggested that the likelihood of chromosome loss may be inversely related to chromosome size, and another set of expected numbers was derived on this assumption.



[Fig. 1. Percentage of cells with forty-six and forty-five chromosomes, by age and sex of patients. •, Male; O, female.

Specifically, we assumed that there are k chromosomes identified by categories, X_i , $i=1,\ldots,k$. If we let Y_i (>0) denote the length of chromosome X_i , and $P(X_i)$ denote the probability of loss of a single chromosome from category X_i , then the hypothesis of loss being inversely related to size states that:

$$\frac{P(X_i)}{P(X_j)} = \frac{Y_j}{Y_i} \text{ for all } i \text{ and } j$$

For example, if an A_3 chromosome is 7·1 per cent of the haploid autosomal complement and if each D is 3·5 per cent of the complement⁶, then the probability of loss of a D group chromosome in a male is 0·045, while the probability of an A_3 being lost is 0·023. That is, a D group chromosome is twice as likely to be lost as an A_3 , because it is half the size of an A_3 . Using this assumption, together with the fact that the probabilities must

add up to $1.0 \binom{k}{i=1} P(X_i) = 1$, it is not difficult to show that the probabilities for the loss of individual chromo-

that the probabilities for the loss of individual chromosomes are given by

$$P(X_i) = \left[Y_i \sum_{j=1}^k Y_{j-1}\right]^{-1}, i=1,\ldots,k$$

Probabilities were calculated using the estimated lengths given in the report of the Chicago conference. The probabilities for individual chromosomes within the appropriate chromosomal groups were then summed. As seen in Table 2, while the expected numbers thus derived improved the agreement with the observed distribution over that given by the random loss expectations, the fit is still not a good one for either sex. In males, G group chromosomes, which here include Y chromosomes, were lost far more often than this hypothesis would explain. In females, G group chromosomes, which here include the two X chromosomes, were lost more often than expected, as were Bs, Ds and Gs. The largest contributors to the lack of fit, in females, were, however, the F, A and D groups, in that order.

These results suggest that the proportion of cultured leucocytes which contain the normal forty-six chromosome complement is relatively constant from the third decade of life. While the Edinburgh group, even in their 3 day cultures, have found from 7 per cent aneuploidy in elderly males to 13 per cent in elderly females, we have found that for both sexes, at all ages, the proportion of aneuploid cells is between 2 per cent and 6 per cent. None of our 329 patients were chromosomal mosaics, nor did any of them have a modal chromosome number other than forty-six. Thus our population was, in terms of chromosome number, an essentially normal one.

While a small percentage of non-forty-six-chromosome cells were hypermodal, with no discernible pattern of addition, most were hypomodal and contained forty-five chromosomes. It seems that loss of a single chromosome is not entirely a random phenomenon. It also appears that small size alone cannot explain the observed patterns of chromosome loss.

With respect to the hypothesis of selective sex chromosome loss, our findings show a marked excess of G group chromosome loss in males which cannot be explained by either the random loss or loss by size hypothesis. G group loss in females, on the other hand, is not consistent, either in magnitude or direction, for the two postulated hypotheses. Thus it seems likely that the probability of loss of a given chromosome is influenced by a number of factors, acting either separately or in combination. These could include a random component as well as the size of the chromosome and the position of the chromosome on the spindle during cell division. To these must be added, in males, the tendency for G group chromosomes, which include the Y, to be lost because of some other, perhaps more selective, mechanism. There may also be prepara-

tive, or other factors, which are characteristic of the laboratory in which the data are collected.

Our data must be qualified by indicating that the culture time of $66-72 \text{ } \hat{\mathbf{h}}$ was sufficiently long to enable both first and second division products to make their appearance. The 2 day culture system which we are now using7 may further reduce the proportion of aneuploid cells and give a more representative picture of the in vivo situation. Certainly this should be the case if chromosome loss occurs primarily in tissue culture. But if loss occurs predominantly during an in vivo division, the proportion of an uploid cells should vary little with culture time.

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PATHOLOGY

Urinary Excretion of 3,4-Dimethoxyphenylethylamine in Parkinson's Disease

There is considerable evidence for the involvement of dopamine in the extrapyramidal system and in its disorders¹⁻⁴. The methoxy derivative of dopamine, 3,4-dimethoxyphenylethylamine (DMPEA), as measured by the paper chromatographic method of Friedhoff and Van Winkles, was excreted in a large concentration in the urine of patients suffering from Parkinson's disease⁶. On the other hand, DMPEA caused a hypokinetic and rigid syndrome in experimental animals7,8, thus further increasing the significance of this compound in extrapyramidal disorders.

It was reported originally that DMPEA determined by this method was unique to the urine of schizophrenic patients, suggesting a close relationship between the compound and the disease^{5,9,10}. It was found, however, that DMPEA was also excreted by non-schizophrenic, normal patients¹¹, and later attempts to detect the compound have been unsuccessful^{12,13}. Furthermore, doubt has been cast on the identification of the compound as DMPEA by the methods used^{14,15}. To increase the sensitivity of the identification of DMPEA, the paper chromatographic system was combined with a fluorimetric examination and many of the pink spots of the paper chromatographic method did not fluoresce^{14,16}.

We have investigated urinary excretion of DMPEA in patients suffering from Parkinson's disease by this new, more accurate method. We studied twenty patients

(eleven female and nine male) with Parkinson's disease, mean age 59 yr. Nine patients (six female and three male) suffering from various neurological diseases without extrapyramidal symptoms were the control group; their mean age was 45 yr, and none was known to have renal or liver disease.

A 24 h urine specimen was collected in hydrochloric acid and deep-frozen until it was analysed. DMPEA was separated by paper chromatography¹⁴ and then examined fluorimetrically by the method of Bell and Somerville¹⁸.

The mean excretion of DMPEA was $4.4 \pm 1.2 \mu g/24 h$ in subjects with Parkinson's disease and 6.8 ± 3.6 $\mu g/24$ h in the control subjects. No DMPEA was detected in two of the nine controls or three of the twenty cases with Parkinson's disease. There was no significant difference between the groups in their excretion of DMPEA. There was also no clear correlation between excretion of DMPEA and the severity of akinesia or rigidity of the parkinsonian patients.

These results show that DMPEA is not unique to the urines of schizophrenic patients as has been suggested5, because it was excreted by non-schizophrenic patients suffering from Parkinson's disease and other neurological diseases. DMPEA treatment had no psychogenic properties17, which casts doubt on the assumed relationship between the compound and the disease. Furthermore, it has been suggested that DMPEA is of exogenous origin 11,12 and that psychotropic drug therapy rather than the disease influences the occurrence of pink spot in the paper chromatographic system used18.

These results do not confirm the views that there are clear changes in the urinary excretion of DMPEA in patients suffering from Parkinson's disease. This difference of results may reflect differences in the methods used and in the population of parkinsonian patients. Recently, the chief end-product of DMPEA, 3,4-dimethoxyphenylacetic acid, has been found in urine from normal and schizophrenic patients19,20, and there were no significant differences between these excretions19. Abnormal methylation in patients with Parkinson's disease might be suggested by urinary excretion of 3,4-dimethoxyphenylacetic acid; further investigation, currently in progress, is necessary to clarify the situation.

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Psychotomimetic Indole Compound in the Urine of Schizophrenics and Mentally Defective Patients

BUFOTENIN (Fig. 1), an indole compound first found in the glandular secretion of certain toad species, has been reported to produce psychotic episodes in physically and mentally healthy individuals. Interest has been focused on this compound in the study of biochemical aspects of schizophrenia because of the possible formation of bufotenin in mammalian tissues from the naturally occurring neurohormone serotonin^{2,3}. A bufotenin-like compound has been reported in the urine of schizophrenic patients⁴⁻⁶. Unfortunately, these findings have not been generally accepted because of some defects in the detection methods employed^{7,8}. Others^{7,9-15} have failed to confirm the presence of bufotenin in the urine of schizophrenics as well as non-schizophrenic individuals.

Serotonin

HO
$$CH_2-CH_2-NH_2$$

N H

Bufotenin

 $CH_2-CH_2-N < CH_2$
 $CH_2-CH_2-N < CH_2$

Fig. 1. Chemical structure of serotonin and bufotenin.

One of us⁸ has developed modifications of paper and thin-layer chromatographic methods which are more sensitive and reproducible for detection of small amounts of indoleamines in the urine. Using these methods and also the recently developed gas-liquid chromatographic method of Capella and Horning18, we have confirmed the presence of bufotenin in the urine of four chronic schizo-phrenic patients in rigidly controlled experimental conditions which included amino-acid loading with and with-out a monoamine oxidase inhibitor¹⁷. The psychotic out a monoamine oxidase inhibitor¹⁷. The psychotic symptoms of these patients worsened about 2 weeks after an increase of the urinary bufotenin first appeared and the aggravation of behavioural symptoms continued as long as the increased concentrations of this compound We therefore suggested before 17 that behavioural worsening in schizophrenic patients is partially mediated by greater concentrations of psychotomimetic indoleamines such as bufotenin in the body fluids during the period of exacerbations of their psychotic symptoms.

In our previous investigation we analysed the urine of schizophrenic patients receiving an amino-acid load and/or a monoamine oxidase inhibitor, and so we could not generalize from the biochemical-behavioural relationship about the implication of bufotenin in unprovoked worsening of schizophrenic patients nor its implication in nonschizophrenic patients who may nevertheless exhibit psychotic episodes. The present study was performed on six male, chronic schizophrenics and four male mentally defective patients as non-schizophrenic controls to learn whether or not the urinary excretion of bufotenin is a biochemical characteristic solely of schizophrenia. ages of the mentally defective patients ranged from 59 to 75 yr and their IQ was between 53 and 66 by the Stanford-Binet mental test. The ages of the schizophrenic patients ranged from 33 to 60 yr. Three of these patients belonged to the catatonic group and the other three had been diagnosed as having paranoid schizo-

phrenia. They were placed on a controlled diet which excluded all preformed catecholamines and indoleamines and neither loading with amino-acids nor the administration of a monoamine oxidase inhibitor was carried out. Psychoactive drugs were withheld from all the patients for at least 7 weeks before the initiation of the study and throughout the experimental period. Collections of urine were made every 24 h and the volume and pH were measured. All daily collections were kept frozen at -20° C until analysed, a period of 1–2 weeks.

Urinary indoleamines were examined by a two dimensional thin-layer chromatographic methods. one third of a 24 h volume of urine was concentrated to 50-100 ml. on a rotary evaporator under reduced pressure at 36° C. The free indoleamines were first retained on a sulphonic acid cation resin and then recovered from the resin with 1 normal ammonium hydroxide in 65 per cent ethanol after the acidic and neutral components of the urine had been washed from the resin with 0.1 normal sodium acetate and 50 per cent The urine and the first water wash effluent ethanol. from the resin were collected and hydrolysed at pH 1.0 for 30 min. The conjugated indoleamines were purified from the hydrolysed urine in the same way as for the purification of the free amine fraction. The amounts of purified sample equivalent to 200 mg of creatinine were spotted on a thin-layer of 'Silica gel G' and the chromatograms were developed two dimensionally with isopropanol, ammonia and water (8:1:1) for the first phase and n-butanol, acetic acid and water (12:3:5)for the second. p-Dimethylaminocinnamaldehyde reagent was used for visualization of indole compounds.

Conjugated bufotenin was detected in the urine of all schizophrenic patients as well as of all mentally defective patients examined (Table 1). Furthermore, total amounts of bufotenin excreted in the urine were determined semiquantitatively according to the size and colour intensity of the bufotenin spot appearing on the thin-layer chromatogram and they were estimated to be about the same, less than 1 $\mu g/24$ h for both schizophrenics and the mentally defective patients in our experimental condi-All schizophrenics excreted both free and conjugated bufotenin, whereas in the patients with mental defectiveness, bufotenin was found only in a conjugated form and no free bufotenin was detected except in one patient in our experimental conditions. In this study, the incidence of the patients who excreted free bufotenin was higher in schizophrenics than in the mentally defective We consider, however, that such differences in the incidence resulted from the small number of the patients examined and that there may be no significant differences in the metabolism of bufotenin between schizophrenics and the mentally defective patients in controlled conditions. We did not find any difference in the excretion of conjugated serotonin between these two groups of the patients and so we feel that the mechanism involved in the conjugation processes for both the serotonin and bufotenin is similar.

Table 1. NO. OF PATIENTS EXCRETING BUFOTENIN/TOTAL NUMBER OF PATIENTS EXAMINED

Schlzophrenia Mental deficiency

F. Free bufotenin; C, conjugated bufotenin.

There does seem to be, however, a relationship between free bufotenin and exacerbation of psychotic behaviour. All our schizophrenic patients experienced exacerbations of the psychotic symptoms and they all excreted free bufotenin in association with behavioural worsening. One mental defective who excreted traces of free bufotenin consistently also developed a psychotic reaction with ideas of reference when he was transferred from the open ward to a closed one for this investigation. In contrast, the other

three mental defectives who did not excrete free bufotenin did not exhibit any psychotic episodes. We therefore suggest that there is a positive correlation between free bufotenin and active psychotic symptoms and it seems that free bufotenin plays some part in the aetiology of the psychotic symptoms in mentally ill patients. too few results to draw a conclusion. Only further work can determine whether the presence of free bufotenin in the body fluids is a characteristic of disturbed schizophrenics or if it also occurs in non-schizophrenics who exhibit psychotic episodes.

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Cytological Studies of the Pathogenesis of Chronic Lymphatic Leukaemia

WHEN blood lymphocytes from patients with chronic lymphatic leukaemia are examined in Romanovsky stained preparations they show remarkable uniformity in size and appearance and resemble small lymphocytes of healthy individuals. On the other hand, as the basis of other criteria, such as transformation by phytohaemagglutinin¹ or retention on columns of polystyrene beads2, there are differences. To explain these differences it has been proposed that normal and abnormal lymphocytes coexist in the peripheral blood in chronic lymphatic leukaemia²⁻⁴. These studies, however, have not ruled out the possibility that the "abnormal" cell is a type that usually occurs in non-leukaemic subjects, but is found in different proportions in this disease.

We have investigated this possibility by using the supravital staining technique and a divisive procedure (the separation of a large group into smaller ones) for evaluating the results. Although this staining technique has been largely discredited as a means for identifying blood cells, our investigations and those of other workers have shown that mitochondrial content (number of mitochondria per cell), as determined in Janus green B stained supravital preparations, is a useful criterion for

classifying lymphocytes and for comparing lymphocytes from different sites and in various experimental conditions⁶⁻⁹. By employing mitochondrial content, each cell can be quantified and the statistical heterogeneity of the cells collected can be calculated by means of a likelihood ratio test and expressed as the probability, P, of whether the group of cells can be adequately accounted for as a single Gaussian distribution 10 . If P is small, that is below a specified significance level, then the possibility that the data can be described by a single Gaussian distribution is small, and the probability that a heterogeneous—that is, a multiple Gaussian—distribution is present must be considered. Each population can be identified by a mean mitochondrial content and a standard deviation. In this way, it can be determined whether the populations of lymphocytes in patients with chronic lymphatic leukaemia resemble those found in controls. The assumption that frequency distribution curves of the number of mitochondria in a cell would be Gaussian seems justified from histograms, two of which are presented here (Figs. 1Aand B).

The stainability of non-mitochondrial cell components in the supravital staining preparations offered another criterion for comparing lymphocytes. Previously (unpublished data), it was found that lymphocytes showed two distinct colour types when stained with Janus green B. Some cells had a purplish cast (dark cells), while others appeared more or less transparent (light cells). Mitochondria were readily recognized in dark cells because of their distinctive blue green colour; the number of mitochondria in these cells did not differ from that observed in light cells.

Lymphocytes were obtained from four male and two female Caucasians with untreated chronic lymphatic leukaemia. They were 58-74 yr of age and their white blood counts were 22,500-115,900 cells/mm³. A sample

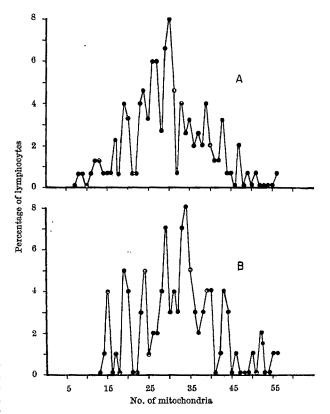


Fig. 1. A, Histogram from a non-leukaemic subject (C.P.) which seems to follow a single Gaussian distribution and which shows a single population by computer analysis (P < 0.90), B, Histogram from a non-leukaemic individual (D.W.) which seems to follow a heterogeneous distribution and which shows at least 5 populations by computer analysis (P < 0.01).



Fig. 2. Blood preparations from a patient with chronic lymphatic leukaemia demonstrating a dark and a light lymphocyte. The dark staining structures in these cells are mitochondria. (× 1,200.)

(1 ml.) of blood was obtained from each patient by venipuncture and the preparation of the samples and the counting of the mitochondria (Fig. 2) were done as described previously^{7,11}. A minimum of 100 cells/subject/examination was studied. Re-examinations, at monthly intervals, for at least 5 months were carried out on two patients (D. and P.). Ten male and ten female Caucasian non-leukaemic individuals from the Cancer Detection Clinic Division of the Michigan Cancer Foundation served as controls. They were 44–76 yr of age and their white blood counts were 4,900–9,900 cells/mm³.

Population analyses were carried out with the aid of an IBM 360 computer at the Wayne State University Computing and Data Processing Center, which was programmed to test each set of data for a maximum of five populations. Because of the large number of analyses, a level of significance of 0.01 was considered to be indicative of, and a level of 0.05 to be suggestive of, multiple populations.

The results indicate that 15 per cent of the control specimens contained multiple populations of lymphocytes (P < 0.01), and that an additional 10 per cent could have (P < 0.05) more than one population (Table 1). For leukaemic specimens the figures were 18 and 29 per cent, respectively (Table 2). With few exceptions, the mean mitochondrial content of the various leukaemic populations fell within the range found in healthy subjects (Tables 1, 2 and 3). Significant differences were observed when the mean mitochondrial contents of lymphocyte populations

Table 1. MITOCHONDRIAL CONTENT AND STAINABILITY OF NON-MITOCHONDRIAL COMPONENTS OF BLOOD LYMPHOCYTES FROM NON-LEUKAEMIC CONTROLS

Mitochondria/cell	Dark cells (%)	Probability of a single population by computer analysis
31-1 (0-6)	52·7	< 0.50
		< 0.50
		< 0.05
		< 0.50
		< 0.02
		< 0.50
		< 0.98
		< 0.70
		< 1.00
26.6 (0.6)	44.0	< 0.20
32.0 (0.9)	38-0	< 0.01
31.8 (1.0)	38.0	< 0.01
30-7 (0-9)		< 0.10
		< 0.10
		< 0.01
		< 0.10
		< 0.90
		< 0.50
		< 0.20
26.4 (0.6)	43.0	< 0.20
	31·1 (0·6) 30·9 (0·9) 30·6 (0·9) 30·2 (0·9) 29·7 (0·6) 29·3 (0·6) 27·6 (1·0) 26·8 (0·6) 26·6 (0·6) 32·0 (0·9) 31·8 (1·0)	(%) 31·1 (0·6) 30·9 (0·9) 51·0 30·6 (0·9) 45·0 30·2 (0·9) 44·0 30·2 (0·9) 44·0 29·7 (0·7) 52·0 29·7 (0·6) 29·3 (0·6) 48·0 26·6 (1·0) 62·0 26·8 (0·6) 42·0 26·6 (0·6) 44·0 32·0 (0·9) 38·0 31·8 (1·0) 30·7 (0·9) 30·6 (0·7) 52·0 30·4 (0·9) 40·0 30·4 (0·9) 40·0 29·3 (0·7) 20·3 (0·7)

Figures are means and standard errors.

Table 2. MITOGHONDRIAL CONTENT AND STAINABILITY OF NON-MITOGHONDRIAL COMPONENTS OF BLOOD LYMPHOCYTES FROM PATIENTS WITH OHRONIG LYMPHATIC LEUKABMIA

29·9 (0·7) 29·3 (0·6) 25·5 (0·6)	Initial values	< 0.001
29.3 (0.6)		4 0.001
25.1 (0.7)	17·3 10·7	< 0·10 < 0·05
30-1 (1-0)	14·0 28·0	< 0.05 < 0.10
, , ,	3.3	< 0.05
36-4 (1-0) 31-6 (0-8) 31-2 (0-7) 31-4 (0-7) 30-9 (0-7) 28-1 (0-6) 27-2 (0-6) 30-6 (0-7) 27-9 (0-6)	18·7 2·0 2·0 5·3 4·0 18·0 4·7 5·3 8·0 1·3	< 0.02 < 0.001 < 0.01 < 0.20 < 0.10 < 0.05 < 0.60 < 0.60 < 0.60
	30·2 (0·8) 36·4 (1·0) 31·6 (0·8) 31·2 (0·7) 31·4 (0·7) 30·9 (0·7) 28·1 (0·6) 27·2 (0·6) 30·6 (0·7)	20·3 (0·5) Re-examination values and the second se

Figures are means and standard errors.

Table 3. PROPORTION AND MITOCHONDRIAL CONTENT OF EACH OF THE POPULATIONS OF LYMPHOCYTES FROM CONTROLS AND LEUKAEMIC PATIENTS WITH MULTIPLE POPULATIONS AS DETERMINED BY COMPUTER ANALYSIS

			ropulat	lons					
	Major		Minor 1		Minor 2	Mir	or 3	Mir	or 4
%	M	%	M	%	M	%	M	%	M
90	29.3 (6.1)	6	47.2 (0.9)	2	10.0	2	56.5		
	29.2 (7.5)	2	55·7 ` ´			-			
	38.4 (6.0)	10	19.2 (0.9)	5	14.8 (0.4)	3	51.3	2	55.5
	31.1 (8.1)	2	66-0 `						
85	31.6 (7.3)	10	16.5 (0.5)	3	52-7	2	13.5		
			Leukaemia	grou	.p				
92	28.2 (6.6)	8	50-3 (5-4)		~				
85	23.3 (5.1)								
96	25.0 (6.0)	2	6.5	2	49.5				
92	19.1 (4.5)	8	33.0 (3.2)						
96	29.2 (8.6)	2	55.7	1	62.5				
	32.5 (8.3)	13	56.1 (4.4)	1	86.5				
	30.0 (8.6)	9	51.4 (3.6)	2	11.3				
90	29.0 (6.4)	10	48.0 (4.3)						
	90 98 80 98 85 92 85 96 92	90 29·3 (6·1) 98 29·2 (7·5) 80 38·4 (6·0) 98 31·1 (8·1) 85 31·6 (7·3) 92 28·2 (6·6) 85 23·3 (5·1) 96 25·0 (6·0) 92 19·1 (4·5) 96 29·2 (8·6) 86 32·5 (8·3) 89 30·0 (6·6)	% M % 90 29-3 (6-1) 6 98 29-2 (7-5) 2 80 33-4 (6-0) 10 98 31-1 (8-1) 2 85 31-6 (7-3) 10 92 28-2 (6-6) 8 85 23-3 (5-1) 15 96 25-0 (6-0) 2 92 19-1 (4-5) 8 96 29-2 (8-6) 2 85 32-5 (8-3) 13 89 30-0 (6-6) 9	Major	Major	% M % M % M % M Control group 90 29·3 (6·1) 6 47·2 (0·9) 2 10·0 98 29·2 (7·5) 2 55·7 80 33·4 (6·0) 10 19·2 (0·9) 5 14·8 (0·4) 98 31·1 (8·1) 2 66·0 Evaluation of the first of the fir	Major Minor 1 Minor 2 Minor 3 Minor 2 Minor 3 Minor 2 Minor 3 Minor 3	Major Minor 1 Minor 2 Minor 3 M Model 4 Minor 3 Minor	Major Minor 1 Minor 2 Minor 3

Figures are either means or means and standard deviations. M, mitochondria/cell.

from different patients were compared (Tables 2 and 3). These differences did not appear to be caused by the disease, for similar variation was also found in controls (Tables 1 and 3). The variation found in chronic lymphatic leukaemia suggests that the leukaemic process was not confined to one population of lymphocytes.

Dark and light cells (Fig. 2) were observed in all preparations (Tables 1 and 2). Various stages in between definitely purple cells and transparent ones were seen, but only those which were definitely purple were scored. In controls, a considerable proportion of lymphocytes were of the dark type (Table 1). In comparison, the proportion of these cells was reduced markedly in patients with chronic lymphatic leukaemia (Table 2).

While the significance and mode of production of dark cells remain unclear, it is known that during the reduction of Janus green B to the leuco form, a violet colour can develop as an intermediate stage¹². Thus it is possible that the dark cells represent lymphocytes incapable of carrying out complete reduction of the dye. Inasmuch as dark and light cells were found within a single population (Tables 1 and 2), we believe that they represent different stages in the life cycle of the lymphocyte. It is interesting to note that the percentage of dark cells found in control and leukaemic specimens resembles the proportion of cells transformed by phytohaemagglutinin which has been reported in the literature¹. Whether this is a coincidence remains to be determined.

We conclude: (a) there are numerous populations of blood lymphocytes in both leukaemic and non-leukaemic individuals; (b) lymphocytes from patients with chronic lymphatic leukaemia are similar to those found in healthy individuals with respect to mitochondrial content; (c) the leukaemic process is not confined to one population of

lymphocyte; and (d) a greater proportion of lymphocytes capable of reducing Janus green B to the leuco form is found in chronic lymphatic leukaemia than in controls. Leukaemic lymphocytes cannot be differentiated from normal lymphocytes on the basis of mitochondrial content as assessed by supravital staining with Janus green B.

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IMMUNOLOGY

Cellular Distribution in the Bone Marrow after Thymectomy

THYMECTOMY of the newborn animal is known to be followed by a striking deficiency of the lymphocyte population¹⁻³. Data concerning myelopoiesis are not consistent. In some cases, extensive extramedullary haemopoiesis was observed in the spleen of thymectomized mice4. An abnormal persistence and even increase of myeloid tissue in the spleen and lymph nodes of thymectomized opossum embryos has been described. Furthermore, there was an increased ratio of immature to mature erythroblasts, not only in the spleen and lymph nodes, but also in the liver, bone marrow and submucosa of the small intestine. No abnormal extramedullary myelopoiesis has been reported in the thymectomized rats. A quantitative investigation⁶ did not reveal any change in the cell population of the bone marrow of rats after thymectomy, which was performed some weeks after birth, when the weight of the animals was about 70 g. Thymectomy is not likely to be very effective when it is performed so late after birth2.

We describe here a study of the cellular distribution in the bone marrow of rats thymectomized 2 days after birth. Nine Wistar albino rats were thymectomized by the method of Miller'. The absence of macroscopic thymic residues was verified in all cases. The animals were killed by decapitation at about 3, 6 or 12 weeks of age, when the body weight was 50 g, 100 g and 200 g, respectively. Nine normal animals of the same strain and body weight were used as controls; the age/ weight relationships were the same in thymectomized and normal rats. The marrow was removed from the femurs immediately after exsanguination and suspended in autogenous serum: smears were stained by the May-Grünwald-Giemsa method. The differential counts were made on 1,000 cells from each animal. The criteria for the classification of blasts were similar to those of Harris and Burkes: transitional cell forms between lymphocytes and blast cells were included in the blast cells. The term lymphocytes was used for small round cells measuring 7-8µ in diameter, with a very narrow rim of pale blue cytoplasm and a relatively large nucleus composed of an almost homogenous chromatin. Erythroblasts were divided into two groups: all smaller cells with a con-densed nucleus were classified as mature erythroblasts, independent of whether the cytoplasm was basophil, polychromatophil or eosinophil. All immature forms of granulocytes were classified as myelocytes; the differentiated forms, including the stab forms, were grouped in the category of granulocytes. The results are summarized in Table 1.

There seemed to be marked changes in the proportion of the red to the white cells between 3 and 12 weeks; our observations agree well with those of Harris and Burke*. The thymectomized rats did not seem to differ in this respect from the normal animals. They did, however, show a much larger number of blasts at all ages. Another feature common to all thymectomized animals was the presence of a larger proportion of immature erythroblasts to the mature ones.

The latter observation is in agreement with the finding of Miller that thymectomy is followed by arrest of maturation in the erythroblasts of the opossum. The abnormal presence of a large amount of blasts in the bone marrow of the thymectomized rats seems to indicate that the thymus may have an even larger influence in the maturation of the mesenchymal cells than could be inferred from the observations in the thymectomized opossum. The thymus seems to play a part not only in the origin of the lymphocytes and in the maturation of the erythroblasts, but also in the early differentiation of the cells with mesenchymal potencies, at least in the bone marrow.

It seems unlikely that the influence of the thymus is confined to the bone marrow: the stem cells differentiating into blood cells in foci of extramedullary haemopoiesis, as well as other fixed cells with unrestricted mesenchymal potencies, are also likely to be sensitive to the influence of thymus. If this is the case, an explanation might be suggested for the increased phagocytic activity which was shown to occur after thymectomy especially after repeated injections of colloid material^{4,9,10}. It may be that, on account of some lack of differentiation of mesenchymal

Table 1. CELLULAR DISTRIBUTION IN THE FEMORAL BONE MARROW OF NORMAL AND THYMEOTOMIZED RATS AT DIFFERENT AGES

	3 weeks		6 V	reeks	12 weeks		
	Normal	Thymectomized	Normal	Thymectomized	Normal	Thymectomized	
Blasts Immature erythroblasts	4-6 (±0-6) 2-8 (±0-6)	9·9 (±0·6) 6·9 (±2·0)	$\frac{4.5}{3.7} (\pm 0.5)$	9-6 (±1-6) 6-2 (±0-4)	2·6 (±0·6) 2·0 (±0·2)	7.4 (\pm 1.6) 4.0 (\pm 0.3)	
Mature erythroblasts	$50.9 (\pm 3.2)$	50·2 (±1·7)	49·1 (±1·3)	42·0 (±2·3)	$34.9 (\pm 4.1)$	$39.9 (\pm 4.4)$	
Myelocytes Granulocytes	5·9 (±0·9) 13·4 (±1·4)	$4.8 (\pm 0.5)$ $12.6 (\pm 1.1)$	6·0 (±0·1) 16·7 (±1·0)	6·0 (±0·3) 19·5 (±2·1)	6·4 (±1·0) 34·0 (±1·7)	$4.2 (\pm 0.7)$ $28.1 (\pm 0.8)$	
Lymphocytes Reticulum cells	18·2 (±1·7) 0-0·5	13·9 (±1·1) 1·0–1·6	19·0 (±0·3) 0-0·3	16·3 (±1·8) 0-0·4	12·4 (±1·5) 0–0·5	14·1 (±1·9) 0·2-1·4	
Plasma cells	0	0-0.2	00-8	0-0-4	0.2-2.0	0.4-1.6	

The average values were derived from counts in three animals. The figures in brackets are stendard errors. Differences in the values of blasts are significant between thymeotomized and normal animals of 3 and 6 weeks (P < 1) per cent or < 5 per cent, respectively). Differences in the values of immature crythroblasts are significant between thymeotomized and normal animals of 6 and 12 weeks (P < 2) per cent and < 0.1 per cent, respectively).

cells, more stem cells with phagocytic properties are available to become macrophages when suitable stimuli are applied.

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PHYSIOLOGY

Susceptibility to Morphine-induced Analgesia in Mice

THE clinical significance of mammalian circadian responses to central nervous system (CNS) drugs has not been adequately established although the implications are impressive 1-11, especially as explanations of unpredictable clinical responses to otherwise thoroughly documented therapeutic agents. This study was designed to determine whether mice, standardized in constant environmental conditions but with alternating 12 h light and dark cycles, would display variations in susceptibility to morphine sulphate along a 24 h scale. Comparisons of susceptibility crests of morphine and other CNS drugs might also provide additional insight into the mechanism of morphine-induced analgesia.

Adult female albino CF-1 mice (Carworth Farms), 26.7 ± 0.2 g body weight, were housed as previously reported¹¹: temperature of $23.3 \pm 1.0^{\circ}$ C; relative humidity of 65 ± 2 per cent; four 40 W incandescent bulbs lit only from 0605 to 1805 h; water and food ad libitum during the fourteen day acclimation period; and dark phase testing with only a photographic safelight as illumination. Preliminary tests indicated that an 8 mg/kg intraperitoneal dose of morphine sulphate most nearly approximated an AD₅₀ (analgesic dose in fifty per cent of tested mice) over a 24 h period. Each mouse was pretested for positive pain response by a modified Haffner test-biting a pinch clamp placed dorsal-ventrally on the base of the tail12. Mice which did not react to the noxious stimulus within 10 sec were rejected.

Beginning at 1500 h and at 3 h intervals over the succeeding 24 h period, groups of eighteen pretested mice were weighed, injected and placed into separate, glass jars. Exactly 20 min after the morphine injection, each mouse was retested. All mice failing to display a positive pain response within 30 sec were considered to be analgesic¹². Responses were transformed into per cent analgesia, relative to the total number of mice injected at that time period. A mean 24 h morphine analgesia response was determined. The methods of Snedecor were used to perform all statistical analyses¹³.

An analysis of variance of the percentage data in Table I revealed that mice are more susceptible to the analgesic

activity of morphine during the dark cycle than during the light cycle of a 24 h period (P < 0.05). A chi-square test of the individual time period data in Table 1 against the mean 24 h morphine response of 49.4+4.4 per cent analgesia indicated that a crest or maximum occurs at 2100 h (P=0.01) and a trough or minimum at 1500 h (P=0.001). The analysis of variance showed a significant difference between the per cent analgesia at each time period within each of the light and dark cycles. This would seem to infer, although not substantiate, the existence of two crests for morphine analgesia within a 24 h period: one crest in each cycle with the greatest reduction in susceptibility (P < 0.05) occurring during the light cycle. Work is in progress to define the exact shape and characteristics of the circadian response pattern for morphine-induced analgesia.

Table 1. MORPHINE SULPHATE, 8 MG/KG INTRAPERITONEALLY, INDUCED ANALGESIA IN MICE

Illumination phase	Time*	Per cent analgesia†
Light	1500	28 (P = 0.001)
Light	1800	50`
Dark	2100	67 (P = 0.01)
Dark	2400	44
Dark	0300	50
Dark	0600	61
Light	0900	56
Light	1200	39

Mean 24 h analgesia \pm standard error of mean $=49\cdot4\pm4\cdot4$ per cent (reference standard for individual time periods)

*Light phases: lights on 0605 to 1805 h; lights off 1805 to 0605, June 30-July 1, 1965.
†Eighteen adult female albino mice per time period. Chi-square analysis performed by the nothods of Snedecor¹³.

Table 2. DRUG SUSCEPTIBILITY IN MICE IN A TWENTY-FOUR HOUR PERIOD

	Crest	Trough	Ref.
Dark cycle: 1800-0600*			
Morphine analgesia	2100	1500	
Lidocaine seizures	2100	1500	11
Flurothyl seizures†	2200	1000	
Acetylcholine lethal seizures	2000	0800	6 8 3
Ethanol lethal depression	2000	0800	Š
Chlordiazepoxide lethal depression	2400	1200	4
Nialamide lethal seizures	Dark	Light	4 8
Light cycle: 0600-1800*			
Ouabain cardiac arrest	1000	2400	2
Pentobarbital sleep †	1400	0200	2 5
Nikethamide lethal seizures t	1400	0200	i
Methopyrapone lethal depression	1600	0800	21
Aurothioglucose lethal depressions	Taght	Dark	10

* Light regimen: lights on 0600-1800, lights off 1800-0600 except as noted

below.

† Lights on 0800-2000, lights off 2000-0800,

† Lights on at sunrise. Lights off at sunset.

§ Lights on 0700-1900, lights off 1900-0700.

Comparison of the morphine crest at 2100h with crests for other CNS drugs (Table 2) suggests that the peak time for susceptibility to morphine-induced analgesia may have more in common with CNS stimulants than depressants. Of the drugs in Table 2 exhibiting a crest during the dark cycle, all but ethanol possess some central stimulant activity. Ethanol precipitates overt stimulant behaviour brought on by initial depression of the reticular formation, then the cerebral cortex, and not by direct CNS stimulation¹⁴. In large doses lidocaine induces clonic seizures without lethality11 whereas in small doses lidocaine is a depressant producing mild intravenous analgesia14, potentiating pentobarbital sleep and inducing minimal sleep by Flurothyl⁶ is a highly potent CNS stimulant producing major seizures in contrast to the relatively mild stimulation precipitated by the mood-elevating amine oxidase inhibitor, nialamide⁸. Acetylcholine in very large intravenous doses precipitates lethal seizures. Chlordiazepoxide in toxic doses in humans precipitates signs of both central stimulation and depression14.

Morphine is a centrally acting drug with mixed depressant and stimulant activity; analgesia most generally considered to be the result of the depressant activity of morphine on the hypothalamus, thalamus and cerebral cortex14,16,17. In man, the depressant activity of morphine dominates, producing both analgesia and sedation, while in the cat, the stimulant activity (sham rage) is manifested

as well as good analgesia. The mixed activity of morphine is also readily seen in the mouse-good analgesia with stimulation evidenced by the presence of a Straub tail. Morphine exhibits other central stimulant activity: induced hyperthermia18 by stimulation of the hypo $that a mic temperature \ regulating \ centre\ ; increased \ release \ of$ antidiuretic hormone from the pituitary gland14 probably caused by hypothalamic stimulation; cortical stimulation following topical or intracarotid administration19; production of electroencephalic arousal patterns in the hypothalamus and reticular formation 16,17; and lowered threshold to pentylenetetrazol-induced seizures20.

Some CNS stimulants are used clinically as depressants although they produce signs of central stimulation (that is, amphetamines in petit mal epilepsy). Amphetamines also, depending on dosage, may potentiate or antagonize barbiturate depressions. The psychotomimetic drug lysergic acid diethylamide (LSD) and the analeptic methylphenidate induce good analgesia in mice without evidence of depression. Simultaneously with deep, surgical anaesthesia, barbiturates induce good analgesia in dogs but not in either rabbits or guinea-pigs. Nitrous oxide induces fair general anaesthesia but good analgesia. Analgesia therefore does not always correlate well with decreased CNS activity.

Mice also exhibit crests at 2100 h in motor activity, rectal temperature and susceptibility to audiogenic seizures. Concurrence of susceptibility to CNS stimulant drugs with the nocturnal activity pattern of mice provides additional support for the suggestion that morphineinduced analgesia might be more related to central stimulant than depressant activity.

Greater diversity of pharmacological activity is exhibited by the drugs with crests in the light cycle, although central or peripheral depression was the terminal effect of four of the five drugs listed in Table 2 (that is, pentobarbital, methopyrapone, aurothioglucose and ouabain). Nikethamide, a unique analeptic, exhibits primary specificity for the baroreceptors in the carotid and aortic bodies with secondary activity on the medullary respiratory and cardiovascular centres. Reliance on natural daylight rather than a progammed lighting regimen detracts from the significance of the nikethamide data, especially because data were collected on more than one day1. Data collected during periods of reduced susceptibility to morphine were so nearly similar (that is, no precise trough) that no attempt was made to attach significance to the trough data in Table 2.

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Neural Release of Brain Serotonin and Body Temperature

FELDBERG^{1,2} has suggested that brain amines are directly involved in the hypothalamic control of body temperature. In cats and dogs serotonin was found to cause a rise in temperature and norepinephrine a fall, whereas in the rabbit3 and sheep4 there is a fall with serotonin and a rise with norepinephrine. Moreover, serotonin antagonizes the normal decrease of temperature of animals under anaesthesia. All these results, however, have been obtained using exogenous amines. We have shown that there is a neurally mediated release of endogenous serotonin in rat brain when the caudal midbrain raphé region is stimulated electrically and we have found that a specific behavioural consequence of such stimulation is a failure of the normal physiological habituation to repetitive sensory stimuli⁵. We have also measured the effect on body temperature of neurally released endogenous serotonin both in awake, unrestrained and in anaesthetized rats.

Male Sprague-Dawley rats (250-350 g) were used. Twelve had chronic electrodes implanted in the midbrain raphé region using a stereotaxic technique. An indifferent electrode was a stainless steel screw embedded in the skull. Both electrodes were connected by 'Teflon' coated wire to female clips and the whole assembly securely attached to the skull by dental cement. Five days or more after the operation rats were electrically stimulated with pulses generated from a Grass S4 stimulator for periods of 0.5 h. The parameters of stimulation were: frequency, 10 pulses/sec; duration, 2 msec; intensity, 1.5-3 V. Observations of the behavioural response to repetitive sensory stimuli were made during this period. Colonic temperature was measured before and after stimulation by means of a telethermometer (Yellow Spring Instrument Some animals were retested after 24 h with reserpine or p-chlorophenylalanine. Rats were decapitated, and brain stems were fixed in 5 per cent glutaraldehyde. Frozen sections were cut and stained for determination of electrode placement.

Table 1. RELATIONSHIP BETWEEN ELECTRODE PLACEMENT AND CHANGE IN BODY TEMPERATURE AFTER 0.5 H ELECTRICAL STIMULATION IN RATS AWAKE AND UNRESTRAINED

No. of animals	Midbrain electrode placement	Mean before	Femperature Mean after stimulation	,
8	Caudal raphé	37.3	38.9	$+1.5 \pm 0.5$
8	Anterior raphé or lateral	37.1	37.3	+0.2+0.2

Sites of electrode placement were the caudal midbrain raphé and adjacent regions. S.D., standard deviation.

The rats with chronic electrodes were electrically stimulated for 0.5 h while awake and unrestrained. Table 1 shows that there was a rise of colonic temperature when the electrode placement was the caudal midbrain raphé. Some animals showing a rise in body temperature were given reserpine (2.5 mg/kg, subcutaneously) or p-chlorophenylalanine (300 mg/kg, intraperitoneally) and restimulated at varying times after the administration of the drugs. It was found that both the drugs reversibly blocked the rise of temperature induced by caudal raphé stimulation. In contrast, certain motor effects of stimulation, a 10 cycles/sec tremor involving whiskers and eyelids, sometimes facial musculature, were not altered by the drugs. As previously reported, stimulation of the midbrain raphé produced a failure of habituation to repetitive sensory stimuli as manifested by a persistence of "startle" responses.

In acute experiments, rats were lightly anaesthetized with chloral hydrate, and stimulated for 1 h with an electrode inserted into the caudal midbrain raphé region. Pairs of these rats were tested in identical conditions of room temperature (23° C) and humidity. Parameters of stimulation were: frequency, 10 pulses/sec; duration, 2 msec; intensity, 6 V. Colonic temperatures were again taken with the telethermometer before and after stimulation. The electrode was present in some rats for 1 h but the animals were not stimulated. After the stimulation, rats were decapitated, brains were dissected out, and the forebrain was removed from the brain stem by a section passing between the rostral border of the superior colliculi and the caudal border of the hypothalamus. The forebrain was analysed for serotonin and 5-hydroxyindoleacetic acid.

Table 2 shows that the stimulated group consistently maintained a higher temperature than control rats (mean difference 1.3° C higher). There was also a significant difference in concentrations of serotonin and 5-hydroxy-indoleacetic acid between the two groups of rats. The group which received raphé stimulation showed a fall in serotonin and a rise in 5-hydroxy-indoleacetic acid.

Table 2. Effect of electrical stimulation of midbrain raphé region on forebrain serotonin, 5-hydroxyindoleacetic acid and body temperature in anaesthetized rats

Ani- Serotonin mals ng/g Change* (No.) ±S.D. (%)	P	ng/g Change* Me	mperature (°C) an ange* P
8 517 + 65		Controls	8 ± 0.4

8 382 ± 62 -26 < 0.001 517 ± 85 +92 < 0.001 -0.5 ± 0.2 < 0.001 *The significance of differences between concentratons in control and stimulated rats was calculated using Student's t test. S.D., Standard deviation.

The results of these experiments show that stimulation of midbrain raphé causes an increase of body temperature (1°-2° C) in the awake unrestrained animal and a reduction in the usual fall in temperature in lightly anaesthetized animals. Indoles in the forebrain were measured in the anaesthetized group and the reduction in fall of temperature with midbrain raphé stimulation is associated with a fall of serotonin and increase of 5-hydroxyindole-acetic acid. These changes in the concentrations of brain indoles after raphé stimulation are in accord with previous results and suggest a release of serotonin. Reserpine and p-chlorophenylalanine reversibly block the rise in tem-

perature with stimulation in awake, unrestrained animals. Both reserpine and p-chlorophenylalanine deplete serotonin in the brain, and reserpine also depletes catecholamines. In contrast, p-chlorophenylalanine does not affect catecholamines appreciably and is in this sense a specific depletor of serotonin'. Together, these facts support the idea that release of serotonin in the brain is linked with the rise of body temperature observed with midbrain stimulation. These data are consistent with Feldberg's hypothesis concerning the role of serotonin in temperature regulation. In particular, there is compatibility between the effects of exogenous and endogenous serotonin both on rise in temperature in awake animals and in opposing the fall of temperature with anaesthesia. Different species were used, however, in the exogenous versus the endogenous experiments.

It is interesting that d-lysergic acid diethylamide (LSD-25) which produces a failure of the normal physiological process of habituation^{8,8} has also been reported to cause an elevation of temperature in rabbits, cats and dogs¹⁰. In man, LSD-25 also increases temperature when it produces its characteristic mental symptoms¹¹. It has been reported previously that LSD-25 causes a fall in temperature in rats¹², but we found that a dose of 200 γ /kg intravenously causes a rise of 1°-2° C in the first 0.5 h

while behavioural effects are maximal. Another related psychotomimetic agent, psilocybin, has also been reported to cause an increase in body temperature¹³. These facts are compatible with the hypothesis that psychotomimetic agents such as LSD-25 may mimic the action of serotonin in brain¹⁴.

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Synaptic Structures at Nodes of Ranvier in Spinal Cords of Mice

Bodian and Taylor¹ described presynaptic structures at nodes of Ranvier in the spinal cord of the monkey, and Andrés² reported a similar bouton from a node of Ranvier in the granular layer of the cerebellum of the albino rat.



Fig. 1. Electron micrograph from the dorsal horn of a spinal cord of mouse. A longitudinal section of a myelinated fibre (AX) passes through a node of Ranvier. The nodal axon (NA) bulges out to form a presynaptic bag (PR) which contains mitochondria and synaptic vesicles (V). This nodal bouton forms synaptic contacts with a dendrite (D) which acts as the postsynaptic (PS) structure. Thickening of membranes is indicated by arrows. Scale, 1/a.

I have found two types of synapses at nodes in the central nervous tissue of rabbits³. In the first type, observed in the parietal cortex, the nodal axon gave rise to a process which terminated with a bouton; in the second, described in the reticular formation of the medulla, the nodal axon bulged out to form a synaptic bag.

In the experiments described here, albino mice narcotized with urethane were perfused with a 5 per cent solution of glutaraldehyde buffered with phosphate to pH 7.4 (ref. 4), through a cannula inserted into the abdominal aorta. The cervical region of the spinal cord was excised and thoroughly washed with phosphate buffer (pH 7.4). Small pieces of the cord were post-fixed in a phosphate buffered 2 per cent solution of osmic acid at pH 7.4 at 4° C for 2 h. The material was embedded in 'Maraglas' by the method of Freeman and Spurlock's. Thin sections from the dorsal horn were stained with a lead salt's.

Fig. 1 shows an electron micrograph of a node of Ranvier. The nodal axon bulges out on one side to form a bag which contains synaptic vesicles and mitochondria. The nodal bouton makes synaptic contacts with a dendrite (arrows). At the sites of contact there is thickening of the membranes and accumulation of synaptic vesicles on the presynaptic side. The bulging of nodal axon to form a bouton corresponds to the second type of synapse which I described³ and which was also reported by Bodian and Taylor¹ and Andrés².

Fig. 2 is another example of the synaptic structures at nodes of Ranvier. The nodal axon gives rise to a process or branch which extends for a distance terminating with a bouton. This terminal bouton contains synaptic vesicles and mitochondria. There is no clear evidence that this structure forms synaptic contacts with either the dendrites or the axon terminals surrounding it. Its presynaptic

Fig. 2. Electron micrograph from the dorsal horn of a cord of mouse. The section passes through the longitudinal axis of a myelinated fibre at a node of Ranvier. The nodal axon (NA) gives rise to a branch (B) which terminates with a synaptic bag (SB). Note the presence of mitochondria and synaptic vesicles in the synaptic bag which is surrounded by axon terminals and a dendrite (D). Scale, 1μ .

nature cannot, however, be doubted. The branching of the nodal axon to produce a bouton corresponds to my first type of synapse³ which seems to be the more frequent: synaptic structures of this type were also found in the frontal cortex of rabbit.

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Capillary Permeability Effects of Synovial Fluid, AMCHA, E-ACA and Prednisolone Acetate

In the past decade, inhibitors and activators of the fibrinolytic system have undergone extensive study. The chief inhibitors are epsilon-aminocapric acid (E-ACA)¹ and *trans*-aminomethylcyclohexanecarboxylic acid (AMCHA)². This communication compares the effect

of E-ACA, AMCHA and prednisolone acetate, alone and combined with synovial fluid, on capillary permeability measured by the diffusion of systemically injected pontamine blue according to the method of Nitta et al.³. The synovial fluid was obtained with a siliconized syringe from patients with rheumatoid arthritis.

In the first experiment, 0·1 ml. of synovial fluid, 0·1 ml. of saline solution containing 2 mg of AMCHA, 0·1 ml. of synovial fluid containing 2 mg of dissolved AMCHA or 0·1 ml. of saline solution, was injected intracutaneously into the depilated skin of the flanks of nine rats which had previously been injected intravenously with a dose of pontamine blue (60 mg/kg) as a 5 per cent solution.

In the second experiment, 0·1 ml. of synovial fluid, 0·1 ml. of saline solution containing 2 mg of E-ACA, 0·1 ml. of synovial fluid containing 2 mg of E-ACA or 0·1 ml. of saline solution, was injected intracutaneously into each of nine experimental rats.

In the third experiment, a series of nine dye-injected rats received 0·1 ml. of synovial fluid, 0·1 ml. of saline solution containing 0·25 mg of prednisolone acetate, 0·1 ml. of synovial fluid in which was dissolved 0·25 mg of prednisolone acetate or 0·1 ml. of saline solution.

The extent to which dye diffusion occurred was determined as described and the average amounts of the diffused dye are presented in Table 1. Twenty-seven rats were used in this experiment.

In the first experiment, there was a positive effect when AMCHA was injected alone, which was dramatically enhanced when the mixture of AMCHA and synovial

Table 1. PONTAMINE BLUE IN THE SKIN OF RATS INJECTED WITH SYNOVIAL FLUID OF PATIENTS WITH BHEUMATOID ARTHRITIS, AMCHA, E-ACA AND PRED-NISOLONE ACETATE

Injected substances	Pontamine blue (µg) extracted from the skin*				
Synovial fluid	7.6	150-2	20.6		
AMCHA + saline solution	13.0	18.0	15.4		
AMCHA + synovial fluid	42.5	443.7	127.8		
Synovial fluid	8.5	130.0	8.5		
E-ACA + saline solution	15.5	8.0	18.0		
E-ACA + synovial fluid	30.2	261.6	36.0		
Synovial fluid	8.7	19.5	20.6		
Prednisolone acetate + saline solution	23.5	20.1	19-9		
Prednisolone acetate+synovial fluid	3.1	13.7	8-0		

^{*} The data presented in the table are the amount of dye which was obtained by subtracting the control amount using saline solution from the experimen-tal values in each animal.

fluid was injected. Similar though less dramatic results were obtained in the second experiment with E-ACA. The animals treated with prednisolone acetate alone showed little difference from those injected with synovial fluid. When, however, the mixture of prednisolone acetate and synovial fluid was injected, the permeability, as indicated by the dye in the injected area, was less than for either alone.

This series of experiments was prompted by the observation that the synovial fluid of patients suffering from rheumatoid arthritis contains markedly larger amounts of anti-fibrinolysin than the synovial fluid of normal individuals. It was reasoned therefore that the antifibrinolysin is a part of a biological compensatory mechanism designed to offset the inflammatory process. AMCHA and E-ACA were therefore administered intraarticularly in a series of rheumatoid arthritis patients to ascertain this assumption. The results of the clinical studies clearly show that this was indeed the case4.5.

Melmon et al.6 have reported a kinin in the synovial fluid of rheumatoid arthritis and suggested that kinins may contribute to the inflammatory symptom?. In fact, the substance producing permeability (probably kinin) in this experiment seemed to increase in the acute cases. We have recently found that the substance in synovial fluid, derived from incubation with AMCHA or E-ACA, causes contraction of guinea-pig ileum.

If a kinin is a mediator of the inflammatory response, the clinical effects of AMCHA and E-ACA are very hard to understand; however, prednisolone acetate, which does not produce the substance which causes the enhanced permeability, is supported by our observation.

Objections to the role of bradykinin as a mediator of the inflammatory response have been raised by Miles, who points out that bradykinin has not been shown to produce the histological picture of capillary damage known to predominate in the late phase of inflammation, and by Phelps et al.8, who suggested that bradykinin is not a mediator of the acute inflammatory response induced by injection of urate crystals into canine joints.

The relationship between the substance in synovial fluid, derived from AMCHA or E-ACA incubated in synovial fluid, and bradykinin remains a matter for conjecture.

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Anaemia, Thrombocytopenia and Reduced Platelet Adhesiveness in Rats fed Bracken Fern, and Protective Effects of Batyl Alcohol

In cattle, the bracken fern (Pteridium aquilinum) produces haematological changes which are characteristic of an aplastic anaemia1. In the horse and rat the syndrome is one of thiamine deficiency caused by a thiaminase enzyme in the bracken²⁻⁴.

Following reports that D-batyl alcohol (D-α-octadecylglycerol ether) protected mice from total body X-irradiation⁵ and patients from irradiation leukopenia⁶, Evans et al.7 administered this substance to three of four bullocks with experimentally induced bracken poisoning and observed a rapid improvement in condition and a return towards normal values of both platelet and leucocyte counts. Recently, Evans and Mason⁸ reported that the prolonged feeding of bracken to rats caused multiple malignant adenocarcinoma of the intestinal mucosa.

In July and August of 1966, approximately 425 lb of bracken was collected in Southwestern Ontario and oven-dried at 40° C until the weight was reduced to about one-third. The dried bracken was then ground and mixed with ground Purina Lab Chow pellets sufficient to restore the bracken to its original weight. A control group of rats was fed Purina Lab Chow in pellet form. All food was available ad libitum, as was the drinking water, all of which contained thiamine hydrochloride (10 mg per litre).

Male, Long-Evans hooded rats (230-280 g) were randomly assigned to one of four groups; forty-four rats (group A) were fed the bracken diet, forty-four rats (group B) received the bracken diet to which had been

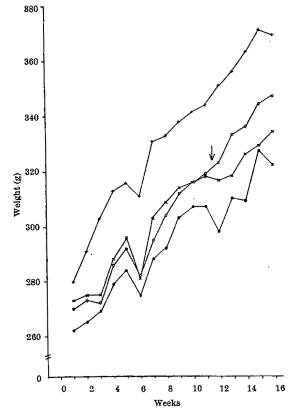


Fig. 1. Growth curves are shown for rats fed a diet consisting of 38 per cent dried bracken fern, with and without added batyl alcohol (75 mg/kg of food). All rats received thiamine in the drinking water (10 mg/l). The rats which were fed bracken gained weight poorly, as compared with rats fed the standard colony diet, regardless of the inclusion of batyl alcohol. — Fed bracken; ×, fed bracken with batyl alcohol; O, fed bracken as far as arrow, then normal diet; +, normal diet.

Table 1. HAEMATOLOGICAL CHANGES IN RATS FED BRACKEN WITH AND WITHOUT ADDED BATYL ALCOHOL (75 MG/KG OF FOOD)

	Group A (bracken only)	(bracken and batyl alcohol)	(after removal from bracken and batyl alcohol)	Group <i>D</i> (standard diet)
Red cells (× 10°/mm³) White cells (per mm³) Platelets (× 10°/mm²) Haematocrit values (per cent) Percentage platelet adhesiveness to glass Mean corpuscular haemoglobin (μμg/cell)	$\begin{array}{ccccc} 7 \cdot 01 \pm & 0 \cdot 025 & (20) \\ 5,388 \cdot 0 & \pm 297 \cdot 64 & (20) \\ 779 \cdot 7 & \pm & 31 \cdot 97 & (20) \\ 44 \cdot 6 & \pm & 0 \cdot 61 & (20) \\ 17 \cdot 1 & \pm & 2 \cdot 71 & (20) \\ 17 \cdot 5 & \pm & 1 \cdot 03 & (11) \end{array}$	$\begin{array}{ccccc} 7 \cdot 03 \pm & 0 \cdot 170 & (38) \\ \mathbf{5.521 \cdot 6} & \pm 228 \cdot 03 & (37) \\ 751 \cdot 3 & \pm & 19 \cdot 18 & (39) \\ 43 \cdot 2 & \pm & 1 \cdot 23 & (39) \\ 24 \cdot 0 & \pm & 2 \cdot 17 & (39) \\ 20 \cdot 3 & \pm & 0 \cdot 57 & (30) \end{array}$	6.84 ± 0.241 (10) 5,914.9 ±434.67 (10) 976.0 ± 48.63 (7) 44.6 ± 0.65 (10) 40.1 ± 5.05 (7) 20.1 ± 1.11 (10)	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

Cell values are given as mean ± S.E.M. Numbers in parentheses refer to number of measurements made.

added baty alcohol (75 mg/kg of food), twenty-four rats (group C) were fed the same bracken-plus-batyl alcohol diet for eleven weeks and were then changed to the standard colony diet, and fifty rats (group D) were given the standard colony diet throughout the experiment. The rats were caged individually and were weighed weekly.

Each week some rats from each group were anaesthetized with sodium pentobarbital and 5 ml. of blood was drawn from the posterior vena cava into siliconized glass syringes containing 7 mg of disodium EDTA in 0.2 ml. of saline. Red blood cells and white blood cells were counted in a Coulter electronic counter. Platelets were counted before and after the passage of blood through a 5 g column of glass beads (0.5 mm diameter). The percentage of platelets remaining in the column was calculated (percentage adhesiveness). Counts were done with Spencer-Brightline haemocytometer chambers using Wright's modification of Reese-Ecker's diluting fluid.

Haemoglobin concentration was measured spectrophotometrically using Hycel, Inc. (Houston, Texas), reagents and the mean corpuscular haemoglobin (MCH) was calculated.

The growth curves (mean weekly weights) are shown in Fig. 1. The data suggest the following conclusions: the rats fed the standard diet gain weight at the fastest rate and reach the highest mean weight at the end of sixteen weeks; the inclusion of batyl alcohol in the bracken does not improve appreciably the rate of weight gain; and there is a rapid improvement in weight gain in the group which was transferred from brackenplus-batyl alcohol to the standard diet.

The results of the blood studies are shown in Table 1. There were no statistically significant differences in red cell counts between any of the groups. White cell counts were depressed in those rats which were fed bracken with or without added batyl alcohol (groups A and B), but the differences were not statistically significant. Platelet counts were significantly (Ps < 0.01) depressed in the rats which were fed bracken regardless of the inclusion of batyl alcohol (groups A and B). Removal from the bracken diet restored the platelet count to above normal (group O). These differences were statistically significant by analysis of variance (P < 0.01). Platelet adhesiveness was drastically reduced in the bracken-fed rats (group A), noticeably improved when batyl alcohol was included in the bracken diet (group B) and restored to near normal if the standard food was substituted for bracken (group C). Again, these differences were statistically significant by analysis of variance (P < 0.01). The haematocrit value of the control group (D) which received the standard diet was significantly greater than that of any of the other groups (Ps < 0.02-0.05), indicating a microcytic anaemia in the bracken-fed rats which was not favourably influenced by the inclusion of batyl alcohol in the diet. The mean corpuscular haemoglobin of the rats which were fed bracken alone (group A) was significantly lower than that of any of the other groups (Ps < 0.02 to 0.05).

These results suggest that the rats which were fed bracken developed microcytic, hypochromic, normocythaemic anaemia with mild leucopenia and more marked thrombocytopenia. The adhesiveness of platelets to glass was drastically reduced: The inclusion of batyl alcohol in the diet did not influence favourably the leucopenia, the thrombocytopenia or the size of the red cells, but it did

restore the mean corpuscular haemoglobin to normal and improved the adhesiveness of the platelets.

To date (July 12, 1967) two rats have shown evidence of malignancy. One of these was from group C, which was removed from the bracken diet; the other was from group B, which was treated with batyl alcohol. Both rats had large, purplish tumours about one inch in diameter in the area of the small bowel, and enlargement of the mesenteric lymph nodes and spleen. Histological sections are being prepared. All of the rats have now been on the standard colony diet for ten weeks, owing to the depletion of the bracken diet.

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Cell Regeneration in **Protein Deficiency**

LESIONS of organs with a high protein turnover are characteristic of kwashiorkor in humans1. These lesions have been reproduced in rhesus monkeys by feeding them protein deficient diets2. On the basis of a study of the cell population kinetics of the mucosa of the small intestine in this primate, it has been suggested that a retardation of cellular proliferation is characteristic of protein deficiency3. In this communication, we describe preliminary observations on the influence of protein deficiency on cell population kinetics in regenerating livers of rats after partial

Twenty-six male albino Wistar rats, 120-150 g, were divided into two equal groups; one group was fed a protein deficient diet containing 1 per cent casein given freely for 5-6 weeks; the other, control group, was fed a diet containing adequate protein (16 per cent casein) for an equal period. Each animal in the second group was pair-fed with a corresponding animal in the protein deficient group. Both groups received other essential nutrients in adequate quantities.

All animals were subjected to partial hepatectomy by the method of Higgins and Anderson. Hepatectomy was always performed at fixed hours in the morning. overcome the differences caused by diurnal variation in

Table 1. EXPERIMENTAL DESIGN

		Animal	s used for labelling inc	lex	No. of an	imals used for mit	otic Index
Groups	Total No. of animals	No. of animals given *H-thymldine (22 h)	No. of animals biopsied/autopsied (24 h)*	No. of animals autopsied (28 h)†	Without colchicine (24 h)	Without colchicine (28 h) ‡	With colchicine (28 h)
Low protein High protein (control)	13 13	12 12	8 7	4 5	8 7	5 6	5 5

* Five animals from each group were given colchicine just after biopsy, and biopsied liver pieces were used for the estimation of mitotic index after 24 h.

† These animals were also used for determination of mitotic index after 28 h.

‡ One animal in each group did not receive *H-thymidine. Time in parentheses indicates hours after hepatectomy.

Table 9	LARRITING	ANT	MITTOTHE	INDICES	IN	PROTEIN	DEFIGIENT	AND	CONTROL	ANTMALS

		No. of labelled cells	s/1,000 hepatocytes	No. of mitoses/1,000 hepatocytes			
Groups		2 h after the injection of ³ H-thymidine and 24 h after hepatectomy	6 h after the injection of 3H-thymidine and 28 h after hepatectomy	24 h after hepatectomy with- out colchicine	28 h after Without colchicine	hepatectomy With colchicine	
Low protein . High protein (control)	Mean Ranges Mean Ranges	$212 (8)$ $135-291$ $107 (7)$ $63-169$ $t=8.764$ $n^*=13$ $P<0.01$	208 (4) 166-261 157 (5) 125-201	0.85 (8) 0-2 0.50 (7) 0-1 t=1.242 $n^*=13$ P < 0.3	15 (5) 7-18 3·5 (6) 1·3-4·5 t=3·737 n*=9 P<0.01	$ \begin{array}{c} 15.7 (5) \\ 7.5-24 \\ 43 (5) \\ 35-50 \\ t=7.280 \\ n*=8 \\ P < 0.001 \end{array} $	

P and t test between labelling indices at 24 and 28 h: low protein, not significant; high protein, t=2.240, $n^*=10$, P<0.05. Figures in parentheses indicate the number of animals used.

mitotic activity, animals from both groups were killed at the same time of the day. The rate of cellular regeneration was assessed by making mitotic counts of liver sections of animals, biopsied or autopsied 24 and 28 h after hepatectomy. The rate of mitosis had begun to increase by 24 h after hepatectomy in both the groups, and so colchicine (0.75 mg/kg) was administered intraperitoneally at this time to five animals in each group and the animals were killed 4 h later. Colchicine is known to arrest mitosis in the metaphase, and so the cumulative mitotic activity was measured in this way for both groups during the 4 h interval. DNA synthesis was assessed by estimation of labelling index 1.5-2 h after the administration of 3Hthymidine (50 μ c./100 g of body weight) intraperitoneally 22 h after partial hepatectomy. (Labelling index: number of hepatic parenchymal cell nuclei which were labelled in each 1,000 cells.) This time interval was chosen because it is known that in normal rats labelling is greatest 20-22 h after partial hepatectomy. The labelling index in some animals from both groups was also determined 6 h after the administration of thymidine. The difference between the labelling indices 2 and 6 h after thymidine would give an idea of the rate at which cells were dividing during the 4 h interval. A total of 2,000 hepatocytes was counted for the estimation of mitotic and labelling indices in each case. (Mitotic index: number of hepatic parenchymal cells undergoing mitosis in each 1,000 cells.) The number of animals used in each group is shown in Table 1 and the results are shown in Table 2.

The significant rise in the labelling index in the control group between 24 and 28 h after partial hepatectomy as

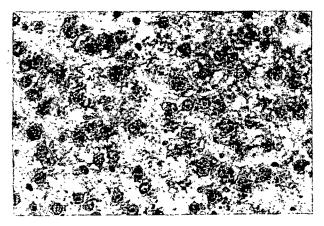


Fig. 1. Autoradiograph counterstained with haematoxylin and eosin 24 h after partial hepatectomy. 3 H-thymidine was administered 2 h before death. Control rat (× c. 275).

compared with the protein deficient group which shows no significant change during this interval indicates poor cellular proliferation in protein deficiency. This feature is revealed independently by comparing the mitotic indices 24 and 28 h after partial hepatectomy in animals given colchicine. Striking differences are found in the rates of accumulation of mitotic figures during this 4 h period between the two groups again emphasizing the inability of the protein deficient animals to keep pace with the control group in cellular proliferative response after partial hepatectomy.

The high mitotic index 28 h after hepatectomy in the protein deficient group is probably the result of slowing down the cell division. The hepatocytes in these animals seem to stay in mitosis for longer than in the controls, thus giving a relatively high mitotic index. At first sight the higher labelling index at 24 h in the protein deficient group may suggest an enhanced cell turnover in this group. Both the mitotic and labelling indices of animals given colchicine, however, suggest a marked depression of the rate of cellular proliferation in protein deficiency. The higher labelling index in the protein deficient group might be explained on the basis of a prolongation of the DNA synthetic phase which in turn would lead to an expansion of the number of cells capable of taking up thymidine and thus of being labelled3.

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α_2 -Macroglobulin as Progressive Antithrombin

For several years we have been interested in the antiprotease activity of human α_e -macroglobulin $(\alpha_2 M)^{1-\delta}$. The antitrypsin and antiplasmin effect of this macroglobulin has already been studied 8-8. During work on the antiplasmin activity of a2M, using a very sensitive fibrinolytic system¹⁰, we found that the interaction between α2M and this protease required several minutes for completion. a2M has a molecular affinity for plasminogen and

fractions obtained by gel filtration on 'Sephadex G-200' are usually contaminated by this proenzyme. Preparations of a2M suitable for studying anti-protease activity should not be contaminated with plasminogen because such α₂M shows less apparent anti-protease activity than an uncontaminated preparation. It is necessary to detect such contamination, for α_2M inactivates plasmin within a few minutes. α_2M should be isolated from plasma because in the serum it has already adsorbed thrombin and

eventually will even take up plasmin. We prepared $\alpha_2 M$ by a technique involving adsorption of contaminating plasminogen by bentonite, but we used less bentonite4 than the original technique. We found that the interaction between a2M and plasmin needed a reaction time of several minutes, and so considered that $\alpha_2 M$ might have the properties of a progressive anti-thrombin. $\alpha_2 M$ proved to be a potent progressive antithrombin (Fig. 1). Human $\alpha_2 M$ inhibits human, bovine, equine and chicken thrombin. The affinity seems to differ from one species to another. Whereas $\alpha_2 M$ inhibits clotting activity of thrombin, it does not inhibit esterase activity, thus showing a behaviour very similar to that with trypsin or plasmin4,7,9.

α₂M has no heparin co-factor activity; using purified human thrombin and fibrinogen we did not observe any immediate antithrombin activity when varying amounts of heparin were added to $\alpha_2 M$. To investigate this lack we submitted human serum to preparative ultracentrifugation. Progressive antithrombin activity was enriched at the bottom of the tube and heparin co-factor activity remained in the top layer.

We also prepared specific antibodies to human α2M from 80 ml. of rabbit anti-a₂M. The concentrated solution of the isolated antibody was added drop-wise to human serum until no more precipitate appeared. After removal of the precipitate progressive antithrombin and heparin co-factor activity were tested in the supernatant: the progressive antithrombin activity dropped (about 30 per cent) whereas co-factor activity was slightly greater than in the untreated material.

Methylamine (0.25 molar) splits $\alpha_2 M$ at pH 7.2, as shown by starch gel electrophoresis. This material shows neither antitrypsin, antiplasmin^{4,5} nor antithrombin activity, which can be restored by dialysis. The dialysed material, however, shows the characteristic double (or

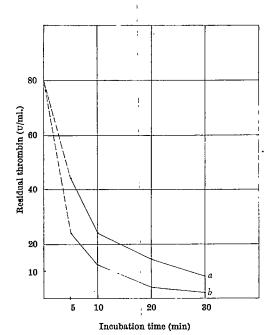


Fig. 1. Antithrombin activity of a_2M . a_1 , a_2M 0.2 per cent; b_1 , serum diluted 1:1.



Fig. 2. Starch gel electrophoresis (microtechnique). (1) Serum; (2) a_2M ; (3) a_2M and thrombin (200 v/ml.). The higher the thrombin concentration the more important is the splitting of the a_2M band.

triple) band of a2M treated with methylamine. Serum containing methylamine at neutral pH to a final concentration of 0.25 molar loses its progressive antithrombin activity—and the activity is only poorly restored by dialysis in the case of whole serum. We have thus found that three different methods—preparative ultracentrifugation of whole serum, an immunochemical method and use of methylamine as a specific inhibitor of α₂M—gave evidence that $\alpha_2 M$ is a natural progressive antithrombin. Heparin co-factor activity seems to correspond to another molecular support.

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Cyanopsin, a Visual Pigment of Retinal Origin

In 1953, Wald¹ reported in vitro synthesis of a new photosensitive pigment. This was made by combining the opsin obtained from extracts of chicken iodopsin2 with 11-cis retinene, in place of the native 11-cis retinene,. The pigment thus formed absorbed maximally at 620 nm instead of the native 562 nm, and was named cyanopsin. It had a number of properties which made it a good candidate for a visual pigment, although it had not been extracted directly from any retina and could not therefore be associated convincingly with the visual system of any animal. Such a pigment has yet to be extracted from a retina; but, in addition to discovering whether a pigment like cyanopsin exists in nature, and, in particular, in cones, it would be satisfying to observe whether there exists a natural iodopsin in situ to which the 620 pigment is related by a simple change of prosthetic group.

Speculation that the synthetic pigment cyanopsin would turn out to be a cone pigment has received some support from microinvestigations3,4; a spectrophotometric photosensitive pigment of λ_{max} of approximately 620 nm was found among the three goldfish cone pigments reported. The retinal changes accompanying metamorphosis of the frog, Rana pipiens, however, would seem to provide a better biological basis on which these matters might be tested, for at meta-morphosis the frog performs Wald's experiment in reverse. In this species, the retina changes at metamorphosis from that of the tadpole, which contained only retinene, to that of the adult, which contains only retinene. Extraction studies have already shown that metamorphosis is accompanied by a conversion from porphyropsin to rhodopsin (presumably in the rods) in the bullfrog, R. catesbeiana^{5,6}. This report describes the pigment content of retinal cones of R. pipiens before and after metamorphosis and compares these with pigments of turtle cones and the pigment pair iodopsincyanopsin Wald has investigated. The observations were made by single cell microspectrophotometry.

Eyes were removed from freshly guillotined 5 cm tadpoles without legs, previously dark adapted for more than 1 h, and, similarly, from adult frogs and turtles. Retinas were removed in 0.7 per cent saline and 1 mm² pieces were further reduced in size on a cover slip and closed with another cover slip for microscopy. These preparations provide

cells which are viewed side on. A microbeam measuring $1\mu \times 4\mu$ was passed through either cone or rod outer segments in the microspectrophotometer for the measurements described. The beam was polarized with its electric vector perpendicular to the long axis of the outer segment. All dissection and manipulations before the actual measurement of absorption spectra were carried out in infrared illumination with the aid of infrared image converters, to prevent bleaching of photosensitive pigments before measurement. Spectra of each cell were scanned automatically from 750 nm to 400 nm. The scanning time and band width of the instrument were previously adjusted to provide an acceptable signal to noise ratio without destroying an unacceptable amount of the visual pigment with the measuring photons. Although this objective becomes very difficult to achieve in the red region of the spectrum because of poorer photocathode quantum efficiency, we have used a photomultiplier of exceptional red response in our microspectrophotometer with satisfactory results.

Figs. 1 and 2 show that the microphotometric recordings of rods from tadpole and frog confirm the demonstration by extraction that porphyropsin is replaced by rhodopsin after metamorphosis. The λ_{max} of the absorption spectrum of a tadpole cone shown in Fig. 3 matches

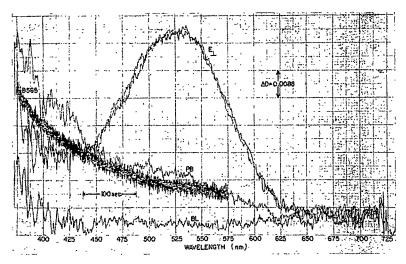


Fig. 1. Tadpole rod outer segment. E_1 , Two successive spectra scanned from red to violet. B 565, Kinetic curve of bleaching produced by steady illumination at 565 nm. PB, Spectrum immediately after bleaching. Note product spectrum absorbing in deep violet, presumed to be retinence. BL, Baseline. The 100 sec time scale applies only to kinetics. Spectra scanned at 30 nm/sec. ΔD , Calibration in optical density units.

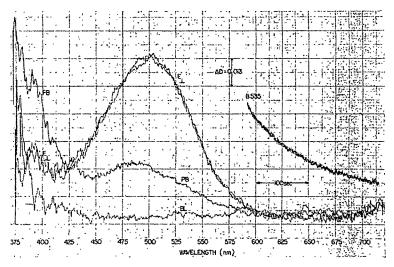


Fig. 2. Outer segment of frog rod. Symbols as in Fig. 1. Note product spectrum absorbing at shorter wavelength than in Fig. 1, presumably retinene₁.

closely that of the longwave goldfish cone as well as Wald's curve for cyanopsin. The absorption spectrum of adult frog cones (Fig. 4) differs slightly from that of chicken iodopsin in that its maximum absorption occurs at about 570 nm instead of at 562 nm. We have found only the 570 nm pigment in all oil drop containing frog cones, and, similarly, only the 620 nm pigment for the tadpole. In collaborative experiments with Dr A. M. Granda (unpublished work), we have found the cones of the freshwater tortoise, *Pseudemys scripta*, to contain the 620 nm pigment while the cones of the seawater turtle, *Chelonia mydas*, contain a 565 nm pigment. (The former uses retinene₂, the latter retinene₁, as the prosthetic group.)

Thus a pigment spectroscopically indistinguishable from cyanopsin has now been found directly in three families of animal—fish, amphibian and reptile—known to use retinene, as a visual pigment prosthetic group. Furthermore, it has been found in two of these that the comparable retinene, based cone pigment is spectroscopically close to or identical with iodopsin. Our results are not therefore inconsistent with Wald's speculation that a pigment like or identical to cyanopsin occurs in nature, that it is a cone pigment, and that the opsin from which it is derived may combine with retinene, to

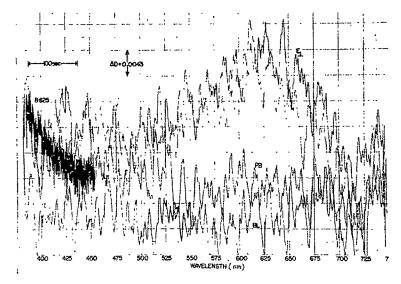


Fig. 3. Outer segment of tadpole principal cone.

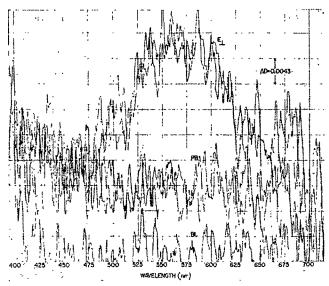


Fig. 4. Outer segment of frog principal cone.

form a functional cone pigment not dissimilar from iodopsin.

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Difference in Swelling and Opacity Formation between Young and Old Lenses

THE present theory of cataract formation in young galactose-fed animals and in young diabetic animals suggests that accumulation of dulcitol or sorbitol in the lens causes

it to swell and that this swelling leads to metabolic upset and opacity formation1,2. Patterson and Bunting³, however, have suggested that another metabolite, the nature of which is still unknown, contributes to swelling and opacity formation in the young lens of the galactose-fed rat. This idea is based on their finding that swelling is greater and opacity formation both quicker and more complete in the young rat lens than in the mature rat lens, although dulcitol accumulation is the same in both. It is true that the presence of another metabolite of galactose, besides dulcitol, could explain these observations, but it is also possible that differences between young and mature lenses could be caused by a physical difference in the elasticity of the lens capsule and fibre membranes rather than to any chemical difference.

To test whether elasticity was involved, lenses from rats of different ages have been immersed in distilled water and their change in weight, final wet weight

to dry weight ratio and protein loss have been measured. Swelling of the lens in the galactose-fed rat is brought about by the accumulation of one or more osmotically active substances within the lens. Swelling of the lens immersed in distilled water is brought about by reduction of osmotic pressure in the medium outside the lens: no metabolic processes are likely to be involved.

Table I shows that there is an even greater difference in swelling and degree of hydration between the young lens and the mature lens immersed in distilled water than there is between the lens of the young and mature galactose-fed rat. In one experiment, the increase of volume and of surface area of the lens was calculated, on the assumption that the rat lens is a sphere. The young lenses showed a 70 per cent increase in volume and a 44 per cent increase in surface area, while the lenses of the mature rat increased 46 per cent in volume and 29 per cent in surface area.

Exposure of the lens to distilled water is a drastic procedure and it was interesting to watch the progressive changes that took place in the two types of lens. In both, nearly all the uptake of water and increase in weight took place in the first 10–30 min. The suture lines became easily visible and over the next 6–8 h the centre of both young and mature lenses became progressively cloudy. By the next morning the two types of lens were noticeably different. The young lens had a dense white nucleus while the mature lens remained cloudy blue grey.

Table 1. SWELLING OF THE LENS OF RATS OF DIFFERENT AGES

(a) Lens immersed in distilled water								
Weight of rat (g)	Normal lens wet weight (mg)	Wet weight (mg)	Swollen lens (percentage increase)	Dry weight (mg)	Wet weight/ dry weight			
50-60 ,, 180 250-300	20 (3)* 20 (1) 20 (2) 29 (4) 45 (4) 45 (2)	34 36 33 49 61 62 65	70 80 65 68 35 41 44	5·8 5·1 5·3 10·4 17·6 16·5 17·3	5·9 7·0 6·3 4·6 3 4 3 7 3·8			

(b) Rats fed 35 per cent galactose in diet*							
Weight of rat (g)	Wet weight lens (mg)	Dry weight lens (mg)	Wet weight, dry weight				
75 250 300	27·8 34·1 39·1	7·5 12·8 15·8	3·7 2·66 2·47				

* The numbers in parentheses are the numbers of lenses.

The ratio of wet weight to dry weight of the lens falls from 2·62-2·34 with increasing age in the normal rat*. The lenses were removed by equatorial incision of the eye in situ, cleansed of tissue with a camel hair brush, weighed on a torsion balance and at once immersed in 1-2 ml. of distilled water for 20 h at 20° C.

Table 2. ESCAPE OF PROTEIN FROM RAT LENS OF DIFFERENT AGES INTO DISTILLED WATER

Weight lens mg	$\mu \mathrm{g/lens}$	Protein diffused into n $\mu g/mg$ wet weight (normal lens)	nedium µg/mg dry weight (swollen lens)
23 (2)	1.580	69	
19 (3)	1.140	60	194
20 (20)	890	44	
22 (2)	1,200	$\bar{54}$	266
21 (3)	1,090	52	
45 (4)	820	18	46
44 (4)	1.050	24	63

Conditions are as described in Table 1. The numbers in parentheses are the numbers of lenses. Protein was estimated by the bluret method of Robinson

Sippel⁴ examined the protein content of the lens in young galactose-fed rats and found that it decreased at about the time that the dense nuclear opacity appeared. This decrease coincided with appearance of protein in the fluids of the eye, probably indicating an increase in permeability of lens fibre membranes and capsule which are normally almost impermeable to lens proteins. Table 2 shows that protein escapes from lenses immersed in distilled water and that this is greater from the young than from the mature lens. The dry weight of the swollen lens is almost entirely protein and the loss from the young lens represents about 20 per cent and that from the mature lens about 5 per cent of the total protein. It is possible that the loss of protein is connected with formation of dense nuclear opacity in the young lens. Waley⁵ noted that a macerated lens remained transparent but that addition of fluid caused turbidity to develop. Charlton and van Heyningen⁷ have evidence that proteins of small molecular size may have been lost from the senile cataractous lens by leakage.

In swelling, in opacity formation and in capsular permeability the behaviour of the young and of the mature lens exposed to distilled water parallels the behaviour in vivo of the lenses of young and mature galactose-fed rats. It seems probable that the cause of the difference is the same in both cases and the simplest explanation of the difference in swelling is that the capsule and fibre membranes of the young lens are more elastic and more permeable than those of the mature lens. It is generally agreed that the human lens becomes less elastic with age as presbyopia develops.

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Incorporation of Sulphur-35 into the Subcommissural Organ and Reissner's Fibre

THE subcommissural organ (SCO) is a specialized area of the ependymal border below the commissura posterior and it occurs in the brain of all vertebrates. In most species the products of apical secretion from the SCO may pass, as does Reissner's fibre, through the ventricles of the brain and the central canal to the posterior end of the spinal cord^{1,2}. The function of the *SCO* is not yet known.

Histoautoradiographic investigations after the injection of **S-cystine (1.2-4.8 µc./body weight, partly repeated) have shown that 35S-incorporation of sulphur-35 into the SCO and Reissner's fibre in the carp (Cyprinus carpio L.) is greater than in most other organs (Figs. 1 and 2).

1. RELATIVE CONCENTRATIONS OF SULPHUR-35 IN THE BRAIN AND OTHER ORGANS OF CARP AFFER SEVERAL INCUBATION PREIODS Table 1.

Time of incubation (days)	Injected ac- tivity/g of body weight (μc.)	sco	RF	NS	σ	L	G	I
$^{2}_{14}$	4·75 4 × 2·5	5·5 2·6	6·4 5·7	3·4 2·3	2·2 2·0	4.0	6.8	8.3

RF, Reissner's fibre; NS, neurosecretory areas in the brain; C, cerebellum; L, liver; G, goblet cells of the midgut; I,β cells in the islets of Langerhans.

The relative concentrations of sulphur-35 in the different tissues were determined photometrically (Table 1) and the only structures with a greater cystine content than the SCO and Reissner's fibre were the goblet cells of the midgut and the islets of Langerhans. When the spinal cords of carp (weight, 530-750 g; brain and spinal cord, 15-20 cm) were fixed and investigated autoradiographically several days after the injections of sulphur-35 the activity in Reissner's fibre was found to have moved daily 2-3 per cent of its total length, giving an absolute measurement of about 0.5 cm/day. Thus it takes about 40 days for substances from the fibre to pass through the central canal of the spinal cord.

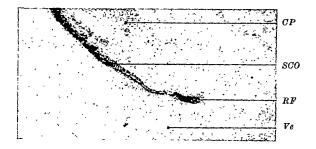


Fig. 1. Autoradiograph of unstained longitudinal section through the SCO and Reissner's fibre. (\times 50.) CP, Commissura posterior; SCO, subcommissural organ; RF, Reissner's fibre; Ve, third ventricle.

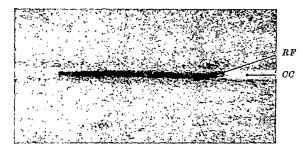


Fig. 2. Autoradiograph of a longitudinal section through the spinal cord with the central duct and Reissner's fibre. (\times 50.) RF, Reissner's fibre; CC, central canal.

These results show that there is intensive metabolism of sulphur containing substances in the SCO, and these must include the complex mucopolysaccharide-protein which has been demonstrated in Reissner's fibre system²⁻⁴.

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BIOCHEMISTRY

Pesticide-induced Enzyme Breakdown of Steroids in Birds

WIDESPREAD and severe decreases in the population of several species of birds of prey have been noted in both North America and Europe during the past decade1-4. It has been thought that these decreases are caused by pesticides, but concentrations of pesticide residues found are often low compared with a toxic dose5. evidence from laboratory studies that gross blockage of the reproductive system does not occur except at concentrations approaching toxic doses^{6,7}. More subtle effects on the breeding cycle caused by changes in hormonal concentrations and neurotoxic effects may be important. The effects of low doses of DDT (1,1,1-trichloro-2,2di(4-chlorophenyl)ethane) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene) on the metabolism of two steroid hormones, testosterone and progesterone, have been examined. It has been shown in several mammalian species that a wide variety of organo-chloride pesticides induce increased concentrations of hepatic enzymes which are capable of hydroxylating the natural steroid hormones^{8,8}. Birds were used in this work and the possibility of additive or synergistic effects of DDT and dieldrin was examined.

White King pigeons, which were about 1 yr old and weighed about 600 g, were used, females for the progesterone experiments and males for the testosterone experiments. DDT (10 p.p.m.) and/or dieldrin (2 p.p.m.) were fed to the pigeons for a week. The amounts ingested were approximately 5 mg of DDT and 1 mg of dieldrin. All pigeons were killed 4 days after the end of the pesticide treatment. The enzyme extraction procedure was essentially that of Conney and Klutch¹⁰. The liver was homogenized in cold 0·25 molar sucrose; nuclei, mitochondria and cell debris were removed by centrifugation at 9,000g for 15 min at 4° C. The microsomal fraction was obtained by centrifuging for 1 h at 105,000g at 4° C and the pellet was washed with 0·25 molar sucrose; 300 mg was then resuspended in 3 ml. of KH₂PO₄-K₂HPO₄ buffer (pH 7·4)

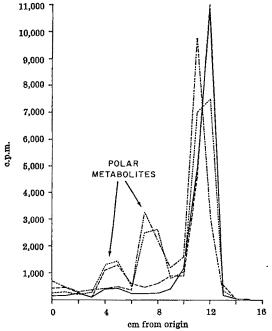


Fig. 1. Chromatographic separation of testosterone and its metabolites. The large peak is unaltered testosterone. Experimental conditions given in text. ..., DDT+dleidrin; ..., ..., dieldrin; ..., DDT; ..., DDT;

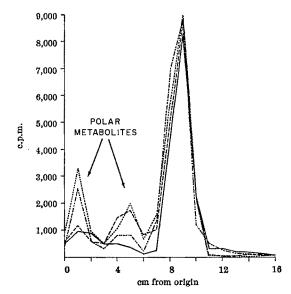


Fig. 2. Chromatographic separation of progesterone and its metabolites. The large peak is unaltered progesterone. Experimental conditions given in text. . . . , DDT+ dieldrin; — , dieldrin; — . . , DDT; — . , control.

by mild sonification for 5 sec. To this were added 500 mumoles of steroid (progesterone-4-14C, 36·1 mc./mmole or testosterone-4-14C, 29-2 mc./mmole), 3 mg of TPN (in 0.75 ml. of 0.05 mole tris), 14 mg of glucose-6-phosphate (in 0.5 ml. of 0.05 molar tris), 0.25 ml. of 0.05 molar MgCl₂ and 5 Kornberg units of glucose-6-phosphate dehydrogenase. The mixture was incubated at 40° C for 30 min with a slow stream of 95 per cent oxygen and 5 per cent carbon dioxide passing through the incubation flask. The steroids were extracted with 30 ml. of dichloromethane, evaporated to dryness under nitrogen and then dissolved in 1 ml. of methanol. The steroids were then separated by paper chromatography using the upper layer of cyclohexane, methanol and water (10:10:1) for testosterone and its metabolites, and decalin, nitromethane and methanol (2:1:1) for progesterone and its metabolites. Each chromatogram was cut into sections I cm long and each section was counted in a liquid scintillation counter using Bray's solution¹¹. Plots of c.p.m. against distance from the origin for the two systems are shown in Figs. 1 and 2. The amounts of metabolites formed were calculated from the areas under the curves, and are summarized in Table 1.

Table 1. INCREASE OF STEROID METABOLISM IN PIGEONS TREATED WITH DDT AND DIELDRIN

a	Amount of polar in mu	netabolites forme moles
_	Testosterone	Progesterone
Control DDT Dieldrin DDT+dieldrin	$28.7 \pm 4.7 (8)$ $75.4 \pm 18.0 (6)$ $111.4 \pm 12.7 (6)$ $168.2 \pm 9.9 (4)$	30·1 ± 8·4 (8) 78·3 ± 8·4 (6) 90·3 ± 6·1 (6) 155·4 ± 17·8 (4)

The mean and the standard deviation are given with the number of birds shown in parentheses. In all cases 500 m μ moles of labelled substrate was present, incubation time was 30 mln, and weight of microsomal fraction used was 300 mg.

The chromatographic patterns of both testosterone (Fig. 1) and progesterone (Fig. 2) after DDT and dieldrin stimulation show that the metabolites formed are different. The data suggest that different enzyme systems are induced by the two pesticides. Pretreatment of pigeons with both DDT and dieldrin gives rise to a curve with two metabolite peaks. The sizes of the peaks are similar to those produced by the individual pesticides alone. Thus it appears that the effects of the two pesticides are additive but not synergistic.

The experiments demonstrate that relatively small amounts of DDT and dieldrin can induce increased rates of metabolism of steroids by induction of hepatic enzymes.

The relationship of elevated steroid metabolism in the liver to the concentrations of circulating steroids has not been established. DDT has been used to decrease concentrations of cortisol in patients suffering from Cushing's syndrome¹² and to perform "chemical adrenalectomy" in the treatment of carcinoma of the breast and prostate13. At least in these abnormal conditions it is possible to alter steroid concentrations by the use of chlorinated hydrocarbons. The effect of induced enzyme metabolism has been demonstrated by in vivo experiments on the metabolism of circulating phenobarbital in the rat. The duration of barbital hypnosis was reduced from 81 min in the control animals to 39 min in rats given 2 mg/kg of DDT intraperitoneally 48 h earlier⁸. In this case, a relatively small dose of insecticide had a profound influence on the effects of a circulating material. There is evidence to suggest that the same enzyme systems are responsible for steroid and barbital metabolism9. It is possible that the feed-back mechanism through the hypothalamus and the anterior pituitary might be able to maintain the normal concentrations of steroids. Radcliffe's14 observation of the decrease in weight in recent years of the eggshell of some birds of prey could be explained by increased metabolism of oestrogen induced by hepatic enzymes. Many studies indicate that overcrowding imposes a stress on animals and causes reduced secretion of gonadotrophic hormones which affects the reproductive organs^{15,16}. If normal regulation of populations depends on a subtle balance of hormone concentrations and related feed-back mechanisms associated with population density, it would not be surprising to find that agents affecting this balance cause declining populations.

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Three Lactic Dehydrogenase Isoenzyme Systems in Pig Spermatozoa and the Polymorphism of Sub-units controlled by a Third Locus C

ELECTROPHORESIS usually reveals five lactic dehydrogenase (LDH) isoenzymes in vertebrate tissues. Each isoenzyme is considered to be a tetramer, usually composed of two sub-units, polypeptide monomers A (LDH₅) and B (LDH₁). Genetic evidence¹⁻³ indicates that synthesis of the polypeptides is controlled by two loci, A and B. In testis and spermatozoa from different animals, with the exception of pigs4,5, six to eight fractions were

The extra bands were named x-bands. discovered. Zinkham and Blanco⁶ found that the LDH_x-band in pigeon testis is polymorphic, and concluded that this is controlled by a third locus C. Ressler et al. suggested that LDHx-band in human spermatozoa is formed from A and B sub-units and some other compound. Zinkham et al.4 and Goldberg5 found that in vitro sub-units of LDH_x can produce mixed forms with sub-units A and B.

In pig spermatozoa, we have found evidence for a third locus C with two different alleles. The polymorphic sub-units C and C' form in vivo a new LDH isoenzyme system with sub-units A and B. Furthermore, a third system, which probably does not involve A, B and C sub-units, was found.

Pig spermatozoa from the tail of the epididymis⁸ were washed with an isotonic glucose solution or 0.9 per cent sodium chloride, and centrifuged (3,000-4,000 r.p.m.) for 15 min. Redistilled water or tris citrate buffer (pH 7.0) $(\mu = 0.05)$ was added in a volume half that of the spermatozoa, and one part was homogenized immediately in a Potter-Elvehjem homogenizer at 0° C and another part was frozen (-20° C) and thawed once. Both types of samples were centrifuged (15,000 r.p.m.) at 4° C for 1 h. The supernatant was used for electrophoretic separation. Both methods gave the same number of isoenzymes, which indicates4,9 that no new spots were produced by this technique.

For electrophoretic separation, a 10 per cent starch gel¹⁶ in 0.015 molar citric acid and 0.05 molar tris (pH 7.2) was used. Vessel buffer consisted of 0.063 molar citric acid and 0.42 molar tris (pH 8.6). The voltage gradient was 4.5 V/cm and electrophoresis lasted about 20 h at 4° C. Staining of isoenzymes was carried out by the Wieme method¹¹ for agar gel zymograms.

Extract of spermatozoa contained the same five isoenzymes which are found in tissues. In each sample investigated, one of three different types of a second LDH isoenzyme system was discovered (Fig. 1a and b). The two types (I and II) are usually composed of five different spots. As Fig. 1 shows, the two types differ in electrophoretic mobility. Type III is composed of several spots with an intermediate mobility. The spot in medium of types I or II (the homotetramers, C4 or C4') usually has the greatest activity and the activities of the other spots decrease markedly towards LDH1 and LDH5.

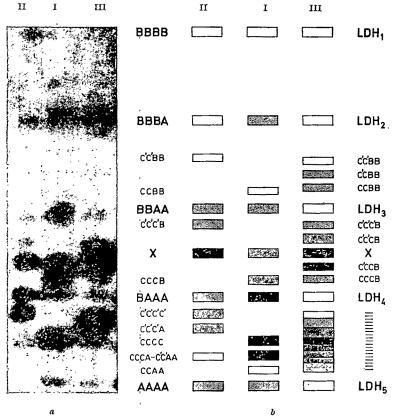
The relative mobility rate of the new system to the LDH₁₋₅ system is decreasing when either ionic strength is lowered or pH is increased in the gel buffer. When different starch gel concentrations (8-16 per cent) are used, and all other conditions are the same, the relative These findings not only mobilities are unchanged. indicate that the extra spots are different from isoenzymes of the LDH₁₋₅ system but also that the mole-

cular size is probably very similar. When more than 5 μ l. of a 50 per cent 2-mercaptoethanol solution is added to 0.1 ml. of samples of types I and II just before electrophoresis, all spots, except the spots C_4 and C_4 , are gradually inhibited (Fig. 2). When 25 μ l. is used, only C_4 and C_4 may be visible and for type III only five spots persist in the area between C4 and Ca

Results of heat inactivation showed that isoenzymes composed of C or C' sub-units are relatively thermostable and when samples are heated for 1 h at 60° C before electrophoresis only these fractions are visible. These findings indicate that the isoenzymes, which are not inhibited, are controlled by a third locus C and are of a tetrameric structure.

Table 1. THE DISTRIBUTION OF TYPES I, II AND III OF A SECOND LDH ISOENZYME SYSTEM FOUND IN BOAR SPERMATOZOA

Phenotypes	I	II	III	Total
No.	32	15	44	91
Percentage	35-3	16-5	48·2	100



second LDH isoenzyme system in boar spermatozoa. b, Schematic picture. Fig. 1. a, Three types of a

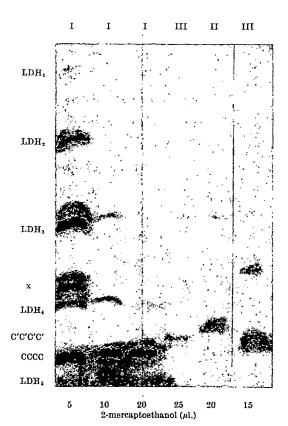


Fig. 2. Inhibition of LDH isoenzymes by using different amounts of 2-mercaptoethanol.

When a recombination technique is used4,5,9,12 involving the addition of a skeletal muscle extract, the activity of homotetramers and isoenzymes from the LDH₁₋₅ system decreases, and spots situated towards LDH, increase, and, furthermore, an extra spot is formed in front of LDH₅. A similar phenomenon can be seen on the opposite site of the homotetramers, when heart muscle extract is used. The same trend was also seen when the heterozygote type (III) was used.

The relative mobilities, number of spots in types I, II and III (see Fig. 1b) and the formation of extra spots when recombination is used, are all in agreement with the hypothesis that isoenzymes of the new system are tetramers composed of sub-units of C, C+B or C+A. The frequency of the polymorph types is relatively high (see Table 1).

A preliminary investigation of the genetic basis of the system has been carried out and so far we have found no evidence against our claim that the different types are genetically controlled.

The behaviour of band x (see Fig. 1b)

differs in some respects from that of the two LDH systems. The mobility of this fraction is the same in all three types (Fig. 1). Activity is not much changed when the recombination technique is used. When phenazine methosulphate is omitted from the staining solution and zymograms are subjected to 37°C for 12 or more hours, a pink spot is seen

in the position of band x. A similar pink spot can be seen in the area of LDH₃, and this spot is relatively stable when butanol is used for inhibition. In these two respects, this x-band shows a behaviour similar to the x-band in rat kidney^{13,14}. When samples are subjected to 60° C for 30 min or when 15 µl. of a 50 per cent solution of 2-mercaptoethanol is added to a 0.1 ml. sample, only the two spots and homotetramers controlled by the Clocus are not inhibited. These facts seem to indicate that these fractions are part of a third system.

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In vivo Interaction of 4-Nitroquinoline-I-oxide and its **Derivatives with DNA**

4-NITROQUINOLINE-1-OXIDE (4NQO) is a highly carcinogenic compound. The reductive pathway, which yields 4-hydroxylaminoquinoline-1-oxide (4HAQO), was thought to be essential for its carcinogenic mechanism^{2,3}. There have been reports of the interaction of 4NQO or 4HAQO with DNA in vitro4-8 and in vivo7. This communication deals with the correlation between carcinogenicity and in vivo interaction with DNA by 4NQO derivatives.

4NQO or its derivatives (1 mg/rat) was injected intraperitoneally into Donryu rats bearing Yoshida ascites hepatoma AH 130. One hour later, the hepatoma cells were collected and DNA was extracted by the method of Kay et al.8.

Fluorescence with maxima at 465-475 mu when excited at 365 mµ was observed in DNA isolated from hepatoma cells of rats injected with carcinogenic 4NQO, 4HAQO or 6-chloro-4-nitroquinoline-1-oxide (6-Cl-4NQO), but not from hepatoma cells of rats injected with non-carcinogenic 4-aminoquinoline-1-oxide (4AQO) or 3-methyl-4-nitro-quinoline-1-oxide (3-CH₃-4NQO). The radioactivity in DNA isolated from hepatoma cells of rats injected with 1 mg of $^{14}\text{C-4NQO}$ (3×10^5 c.p.m./mg) shows that 1.7 moles of quinoline compound was bound with a mole of DNA, if the molecular weight of DNA is 6.7×10^8 (Table 1).

The fluorescent compound in DNA was not eliminated from single strand DNA by heating and rapidly cooling native DNA (Table 1), which suggests that there is a covalent bond between the quinoline compound and the DNA molecule.

Table 1. Fluorescences of native and heat-penatured dnas from AH 130 treated $in\ vivo$ with 4ngo and 7ts derivatives

Injected	Carcinogenicity	Fluorescence (relative intensity/ E_{zee})		
		Native	Heat-denatured	
4NQO	+	10.4	10.1*	
4NQO	+	14.3	13.3†	
14C-4NQO	+	11.8		
4HAQO	+	15.3	15-8*	
6-Cl-4NQO	+	19.0	-	
4AQO	<u> </u>	Ō	0*	
3-CH ₂ -4NQO		Õ		
Salina		ň	n•	

* DNAs isolated by ethanol precipitation. † DNAs isolated on a column of 'Sephadex G-25'.

The reductive pathway to yield 4-hydroxylamino derivatives seems to be necessary to form fluorescent compound in DNA and exert carcinogenic action, because 3-CH₃-4NQO, which cannot be reduced by an enzyme, did not produce fluorescent compound. The more reduced compound 4AQO, which is not carcinogenic, did not produce fluorescence in DNA.

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Isonicotinic Acid Hydrazide as an Inhibitor of Transpeptidation: Relevance for Blood Coagulation

WE have found that isonicotinic acid hydrazide, a widely used drug1, is a potent inhibitor of the crosslinking of This reaction constitutes the terminal step in normal blood coagulation and proceeds by way of an enzyme catalysed transpeptidation by forming inter-fibrin γ -glutamyl- ϵ -lysine bonds^{2,3}. Compounds which resemble the donor (amine) or the acceptor (carbonyl) side-chain functions of fibrin specifically inhibit the crosslinking reaction without, however, interfering with gel formation -that is, clotting time—itself.

Table 1 Type of label and ref. Amine tracer Carbon-14 isotope^{4,5} Chemical⁵ Glycine ethyl ester Hydroxylamine
Hydrazine
N-(5-aminopentyl)-5-dimethylamino-Chemicals Fluorescences

The compounds listed are sufficiently different in the basicity (nucleophilicity) of amines and in the substituent groups to permit the conclusion that most, if not all, amine inhibitors $(H_2N.R)$ would block the crosslinking sites of fibrin in a similar manner

$$H_2N \cdot F \cdot CO \cdot Y + H_2N \cdot F' \cdot CO \cdot Y \xrightarrow{\theta} H_1N \cdot F \cdot CO \cdot NH \cdot F' \cdot CO \cdot Y + HY$$
 (1)

$$H_2N \cdot F \cdot CO \cdot Y + H_2N \cdot R \xrightarrow{e} H_2N \cdot F \cdot CO \cdot NH \cdot R + HY$$
 (2)

where reaction (2) competes with the crosslinking process (1), shown for dimerization by a single amide bond (k and k' represent fibrin; e stands for the crosslinking enzyme, that is, the thrombin-activated fibrin stabilizing factor."; Y is the leaving group in transpeptidation).

Highly potent inhibitors were found among primary amines, aminoxy compounds and hydrazine derivatives. Apart from usefulness in elucidating the mechanism of crosslinking, such inhibitors may be of pharmacological significance. Thrombi formed in the presence of these compounds are very much more susceptible to attack by lytic enzymes.

Details are available of the mode of action of the donor type of inhibitors. Four of these (Table 1) were shown to serve as substrates for the crosslinking enzyme. As such, they could be incorporated into fibrin and were, in fact, indispensable for determining the nature of acceptor and donor sites on the protein.

Isonicotinic acid hydrazide

1-naphthalenesulphonamide

$$\left[R' \cdot \text{CO} \cdot \text{NH} \cdot \text{NH}_2; \right] = R'$$

is the most potent inhibitor found so far. In the isolated bovine fibrin crosslinking test system (Fig. 1), its inhibitory effect may be readily demonstrated even in concentrations close to those obtained in plasma during the pharmacological administration of the drug1.

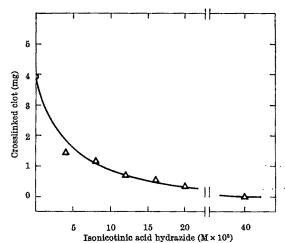


Fig. 1. Inhibition of fibrin crosslinking by isonicotinic acid hydrazide (Calbicohem, lot 105421). Ordinate denotes the amount of fibrin which cannot be dissolved in 1 per cent monochloroacetic acid. For detailed methodology see refs. 4 and 14.

There are clearly several ways in which isonicotinic acid hydrazide could exert an inhibitory effect on fibrin crosslinking, but only a few possibilities will be indicated here, pertaining to the assumption that it serves as a transpeptidating partner. Isonicotinic acid hydrazide might react either as a pseudodonor or as a pseudoacceptor substrate with fibrin, for example

 $R' \cdot CO \cdot NH \cdot NH_2 + H_2N \cdot F \cdot CO \cdot Y$ $H_*N \cdot F \cdot CO \cdot NH \cdot NH \cdot CO \cdot R' + HY$ $R' \cdot CO \cdot Y + H_2N \cdot F \cdot CO \cdot NH \cdot NH_2$

 $R' \cdot CO \cdot NH \cdot F \cdot CO \cdot Y + NH_{\bullet} \cdot NH_{\bullet}$

Future work is planned to decide which, if any, of these possible reactions might have occurred. Meanwhile, preliminary experiments indicate that isonicotinic acid hydrazide also has an effect on reactions involving other transamidating enzymes—for example, guinea-pig liver transglutaminase⁸ and a lobster tail muscle enzyme^{10,11}. A general effect of this sort raises the question whether the antitubercular activity of the drug might not be related to inhibiting a critical transpeptidase reaction in mycobacterium. It is interesting to note that another antibiotic, penicillin, functions through inhibition of a cell wall transpeptidase12,13.

This work was aided by a US Public Health Service research career award and by grants from the American Heart Association, and the National Heart Institute, US National Institutes of Health. L. LORAND A. JACOBSEN

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Rate of Release of Iron from Ferritin to 1,10-Phenanthroline

Although the uptake and release of iron from ferritin, the principal form in which iron is stored by mammals, have been examined qualitatively and the rate of uptake has been studied previously2, there have been no previous studies of the rate of release of iron. We report here the results of a study of the rate of in vitro release of iron from ferritin in the presence of 1,10-phenanthroline. The effects of ascorbic acid, as well as concentration and temperature, were determined.

Thermostatically controlled solutions of ferritin (cadmium free) and 1,10-phenanthroline were mixed with care to avoid frothing or excessive shaking of the solutions. The mixtures were then returned to the constant temperature bath and the absorbance at 510 mµ was measured as a function of time. The solutions of iron(II) 1,10-phenanthroline complex which are formed, obey Beer's law for the concentrations studied $(1 \times 10^{-5} \text{ to } 10 \times 10^{-5})$ molar) with an absorbancy of 1.15 × 104. The reactions were followed until the absorbances were greater than 2. This represented a release of 12 per cent of the iron from ferritin and required a minimum of 60 days at 25° C for the systems of lowest ferritin concentration. pH was measured periodically and showed a very slight increase during most runs but remained in the range 7.65 ± 0.30 . Vigorous shaking of the ferritin solutions accelerated the release of iron.

Data were obtained in the form of the concentration of released iron as a function of time. The total amount of iron was known from an analysis of the ferritin used. The absorbance increased only to a point at which about 12 per cent of the iron was released, and this apparently represents an equilibrium state. The logarithm of the amount of iron remaining in the ferritin was plotted against time. An example is shown in Fig. 1. There is an initial portion of the reaction which corresponds to the rapid release of a small amount of loosely held iron followed by the slower release of a much larger amount. It was concluded that the rate law for the release of iron from ferritin was

$$\frac{\mathrm{d[Fe]}}{\mathrm{d}t} = k[ferritin iron]$$

where [Fe] represents the concentration of iron in the solution and [ferritin iron] represents the amount of iron remaining in the ferritin.

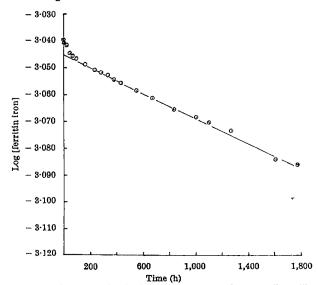


Fig. 1. First order plot for the reaction of 1,10-phenanthroline with ferritin at 25° C. Log [ferritin iron] is the logarithm of the concentration in moles/l. of Iron which remains with ferritin and is uncombined with 1,10-phenanthroline at time t. Initial concentrations: 1,10-phenanthroline (0.0063 molar); ferritin (0.000914 moles of Iron/l.).

The rate constants for the release of iron from ferritin in the presence of 1,10-phenanthroline are given in Table 1. Average values of the rate constants at various temperatures were used to obtain the activation energy for this process, which was 13 ± 2 kcal/mole. The frequency factor was found to have the extremely low value of about 10¹ sec-1. In solutions where the initial concentration of 1,10-phenanthroline was varied twenty-five fold and the initial concentration of ferritin was kept constant, the rate constants showed no change within the experimental error. This requires a mechanism which is independent of the concentration of the 1,10-phenanthroline. It is probable that the rate determining step is the slow release of iron from micelles in the interior of the ferritin macromolecule and its transport to the surface of this species. In this case it is followed by two much more rapid reactions: the complexing of the iron(III) by the 1,10-phenanthroline and the photoreduction of this to the iron(II) complex3. The rate of this photoreduction is more than thirty times greater than the rate of the ferritin-1,10-phenanthroline reaction at 25° C. This explains why the reagent is so effective in removing iron from ferritin as well as the fact that the overall reaction is zero order in this reagent.

A mechanism for this process which is consistent with the data obtained is as follows

iron (III) in phosphate slow iron (III, II) at 1,10-phenanthroline micelles in ferritin → surface of ferritin in releasable form

iron (II) complex with 1,10-phenanthroline.

The rate of release of iron is greatly increased by the presence of ascorbic acid (compare Table 1).

Table 1. First order rate constants for the release of iron from ferritin system

		W-2250-2-0		
Tem- perature °C	Initial ferritin (moles of iron/l. × 104)		Initial 1,10- phenanthroline (moles/l. × 10 ⁴)	$k \times 10^4$ h ⁻¹
25.0	4-89 9-14 14-66 18-28 27-42		63	0.28 0.53, 0.56, 0.47 0.53 0.34, 0.29, 0.30 0.32
37.5	4·89 9·14 14·66		63	Mean 0.40 (±0.11) 1.21 1.33, 1.04 0.97, 0.88
50.0	4·89 9·14 14·66		63	Mean 1·1 (±0·1) 0·79 1·99, 4·48, 1·82 1·49, 1·85
25.0	9·14		2·5 12·6 25·0 37·8 50·4	Mean 2.1 (±0.8) 0.48 0.29 0.35 0.27 0.31
37·5 25	9·14 4·89	0·03 0·06 0·48 0·60	12·6 63	Mean 0.34 (±0.06) 1.49 0.63 2.52 16.0 24.3

A plot of the rate constants of Table 1 against the concentrations of ascorbic acid is roughly linear, indicating that the reaction in this case has the rate law

rate =
$$\frac{-\text{d[ferritin Fe]}}{\text{d}t}$$
 = k[ferritin Fe] [ascorbic acid]

These results provide detailed expressions for the rate of release of iron from ferritin which are consistent with the known properties of this species. They also provide a method for measuring this release which can be used in other quantitative rate studies of this process.

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Radiotracer Determination of Supernatant Liquid entrapped among Centrifuged Red Blood Cells

WE are conducting experiments to measure the rate of permeation of glycerol through the red blood cell membrane. The procedure involves mixing glycerol solutions with red blood cells and letting them stand for different periods of time, then centrifuging the mixtures to separate supernatant glycerol solution from the red blood cells containing glycerol. The red blood cell fractions are then analysed chemically for glycerol content. The question arises as to how much glycerol solution is entrapped among the red blood cells after centrifugation. The answer to this question would have some effect on the results of analyses for glycerol in the red blood cells with subsequent effects on the permeation rates; a knowledge of the amount of extracellular glycerol will enable us to make proper corrections. The present experiment was carried out to determine the percentage of extracellular glycerol.

The experimental procedure involves mixing a radioactive carbon-14 sucrose solution with packed red cells. A count of this original solution is taken. Sucrose does not penetrate the cell wall. The mixture is then centrifuged, and the supernatant solution is removed. An equal mass of non-radiactive sucrose solution is added to the red cells and another count taken. The geometries of the original and final solutions are similar; this ratio of the final count to the original count gives a measure of the fraction of supernatant liquid entrapped among the red

In our first experiment, a 4.0 g sample of packed red cells in a 15 ml. test tube was mixed with 4.0 g of a 7 per cent sucrose solution containing carbon-14. The specific gravity of the red blood cells is about 1.09 (ref. 2). The specific gravity of the 7 per cent sucrose solution at 60° F is 1.03, about the same as blood serum. sample (1 g) of this mixture was counted with an endwindow Geiger counter. The net count, averaged for a 10 min period, was 4,293 c.p.m. The mixture was then centrifuged in a clinical model centrifuge at 3,300 r.p.m. (top speed) for 10 min. The centrifugal force was 1,720 times the force of gravity.

Next, the supernatant sucrose was removed, together with the uppermost portion of the red blood cells, by aspiration. The remaining red cells were weighed and an equal mass of non-radioactive sucrose was added. This mass was then mixed for 1 min. A sample (1 g) was removed and counted. The net count, averaged for 10 min, was 339 c.p.m. From these data, the amount of sucrose entrapped among the red blood cells after centrifugation is calculated to be 7.9 per cent.

This experiment was repeated. The radioactivity of the original mixture, averaged for a 10 min span, was 4,658 After centrifugation, removal of supernatant liquid and dilution, the radioactivity was 334 c.p.m. In this second experiment, the amount of sucrose entrapped among the red blood cells after centrifugation was 7.2 per cent. The average of the two experiments is 7.6

A test was made to evaluate the possibility that some sucrose had permeated the red cell wall, changing the meaning of the results. The final blood and sucrose solution from the first experiment was diluted with a large amount of non-radioactive sucrose. If the radioactive sucrose were inside the red cell, the dilution and subsequent removal of supernatant would not substantially reduce the radioactivity of the red cells; the radioactivity would remain at approximately 339 c.p.m. On the other hand, if sucrose was entirely extracellular, the dilution, centrifugation, removal of supernatant and redilution to 1:1 should reduce the radioactivity substantially. It was found that the radioactivity was reduced to a very small

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reading, just slightly above background, after these steps were taken. It is concluded, therefore, that sucrose does not permeate the red cell wall.

Thus centrifugation of blood at top speed in a clinical laboratory centrifuge for 10 min can leave as much as 7.6 per cent of the supernatant liquid entrapped among the red blood cells. Sucrose does not permeate the red blood cell wall.

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Isoenzymes of Phosphoglucose Isomerase in Mice

A PROCEDURE has been developed for the visualization of the glycolytic enzyme, phosphoglucose isomerase, after electrophoresis in a starch gel. The staining mixture applied to the sliced gel surface consists of fructose-6phosphate (free of glucose-6-phosphate), glucose-6-phosphate dehydrogenase, NADP, phenazine methosulphate and the tetrazolium salt MTT. The formation of glucose-6-phosphate by the isomerase is coupled to the reduction of the tetrazolium dye, which turns blue.

No heterogeneity has yet been observed for phosphoglucose isomerase in human haemolysates, but variant isoenzymes were found when haemolysates from a number of wild mice (Mus musculus) were examined, and a similar type of polymorphism was also encountered in haemolysates and muscle extracts from various strains of laboratory mice. Inserts (a) and (c) in Fig. 1 are from a pair of wild mice, and it can be seen that insert (a) gave a single enzyme band migrating significantly nearer to the anode than the single more cathodically migrating band seen in insert (c). When the mice of this pair were crossed they produced progeny of both sexes, all of which gave triplet pattern shown in insert (b); the most anodically migrating band of the triplet was indistin-

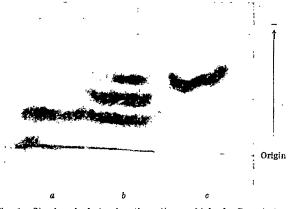


Fig. 1. Starch gel electrophoretic patterns obtained after electrophoresis of mouse haemolysates and specific staining for phosphoglucose isomerase. Haemolysates were made and electrophoresis was carried out as described befores, but metal cooling plates were used with a potential difference of 20 V/cm for a period of 4 h. The staining mixture applied to the gel surface was made by mixing 0.3 molar tris buffer at pH 8·0 (1·5 ml.), 0·1 molar magnesium chloride (0·5 ml.), 18 mmolar sodium fructose-6-phosphate (0·2 ml.), 6 mmolar NADP (0·1 ml.), purified glucose-6-phosphate dehydrogenase (0·2 ml. of a solution containing 10 V/ml.), MTT (1 mg) and phenazine methosulphate (0·5 mg), just before use. It is important for the fructose-6-phosphate to be free from glucose-6-phosphate, and in this laboratory it is prepared as needed by the acid hydrolysis of crystalline trisodium fructose diphosphate. Inserts (a) and (c) contained haemolysates from a breeding pair (see text), and insert (b) contained haemolysate from one of the progeny.

guishable in mobility from that appearing on its own at insert (a); the most cathodically migrating band was likewise indistinguishable from the one appearing on its own at insert (c), and the third band of the triplet had intermediate mobility. This situation, which is analogous to that found for the enzyme phosphogluconate dehydrogenase¹⁻⁴, and for certain other enzymes⁵⁻⁷, is consistent with a dimeric structure for the enzyme phosphoglucose isomerase, and indicates that this enzyme in mice is likely to be controlled by an autosomally linked locus.

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Fractionation of Protein Solutions by Membrane Partition Chromatography

HIGH flux preformed ultrafiltration membranes, serving as diffusive barriers to the transport of macromolecular species, have been found to be extremely useful for: the non-denaturant concentration of dilute protein solutions1; as a practical means of rapid dialysis and evaluation of micro-ion binding phenomena; for separating the products of proteolysis during digestion (unpublished work of Blatt, Robinson, Zipilivan and Hudson); and in the determination of lower molecular weight moieties2. These membranes are hydrous gels formed from the complex interaction product of polyanions and polycations and are marketed under the name of 'Diaflo' by the Amicon Corporation, Cambridge, Mass. The molecular weight retentive capacities of these membranes, 'UM-2' and 'UM-1', have been arbitrarily fixed at 500 and 10,000, respectively. In a recent study, a gel membrane with a more expanded structure (exclusion limit molecular weight 35,000) was investigated as a partitioning device in the removal of the albumin impurity from crystalline alpha lactalbumin3. Whereas these membranes are non-porous hydrous gels, ultrafilters with exclusion limits in excess of 40,000 require pored structures for formulation.

Five membranes (arbitrary molecular weight exclusion limits in parenthesis) were evaluated for their protein retentive properties: (a) 'XM-100' (100,000); (b) 'XM-50' (50,000); (c) 'XM-4a' (35,000); (d) 'UM-1' (10,000) and (e) 'UM-2' (500). A modified ultrafiltration cell', similar to that used for the evaluation of proteolysis, was used (Fig. 1). In this system, a non-pulsatile buffer flow is established and the ultrafiltrate is passed through the ultraviolet monitoring system until baseline stability is achieved. The pump and ultraviolet analytical system are components of the 'Spectrochrom' apparatus, Beckman Instrument Co., Fullerton, California. After equilibration the protein solution is injected into the sampling valve and the absorbance of the ultrafiltrate recorded at 280 mu. In all studies, 1 ml. of 1 per cent protein solutions,

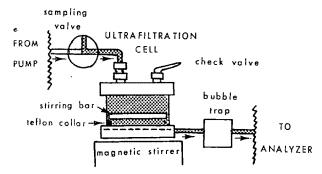


Fig. 1. Constant flow ultrafiltration cell (16.5 ml. capacity) with buffer and sample introduction valve, ultrafiltrate egress port, bubble trap and entry to the ultraviolet analytical system. The dashed line above the stirrer denotes the ultrafiltration membrane.

in phosphate buffered saline, pH 7·4, were first injected into cells without membranes and then through each membrane system (vide supra). The amount of protein is calculated from the differential peak areas of the ultrafiltrates relative to the area of the non-membrane system. With bovine serum albumin (Fig. 2), no retention was observed when a filter with a limit of 100,000 was used. With the 'XM-50' filter, however, there was considerable retention of albumin.

The retentive capacities of the different ultrafilters for a variety of proteins are summarized in Table 1. Because many of the commercial preparations contain impurities which could partially invalidate the results, we selected protein standards which have been used in the determination of molecular weights by gel diffusion chromatography. The heavy line serves to bracket the principal exclusion range (>60 per cent retention). From the data, it is apparent that the selected exclusion limits, based upon other model compounds, are only arbitrary and true characterization of retention entails more than the gross molecular weight. In a further series of studies using polyethylene glycols of graded molecular weight, complete diffusivity through the membranes was observed for molecules far in excess of the limits stated. This passage we attributed to the linear structure of the polymer, which makes it possible for properly oriented molecules to pass through the membrane structure. The limited diffusivity of essentially uniform proteins (for example, albumin and haemoglobin on the 'XM-50' filter) may therefore be caused by a portion of the protein being affixed to the surface or entrapped within the membrane matrix thus altering its retentive capacity.

On the basis of the observed exclusion limits, a tandem assembly was devised consisting of three of the ultrafiltration units shown in Fig. 1 placed in series, each with

a progressively lower exclusion filter (50,000: 10,000:500). In evaluating this system, 80-100 mg of the following mixtures in phosphate buffered saline, pH 7.4, was used: (a) albumin, cytochrome c, cyanocobalamin (vitamin B₁₂), and phenylalanine; (b) aldolase, ovalbumin, cytochrome c and cyanocobalamin. These mixtures were introduced into the upper cell (the other cells were previously filled with buffer) and a buffer reservoir attached. Nitrogen pressure (50 p.s.i.) served to pump buffer through the fluid filled system to effect parti-With these mixtures, flow rates of 10-15 ml./h were achieved. Separations were done at 5° C using total ultrafiltration volumes of 150-175 ml. The final ultrafiltrate (<500) was concentrated by flash evaporation; material found in each of the cells was reduced to 3 ml. by concentration on its own filter. These fractions were then evaluated by reverse flow chromatography on 'Sephadex

 $G\text{-}100^\circ$ (1·0 × 22 cm column) using the 'Spectrochrom'. Admittedly, a longer column would have afforded better resolution, but in the interest of easy handling the smaller column was used. The partition schema and the chromatographic analysis of starting materials contrasted with the filter separated fractions are shown in Figs. 3 and 4. A transition of high to lower molecular weight species was accomplished by the graded retention filters. Furthermore, these separations were in accord with what had been predicted on the basis of the single system evaluation (Table 1). Diffusivity of a given protein through these membranes does not seem to be altered by using a protein mixture.

Table 1. PROTEIN RETENTIVE CAPACITY OF SIZE SELECTIVE MEMBRANES

		Percentage retention				
	'XM-	'XM-	'XM4-	'UM-	UM-	
			a'	1'	2'	
weight	(100,000)	(60,000)	(35,000)	(10,000)	(500)	
480,000	100*	100				
160,000	100*	1.00				
142,000	0	85	100	100	100	
67,000	0	68	100	100	100	
64,500	0	622	70	100	100	
45,000	0	77	98	100	100	
25,000	0	0	0	90	100	
24,500	0	0	0	95	100	
20.000	0	0	0	95	100	
17,800	0	0	0	88	100	
12,400	0	0	0	85	100	
1,355	0	0	0	0	0†	
165	0	0	0	0	0	
	160,000 142,000 67,000 64,500 45,000 25,000 24,500 20,000 17,800 12,400 1,355	lar weight (100,000) 480,000 100* 160,000 100* 142,000 0 67,000 0 45,000 0 25,000 0 24,500 0 24,500 0 27,800 0 17,800 0 1,355 0	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	

Values in parentheses represent arbitrary cutoff limits ascribed to the various membranes.

* Membrane plugging was observed with these compounds as evidenced by a considerable increase in pressure during ultrafiltration. This was not observed in the non-porous membranes (35,000 and less).

† With the other compounds, diffusivity, where present, was effected at a rate comparable with the clearance observed in a non-fliter system. With cyanocobalamin, however, a slow escape was noted and, given sufficient time, complete passage through the membrane was obtained.

Systems using the 'UM-100' filter with different mixtures have been evaluated, but with less success. The larger molecular weight materials (for example, gamma globulin and apoferritin) show a tendency to plug the filter when used for protracted periods, and, as such, interfere with passage of material usually diffusible through these membranes. If charge sites could be included within the structure of the membrane, selective rejection of gamma globulin with admission of the species of different charge and of lesser size might be effected. In addition, because these membranes can be tailored to different exclusion limits, tandem systems can be devised for the separation of proteins more similar in molecular weight than those selected for evaluation.

The chief advantage of this ultrafiltration technique is its ability to resolve complex mixtures into size graded

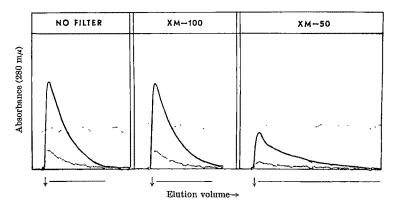


Fig. 2. The absorbance (logarithmic) at 280 m μ of the ultrafiltrate of bovine serum albumin through filters graded at 100,000 and 50,000 exclusion limits, respectively, contrasted with a non-filter system. Flow rate was 54 ml./h and total protein was approximately 10 mg.

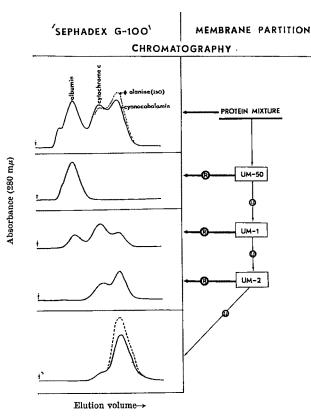


Fig. 3. The membrane separation schema and the 'Sephadex G-100' separation patterns of the following protein mixture: albumin, cytochrome c, cyanocobalamin, and phenylalanine. Flow rate was 10-15 ml./h, and temperature was 4° C. R, Retentate; U, ultrafiltrate.

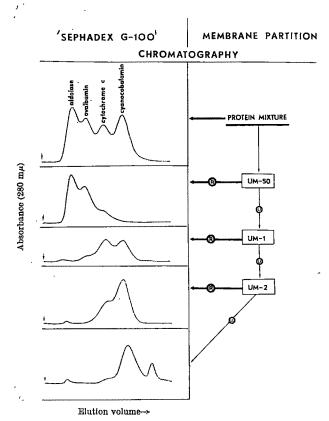


Fig. 4. The membrane separation schema and the 'Sephadex G-100' separation patterns of the following protein mixture: aldolase, oval-bumin, cytochrome c, and cyanocobalamin. Flow rate was 10-15 ml./h, and temperature was 4° C. R, Retentate; U, ultrafiltrate.

classes without the use of precipitating agents, cumbersome electrophoretic methods or the necessity for column chromatography. Increases in the separation rate, as well as the amount fractionated, can be obtained by increasing the surface area of the filter. In that limited fractionation rather than total resolution is achieved, "membrane partition chromatography" seems to be an apt title for this preparative technique.

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Possible Conformations of 5S Ribosomal RNA

THE determination of the nucleotide sequence of two 5S ribosomal RNAs by Brownlee, Sanger and Barrell represents a major advance in our knowledge of the structure of ribonucleic acids¹. This RNA contains only the four normal bases A, U, C and G, and so it is an ideal system on which to test some of the physico-chemical techniques known to be sensitive to the conformation of nucleic acids in solution²⁻⁴. There is a wide range of plausible secondary structures which can be devised for 5S ribosomal RNA. The model conformation suggested by Brownlee et al. contains a relatively small percentage of base paired residues. It is possible, however, to design other models in which a much larger number of nucleoside residues is formed into double strand helices. One such model is illustrated in a two dimensional projection in Fig. 1. The remarkable property of this hypothetical conformation is the large fraction of residues in uninterrupted double strands. The number of residues involved in base pairing varies from 82 to 98 out of a total of 120. depending on the identity of the base in position 13 and on whether G-U pairs, two single base pairs and one loop with only two residues are included. The base composition of the 5S ribosomal RNAs would permit a theoretical maximum of 112 base paired residues. In our model as much as 87 per cent of this maximum is attained. This is a much larger percentage of double strand sections than seems possible for any of the transfer RNAs of known sequence5-8

The hypothetical 5S ribosomal RNA structure shown in Fig. 1 is consistent with the general picture of RNA conformation which has emerged from studies on smaller model compounds. Most of the base paired sections contain four or more nucleotide residues. Shorter double or triple strand oligonucleotide complexes have been observed to form only in extreme environmental conditions!. The number of bases looped out of helical regions has been kept to a minimum. This takes into account the strong tendency of single strand oligonucleotides or even individual nucleosides to form stacked arrays^{8,10}. Uridylic acid occurs at almost all places in the structure where an abrupt bend, hair pin turn or junction of two helices is

postulated. The vertical stacking interactions of U are much weaker than the other bases, and so its presence at positions number 14, 25, 32, 40, 65, 80, 89 and 111 should minimize the energy necessary to interrupt the helices at these points. One double strand section is connected by a loop containing only two residues. This may be possible if one of the base paired residues adjacent to the loop has the syn conformation found occasionally in crystals11 instead of the anti conformation usually thought to exist in solution. Alternatively, a larger loop may be formed instead of the extra base pair.

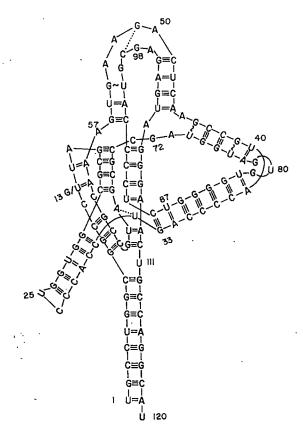


Fig. 1. Two dimensional projection of a possible conformation for the 5S ribosomal RNA in which up to 98 of the 120 nucleoside residues are base paired. Normal G-C and A-U pairs in helical regions are connected by three or two lines, respectively. G-U pairs are indicated by a tilde and two possible single base pairs are shown by a dotted line.

Spectroscopic techniques are available which make it easy to distinguish a mostly double strand structure such as the one shown in Fig. 1 from a model in which little base pairing occurs. As an example, the calculated optical rotatory dispersion of three possible conformations for the 5S ribosomal RNA is shown in Fig. 2. These calculations are performed as described previously for transfer RNA except here the problem of how to estimate the contribution from strange bases does not arise2. The three models used to compute the spectra in Fig. 2 are: (a) a completely single stranded RNA; (b) an RNA containing the twenty-three base pairs suggested by Brownlee et al.; and (c) the model with forty-nine base pairs shown in Fig. 1. The optical properties of G-U pairs are unknown, and so they were neglected in the calculations. should have only a very small effect. Models (b) and (c) contain only three G-U pairs each. The large difference in the calculated optical rotatory dispersion for the three models is best illustrated by the position of the longest wavelength cross over. This occurs at 279 mu, 273 mu and 263 mu for, respectively, zero, twenty-three and forty-nine base pairs. Thus even a qualitative measurement of the optical

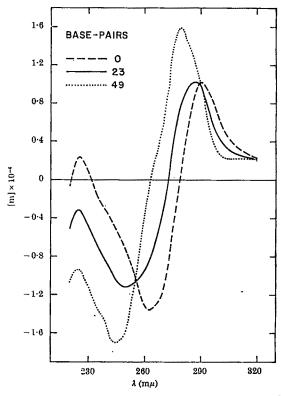


Fig. 2. Calculated optical rotatory dispersion curves for three possible conformations of 5S ribosomal RNA.

rotatory dispersion of the 5S ribosomal RNA should permit the approximate degree of base pairing to be established.

Note added in proof. Preliminary measurements of the optical rotatory dispersion of 5S ribosomal RNA indicate a crossover at 264 mu. This strongly suggests a conformation in which extensive base pairing occurs.

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Coupling of Enzymes to Cellulose using Chloro-s-triazines

Many methods are now available for coupling enzymes and other biologically active compounds to solid supports¹⁻⁴. Most involve the preliminary preparation of a special support or of a special derivative of a general support such as cellulose; for example, the carboxymethyl or p-amino-benzyl ether derivative. We have therefore sought coupling reagents which allow a more direct approach and have developed the use of cyanuric chloride and dichloro-s-triazines. The latter can be prepared as stable compounds with solubilizing groups such as carboxy-methoxy or carboxy-methylamino, which make them very convenient reagents.

Table 1. condition for the reaction of chloro-8-triazine with cellulose and of the product of this reaction with chymotrypsin

Any unreacted chlorine should be reacted subsequently with 1 molar ammonia-ammonium chloride buffer.

One of the chlorines is sufficiently reactive to react in a few minutes with cellulose in aqueous solution at pH 9-11 (Table 1) to give good yield of the monochloro-s-triazinyl-cellulose complex. Stronger nucleophiles, such as the —NH₂ group of amines, amino-acids and proteins, will then react with the second chlorine in mild conditions, 0°-20° C and pH 8·6 in times of the order of 16 h, that is

Using this method we have been able to couple chymotrypsin to prepared cellulose (Whatman Standard Grade Cellulose powder) so that the product contains 1-2 per cent by weight of enzyme protein with 25 per cent of its free solution activity. Such products retain up to 60 per cent of their initial activity for 2-2.5 yr when stored as suspensions in distilled water at 2° C.

We have also developed conditions in which it is possible to react cyanuric chloride with cellulose and still retain two of the chlorine atoms. Cellulose is treated with normal sodium hydroxide, the excess removed by filtration and the product added to an acetone solution of cyanuric chloride. Water is at once added and the acid generated by the hydrolysis of cyanuric chloride reduces the pH of the reaction mixture sufficiently rapidly to prevent the hydrolysis of a second chlorine. After a contact period of 10–15 sec, the mixture is quenched in 20 per cent acetic acid and washed in cold acetone and water mixtures. The dichloro-s-triazine cellulose so formed is extremely reactive towards proteins. It will react with chymotrypsin in a few minutes at pH 7.

Any of the second chlorines remaining together with all of the third chlorines may be removed by reaction

Cell—OH + Cl
$$N$$
 \rightarrow Cell—O— N \rightarrow Cl N \rightarrow NH—Protein \rightarrow Cell—O \rightarrow NH—Protein

with an amine; for example, molar ammonium hydroxide-ammonium chloride buffer, pH 8.6. The rather lower reactivity of the enzyme obtained by this method (5 per cent of its free solution activity) could almost certainly be improved by using shorter contact times.

Because of the mild treatment that the cellulose undergoes in all these processes, the method can be used for all forms of cellulose, for example, paper and cotton cloth. It may be applied also to other nucleophilic polymers such as crosslinked dextrans, starch, polyvinyl alcohol, wool and possibly nylon. Work on these problems is continuing.

Processes described in this communication are the subject of UK patent application 30615/67, rights in which have been assigned to NRDC.

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PSYCHOLOGY

Pupil Size as a Measure of Arousal during Information Processing

Following the work of Hess^{1,2}, and in parallel to other recent work³ where changes in the size of the pupil of human subjects' eyes were found during information processing tasks, a number of further findings are reported here. Variations in the level of arousal are presumed to be the intervening variable. A cinematographic technique for recording pupil size has been developed in which very high film emulsion speeds have been attained, in excess of 5,200 ASA from 650/800 ASA Ilford HPS 16 mm ciné film.

In the experiment, two kinds of factor were varied: the type of problem presented, and the required motor response of the subject. The latter included immediate verbal answering on solving the problem, storing the answer until a signal is received, and a substitute activity such as pressing a button. All stimulus material was presented visually on a back-projection screen.

Several findings were made with material involving novel and familiar, comprehensible and incomprehensible cartoon drawings. There was, however, no evidence to link pupillary changes with a like or dislike of the material, nor with the degree of complexity of the stimulus or the information contained in abstract and geometrical drawings. This perhaps conflicts with previous pupillary² and other autonomic^{4,5} findings.

More important, it was found that a peak in the dilation of the pupil was followed by a sharp post-solution drop, when single-solution anagrams and mental-arithmetic division sums were solved. That this drop was associated with a reduction in arousal at the end of a job was demonstrated by the presence of the drop, if not the peak, when the subject was instructed to answer and halt his attention on multi-solution anagram-type word-game problems on receiving a signal. Otherwise, there was no such pupillary drop with the multi-solution problems. Unsolved material, on the other hand, both arithmetic and single-solution anagrams, was found to produce a high plateau of maintained dilation, usually just below the peak attained at solution.

Similarly, difficult arithmetic problems, solved or unsolved, were found to produce an overall higher level of dilation than easy ones. Moreover, the same phenomenon occurred when the same kind of arithmetic material (with answers consisting of two digits) was to be answered by two different techniques, one easier than the other. Thus the cognitively easier response, of giving each of the two digits of the answer as attained, was found to result in a lower level of dilation than when the first digit had to be remembered while the second was being found, and then both being answered verbally together.

The absence of solution peaks with the multi-solution word-game material, the overall relatively low levels even when many responses were made, the lack of differences noted here between material found easy or difficult, or when many or few responses were made, suggested one of two things. Either verbal answering did not account solely for the solution peak (some other factor such as success was perhaps responsible), or the potentially easier multi-solution problem was different in its effect from the single-solution one.

The importance, however, of the contribution of verbalization to the response peak of the pupil was demonstrated in several ways. Verbalization of a stored response, either at a pre-arranged moment, or after receiving a signal, resulted in an increase in the dilation of the pupil during those periods, as compared with control situations when verbalization occurred elsewhere. The moment of actual as opposed to verbalized solution of a problem was still marked by a peak, although often of a somewhat lower level. Other motor activities, however, such as pressing a button on solving the problem, proved sufficient to bring the solution peak up to the same level as when con-Nevertheless, while comitant verbalization occurred. pressing a button proved equivalent to verbalization in forming the peak, it did not replace it. Thus subsequent additional verbal answering of the response resulted in a dilation level equal to that which occurred in a similar condition where there had been no such pressing of a button. It has already been noted here, however, that verbalization was not an inevitable inducer of relative dilation of the pupil, as with the multi-solution word-game material.

There were a number of subsidiary findings. There was a tendency for an overall "down-drift" in the pupillary baselines during the course of the experimental runs, possibly associated with a decline in arousal. These would usually be reset to earlier levels by switching stimuli or response types, or when a number of successive difficulties or failures were encountered. Similarly, overall baselines appeared to be pre-set to blocks or runs of similar stimuli. Thus, during inter-stimulus or inter-task intervals within such blocks of similar material, a baseline pupillary diameter would be found which differed from that encountered with blocks of different material.

Our intention now is to add measurements of blink-rate to the studies of the pupil in information processing tasks.

The latter will include reaction time and vigilance situations, and comparisons of performance under established sets such as speed and accuracy. The effects of drugs, stress and fatigue could also be studied, as well as situations using auditory material such as the Wittenborn⁶ tests of attention, with a button pressing response and a variable rate of presentation.

This work was carried out while I was at the Department. of Psychology, University of Sheffield.

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GENERAL

Gymnoplasts instead of "Protoplasts"

MICROBIOLOGISTS call yeast cells and bacteria devoid of their cell wall "protoplasts". According to a 100 yr old definition the protoplast represents the totality of the living cell constituents quite independent of whether the cell is coated by a wall or not. The general cytological term has therefore unfortunately been narrowed by considering the "protoplast" as the result of the removal of the cell wall. In seminars and symposia on "protoplasts", the term is used for a cytological speciality which covers only a small sector of the wide classical concept, as represented by the publications in the journal Protoplasma and the monographs of Plasmatologia which are concerned with all aspects of living matter, and not only with the problem of whether and how the lost cell wall can be regenerated.

The first cytologist to report naked protoplasts of higher plants was Küster², who called them gymnoplasts, in contrast to the dermatoplasts, the normal plant cells with their cell wall. These are clear and logical terms which tell us that there are cells with walls, the dermatoplasts, from which naked protoplasts, the gymnoplasts, can be prepared.

Another scientific misnomer is "spheroplast" for a gymnoplast the cell wall of which is incompletely removed. Most gymnoplasts are perfect spheres as well, and so it is illogical to give to a neutral morphological term such a special meaning. A term ought to express what it means. An incompletely naked gymnoplast could, for example, be called a semi-gymnoplast. Morphological terms are necessary for the description of observed objects, and they should not be used for the characterization of their ontogenetic or functional features. For example, spherosomes which contain hydrolytic enzymes are lysosomes, but probably not all spherosomes belong to that category, and, of course, not all spherical cells are "spheroplasts", that is, cells with incompletely removed cell walls.

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BOOK REVIEWS

STATISTICAL MECHANICS

Statistical Mechanics, Thermodynamics and Kinetics By Oscar Knefler Rice. (A Series of Books in Chemistry.) Pp. 586. (San Francisco and London: W. H. Freeman and Company, 1967.) 90s.

In 1930, few chemists were familiar with the calculation of the thermal properties of gases from spectroscopic energy levels through the methods of statistical mechanics. Giauque wrote an explanatory paper in that year in which he showed that it is possible to go from the equations of classical thermodynamics, which he assumed that his readers understood thoroughly, to expressions for the quantal partition functions of the rotational and vibrational motion of molecules. Professor Rice has adopted the same point of view; he says that "Chemistry students, although their mathematical sophistication has increased in recent years, are generally less familiar with probability theory than with thermodynamics". It is not an approach that is widely used, and, indeed, the current fashion is for a combined textbook of statistical mechanics and thermodynamics in which the latter is derived from the former. No doubt Rice, a teacher of long experience, has followed Giauque's route successfully with his senior and graduate students, but it is not one that they will encounter in their outside reading.

The approach from classical to statistical thermodynamics is open to question on several grounds. First, it may not be easier than the conventional route. Many students will follow readily and often blindly quite elaborate mathematical derivations but have difficulty with the abstract notions behind the apparently simple equations of classical thermodynamics. Secondly, the route starts from an equation that is not one of the basic equations of thermodynamics, namely that between an equilibrium constant K and a standard change of free energy, $\Delta G^0 = -RT \ln K$. The transition to statistical mechanics now requires the assumption that "under standard conditions, the entropy per mole is the same for all quantum species". This assumption raises difficulties in the mind of the student who knows that, classically, entropy is defined only for a system in equilibrium, with a Boltzmann distribution over the energy levels. statement can be justified for internal quantal states, but is, of course, quite inapplicable to translational states. Thus the author has to revert to the method of the canonical ensemble to treat the translational motion of monatomic gases in the third chapter, after he has dealt with the complexities of ortho- and para-hydrogen in the second chapter.

Those who are happy with this approach, however, will find here a wealth of illuminating and occasionally unorthodox ideas. The discussions of the topics on which Rice has worked himself are very extensive and in two cases, quantal liquids and chemical kinetics, at a level appropriate to a monograph.

J. S. ROWLINSON

DISTRIBUTION THEORY

Mathematics for the Physical Sciences

By Laurent Schwartz. (Adiwes International Series in Mathematics.) Pp. 358. (Paris: Hermann; London: Addison-Wesley Publishing Company, 1966.) 94s.

This book is a translation from the French original, with modification in various chapters. It is not a book on

"mathematical methods" in the sense of so many English books, but a work in which there is a careful emphasis on the meaning and implication of various mathematical concepts and procedures. It is written by the founder of the theory of distributions, so it is no surprise that this theory is introduced almost at the beginning and used throughout. It must be said that the contact with the physical sciences is disappointingly slight. The first two-thirds of the book is concerned with pure mathematics; and, of the remainder, only one of the three chapters comes within the realm of applied mathematics. Thus mathematical physicists will turn to the book in order to improve their knowledge and understanding of the mathematics they use, rather than to learn that mathematics in the first instance. As an example it is pointed out that there is no such thing as a "Dirac δfunction". The text says: "The properties of the Dirac function are contradictory and such a function could not exist. . . . Naturally, the physicists know very well that they are concerned with a symbolic device and not a 'true' function. It is more prudent to keep the concept of distribution . . . if we wish to do more than find results intuitively which still have to be proved rigorously in terms of distributions".

The first chapter is concerned with preliminary results on series and integration, including Lebesgue integration. The second chapter deals with distributions, including their definition, differentiation and multiplication; and there are sections on topology in distribution space, convergence of distributions and distributions with bounded supports. Then there is a chapter on convolution; this includes examples of convolutions arising in the theory of electric circuits. The fourth chapter, on Fourier series, deals with the convergence of the series in the distribution sense and in the function sense; and it includes a section on Hilbert space. The next two chapters are given to Fourier and Laplace transforms. Chapter seven, the only one dealing strictly with applied mathematics, is concerned with solving equations of vibration, namely, those for strings, membranes and vibrations in three dimensions. The treatment is quite thorough. A short section is also included on the solution of the equation of heat conduction. The book concludes with short chapters on the gamma function and on Bessel functions.

There is a fair sprinkling of exercises throughout the text, these being taken partly from French examination papers. The book will be of value to the advanced student who is strong in pure mathematics. Its chief value lies in the point of view adopted—that, for a proper understanding of the place of mathematics in various physical contexts, an appeal to the theory of distributions is indispensable.

L. S. GODDARD

FOR STUDENTS OF CHEMISTRY

Introduction to Quantum Theory

By Hendrik F. Hameka. Pp. x+276. (New York and London: Harper and Row, Ltd., 1967.) 55s.

The-flow of textbooks on quantum mechanics increases from year to year. This is all to the good and simply a sign of the vitality of the subject which has grown to such an extent that different specialities—solid state physics, quantum chemistry, atomic physics, elementary particle physics and so on—require a preparation which at least differs in emphasis if not in content. At the same time, the universities are trying to have the subject taught for the first time in the earlier years of the undergraduate curriculum. This again is simply a well understood necessity because of the more recent considerable developments in every branch of quantum physics which require that

the student be brought up to the research level in several

steps, extending over two or more years.

Hameka's book is such an attempt to shift the teaching of quantum theory to earlier parts of the curriculum. One feature of this is the care taken to develop all the required mathematics in some detail, witness chapters on differential equations and matrices and determinants. The ground covered is quite standard with a bias towards the needs of quantum chemistry: the final two chapters deal with two electron systems and atoms respectively. The general emphasis is on the Schrödinger equation, its solution in one dimensions, the eigenvalue problem associated with it and the hydrogen atom. A noteworthy feature of the book is the careful treatment of perturbation theory, the variation theorem and the connexion between them. This will be a most useful book, perhaps mainly for those students who wish to go on to do quantum chemistry.

S. ZIENAU

CONTINUUM MECHANICS

Modern Developments in the Mechanics of Continua Edited by Sılamon Eskinazi. (Proceedings of an International Conference on Rheology held at the Pinebrook Conference Centre of Syracuse University, Pinebrook, New York, August 23–27, 1965.) Pp. xii+203. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 80s.

This book contains the text of sixteen papers presented at a rheology conference sponsored by Syracuse University in August 1965. The authors are drawn mainly from the United States, but there are also contributions from Canada, France and Mexico. Five of the papers are concerned wholly or in part with experimental work, while the remaining eleven are entirely theoretical.

Of the experimental papers, two (P. S. Virk et al. and

Of the experimental papers, two (P. S. Virk et al. and A. G. Fabula et al.) are concerned with the Toms effect of drag reduction produced by very low concentrations of certain polymers in solution; the paper by Virk et al. amounts to a brief review article on the subject. A further two experimental papers deal with very different topics in the mechanics of mixtures. D. R. Axelrad and R. N. Yong consider flow of two phase media such as clay, and V. C. Behn and G. A. Strobel describe work on the diffusion of benzoic acid into kaolite slurries. The last paper in this group, by G. B. Thurston, describes experiments on shear wave propagation in birefringent viscoelastic liquids.

Turning to the theoretical contributions, we find that the conference began with some characteristically controversial remarks by C. Truesdell on the subject of thermodynamics. The greater part of Truesdell's paper is devoted to an exposition of work by Bowen and Wang on the formulation of a thermodynamic theory for a class of materials called materials with quasi-elastic response. The next two theoretical papers, by F. W. Smith on the flow and deformation of aggregates and A. J. A. Morgan on constitutive equations in implicit form, are brief accounts of work published more fully elsewhere. Jaunzemis contributes a short but clear description of Cosserat continua, and S. K. Datta investigates numerically the stability of second order fluids in Couette flow. I found the article by A. C. Pipkin on approximate constitutive equations one of the most rewarding in the This discusses the various approximations (for small motions, short motions, slow motions) which can be introduced into the general constitutive equations for non-linear visco-elasticity, and shows clearly their relation to each other and the general theory, as well as pointing out some of the pitfalls awaiting those who apply the approximate equations uncritically. Another fascinating paper is that by R. L. Fosdick on strain compatibility. This not only gives new forms of the compatibility

conditions for finite strain, but also, by a counter-example, disproves Ericksen's conjecture that the only deformations of incompressible materials with constant strain invariants are homogeneous deformations, and so reopens the question of whether there exist further deformations which are possible in all incompressible elastic materials. This paper has been somewhat overtaken by subsequent events, and its value would have been even greater if it had been published earlier. In the remaining papers, C. C. Wang proves a kinematic theorem for a class of motions (motions with constant stretch history), B. D. Coleman and M. E. Gurtin present a simplified onedimensional account of their work on acceleration waves in non-linear materials, and J. Barberan and I. Herrera prove uniqueness theorems for dynamic linear visco-Somewhat out of place is a paper by elasticity. R. Goethals and Th. A. de Roquefort on the theory of jets.

The level and standard of the papers are as diverse as their subject matter. As indicated above, some of the papers are brief accounts of work published elsewhere, some are original research papers, and others are short review articles. This variety makes for a rather untidy volume. Yet again the question arises as to whether any useful scientific purpose is served by the publication of books of this kind. I believe that the best of these papers would have been more accessible more quickly if they had been published in appropriate journals in the usual way, and that some of the papers would have been improved if they had been subject to the normal refereeing processes. This is not to deny the value of the conference, which evidently succeeded in bringing together people with a variety of interests and, so participants inform me, was both interesting and enjoyable. A. J. M. SPENCER

MANY-BODY THEORY

Field Theoretical Methods in Many-Body Systems By D. A. Kirzhnits. Translated by A. J. Meadows. Edited by D. M. Brink. (International Series of Monographs in Natural Philosophy, Vol. 8.) Pp. xvi+394. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 110s. net.

This book was published in Russian in 1963. The author has not attempted to give a comprehensive survey of many-body systems, but has shown in detail how methods developed originally for problems in the quantum theory of fields can be used to get systematic approximations for many-fermion systems such as nuclei, atoms and plasmas. Most of the book is concerned with the properties of the ground state and low lying excited states of such systems, but there is a short final chapter on the use of these methods at non-zero temperatures.

In many treatments of many-body theory approximations such as Hartree-Fock theory, the random phase approximation or Brueckner theory is introduced by using arguments the range of validity of which is far from obvious. Kirzhnits restricts his attention to systems in which certain parameters are small, and uses perturbation theory to obtain systematic expansions in these small parameters. He considers, for example, systems with forces of a very short range, very weak long-range forces, and systems the density of which varies very slowly in space. The difficulty with this approach is that for real systems these parameters are not small enough to be sure that the approximation is good. The table of results on page 182, calculated for nuclear masses and shapes, is very much more impressive than anything that has been obtained by using Brueckner theory, and might justify the dismissal of Brueckner's method as "highly complicated, requiring very extensive numerical calculations". There is, however, no indication of the sensitivity of the results to the form of the interaction between the nucleons

which is assumed. If the calculations based on Brueckner theory are correct, the results are so sensitive to the form of the interaction that the good agreement with experiment must be regarded as a coincidence.

The book is not easy reading, but contains a systematic development of a particular approach to many-fermion problems, and is a valuable addition to the literature.

D. J. THOULESS

RAMAN SPECTROSCOPY

Raman Spectroscopy
Theory and Practice. Edited by Herman A. Szymanski. Pp. ix + 255. (New York: Plenum Press, 1967.) \$12.50.

This book consists of seven review articles on particular aspects of Raman spectroscopy by specialists in certain fields. A general introduction by L. A. Woodward is designed to give an account of the theory of the Raman effect and to indicate the types of application in which it has been found to be of value. This is very well done, but the treatment of symmetry properties in the nomenclature of group theory is so terse that it might prove a deterrent to further study rather than the stimulant intended. Chapters by J. R. Ferraro and by J. A. Koningstein describe respectively modern Raman instrumentation and techniques (using commercial instruments in the main) and the application of lasers to Raman spectro-A fairly detailed account of the relationships between Raman intensities and the derivatives of bond polarizabilities is given by R. E. Hester. J. Behringer describes the problems associated with observations of Raman scattering of light close in wavelength to absorption features (resonance Raman scattering). the scattering is very strong in these conditions the interpretation of the observations can be more difficult than in normal Raman scattering.

The remaining chapters deal with the more specific problems of ionic melts (G. J. Janz and S. C. Wait, jun.) and complex ions in solution (D. E. Irish). They provide interesting examples of the scope of Raman spectroscopy and both include a brief but clear discussion of

some selection rules in group theoretical terms.

This is a rather patchy book, but nevertheless interesting and should be of particular value to those with some experience in general spectroscopy.

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SEMICONDUCTORS

Semiconductors and Semimetals

Edited by R. K. Willardson and Albert C. Beer. Vol. 1: Physics of III-V Compounds. Pp. xii + 500. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 152s.

This is the first of a series of volumes to be devoted to the physics of semiconductors and semimetals. The first three volumes of the series review the properties of III-V compounds, and this first volume is concerned particularly with band structure, magnetic field phenomena and plasma effects in the III-V compounds.

The authors have attempted to provide a reference work which combines a review of material already available, together with a substantial proportion of new material. Thirteen authors contribute to this volume, which begins with an introductory chapter by C. Hilsum, who provides a clear and concise outline of the present status of work on III-V materials.

The following chapters are grouped under the three headings of "Band Structure", "Magnetic Field Effects"

and "Plasma Effects". Two chapters are devoted to methods of band calculation for III-V compounds, and these underline the difficulty of the analytic approach in determining data on band structure which can be compared with experimental results. Much useful general information on band shapes can, however, be derived. The rest of the section on band structure deals with effects of heavy doping, which is becoming of increasing importance in the design and understanding of many experimental devices, and on the band structure of alloys, in particular the variation of band gap with composition.

The section on magnetic field effects includes two chapters on magnetoresistance, outlining the use of this phenomenon as a diagnostic tool in examining band characteristics and conduction mechanisms. The chapter by Becker includes a useful survey of results on a range of III-V compounds.

The final chapter describes plasma effects in III-V compounds, including a survey of equilibrium plasma effects undertaken to determine band parameters, and a description of the difficult area of non-equilibrium plasma investigation.

This volume seems to me to achieve its aim to be a comprehensive reference work for the specialist working in the field of III-V compounds. It is not appropriate as an introduction to the physics of these compounds, however, and, as such, its suitability for graduate courses is questionable. It is clearly very difficult to combine separate contributions from many authors, and although the editors have selected authoritative articles to include in the book, it remains a collection of separate articles rather than a cohesive whole. Nevertheless, it is obviously the most complete survey of the physics of III-V compounds which has appeared so A. A. SHEPHERD

UNIFIED MACHINES

The Unified Theory of Electrical Machines By Charles V. Jones. Pp. x+542. (London: Butterworth and Co. (Publishers), Ltd., 1967.) 90s.

ELECTRICAL machines, as a subject, is perhaps not as much "out of fashion" at the present time as the authorsuggests in his preface. Indeed, one might argue that the particular aspect which is the subject of the suggests in his preface. whole book has become very much more fashionable duringthe past few years and the author need have no fears that his book will seldom see the light of day, for it is an excellent treatise which is certain to be read with enthusiasm by generations of students who are interested in electric motors and generators.

One of its main attractions is that the student needs so little in the way of background knowledge before he can begin to read it intelligently. The author spells out every step carefully, never accelerating too rapidly over what many authors might consider too well trodden ground, and yet progressing in depth into the subject, certainly up to degree finals standard. It is clearly written by a practising engineer and cannot be criticized as the work of an abstract academic. The arguments used to develop the subject are both logical and persuasive and many of those hitherto prejudiced against generalized theory cannot fail to be converted.

The book is unusual in that many sets of experimental results are quoted in support of the theory, as might be the case with a paper in proceedings of learned societies. or in technical reports, and this is refreshing in a textbook. It might even be said that it needs courage to include material of this kind, as could also be said of paragraphs. when the author gives a brief account of his own thought processes leading to particular developments. It is a great pity that more standard textbooks are not written in this attractive style.

Although originally intended for final year undergraduates, much of the material could be absorbed by second year students and would form an excellent introduction to third year studies, because the material is so clearly presented. The author suggests sections of the book which might be used as a short course.

E. R. LAITHWAITE

ELECTRICAL TEXT

Electrical Technology

By Edward Hughes. Third edition Pp. xvii+709. (London: Longmans, Green and Co., Ltd., 1967.) 25s. paper; 35s. cased.

Ir is difficult to say immediately why Electrical Technology should be a depressing book. The exposition is lucid, the printing and layout clear, and although in a book of this size there must be some misprints they are not obvious. The selection of questions, too, is exactly right for passing the examinations of the Ordinary National Certificate. Perhaps it is the line drawings which make it look older than its first edition of 1960. Marconi himself would have recognized the valve on page 517, and the drawings in general have an Edwardian look. But, of course, blocks are expensive to make and the book is good value at 35s. Any book aimed at a particular examination has the limitations of the syllabus and, though this has recently been changed (hence this third edition), it remains out of date. Although it is nice to see an electronics section, thermionic valves play too big a part, while the transistor section is rudimentary and there is no hint of digital methods. Authors have no control over the syllabus of the examinations for which their books are used, and publishers often feel that extraneous material adds to the printing cost without enhancing sales. I wonder whether the omission of current research work, such as that on linear motors or direct current machines without commutators, really diminishes the cost of a book by a significant amount. Its omission deprives the inquiring student of a sense of progress and excitement in his L. MOLYNEUX subject.

HETEROCYCLIC ADVANCES

Advances in Heterocyclic Chemistry, Vol. 7 Edited by A. R. Katritzky and A. J. Boulton. Pp. xiv+511. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 176s.

Advances in Heterocyclic Chemistry, Vol. 8 Edited by A. R. Katritzky and A. J. Boulton. Pp. xiv+404. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 152s.

THE latest two volumes of this semi-annual publication fully maintain the standards established during the past four years, with a selection of timely reviews on both general and specialist aspects of the subject. No one, however Philistine, can fail to find interest in at least some of the material, while to the research worker active in heterocyclic chemistry this series must be an important part of his library.

The seventh volume includes an invaluable and comprehensive review of more recent aspects of the chemistry of furans, tailored to act as a supplement to existing publications. The mass spectrometry of simple heterocycles is also summarized briefly in a chapter which is mainly devoted to the mass spectra of alkaloids. Methods available for the halogenation of heterocycles are discussed

in practical as well as mechanistic terms and the influence of reaction conditions, catalysts or modern halogenating reagents on the orientation of the product is summarized in tabular form. The mechanistic complexities of the process are also underlined.

Not the least attractive contribution to this volume is a review of reviews on heterocyclic systems. This systematic compilation of references to recent books, reviews and articles spans the entire range of heterocycles together with related natural products, drugs and pigments. This most valuable source of information will be widely used and widely appreciated. Of less general interest are chapters on diquinolyl-methanes, a "recent advances" review of 1,3,4-oxadiazole chemistry and an extensive account of the chemical and spectroscopic features of the quasi-aromatic 1,2 and 1,3-dithiolium salts.

On the whole, the eighth volume in the series will be appreciated by specialists more than by general readers. A chapter on the formation and behaviour of cyclic peroxides (which are formally heterocycles) includes the process of ozonolysis, and will be of more general interest. I was also captivated by an exhaustive survey of thio-, thia- and thio-thia-pyrones (α and β) which reviews their preparation and properties (chemical and spectroscopic) and the inter-relation of structure and properties. Equally interesting and equally comprehensive is an account of the chemistry of indoxazenes and anthranils, that is, the benzo-analogues of isoxazole. This volume also contains shorter reviews of the Claisen rearrangement in N-heterocycles, heterocyclic diazo-compounds and the Hilbert-Johnson reaction. The fundamental chemistry of the phenoxazine nucleus, the parent system of the actinomycin antibiotics, is also reviewed, and a thorough painstaking survey of 1,2, 1,3 and 1,4-diazepines provides the first English account of these systems.

G. L. BUCHANAN

ROTATORY DISPERSION IN BONN

Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry

Including Applications from Inorganic Chemistry and Biochemistry. (Proceedings of a NATO Summer School held at Bonn, 24 Sept.-1 Oct., 1965.) Pp. xvi+416. (London: Heyden and Son, Ltd., 1967. Distributed in the USA by Sadtler Research Laboratories, Inc., Philadelphia.) 96s.; \$13.50.

In the summer of 1965 Dr Günther Snatzke organized a NATO Summer School at Bonn on optical rotatory dispersion and circular dichroism. The present handsome volume, which he edited, emerges as a substantial result of a most enjoyable meeting. There are seventeen contributors and twenty-three chapters and, happily, the intermittent maelstrom of discussion has not been put on permanent record.

One of the aims of the meeting was to introduce the subject matter to chemists unfamiliar with it. Its success in this respect is reflected in the proceedings, a notable achievement being the variety of approaches, summed up by experts in the field, offered to the student. Moreover, the treatment is balanced in the sense that, although the bulk of the material refers to organic chemistry, there are substantial sections on inorganic and biological aspects. After general introductory chapters by P. Crabbé and by C. Djerassi, the theoretical background is outlined first by A. Moscowitz and then by S. F. Mason, who also has a chapter on inorganic applications. So, too, does F. Woldbye who, for his second barrel, has a useful section on instrumentation. Several contributors discuss the relative merits of ORD and CD, and P. Crabbé devotes a chapter to this: not surprisingly, it is best to have both facilities. But overall one gets the impressionwhich has become stronger rather than otherwise in the intervening two years—that, given equal coverage of the spectrum, then CD has a decided edge on its competitor, especially in dealing with complicated chromophores.

As usual, the carbonyl chromophore comes in for a lot of attention (saturated compounds—W. Klyne; unsaturated ketones—G. Snatzke; carboxylic acids, lactones, lactams—W. Klyne, H. Wolf), but, in addition, a wide variety of chromophoric derivatives of "colourless" substrates are discussed (B. Sjöberg) and there are sections on sulphur compounds (K. Kuriyama) and twisted conjugated systems (K. Mislow).

Temperature and solvent effects are not well understood in detail, but are important because they may tax structural correlations. The editor has a chapter on the former, while solvation studies are discussed by A. Rassat and A. Moscowitz. A section by J. Badoz on magnetically induced optical activity is particularly interesting because, unlike many of the other topics in this volume, this is not given much prominence in standard texts. The possibility of using this effect, discovered by Faraday in 1845, to obtain useful information about optically inactive molecules is an intriguing one, but, from what has appeared so far, it seems that progress has been rather slow. Badoz remains stoical about this, and studies are evidently continuing.

The general impression left by this volume is favour-Two criticisms should be made, however. first is that the proceedings appear about two years after the conference: to my mind it seems better that proceedings, if published at all, should be produced rapidly and treated as ephemera, the substance of the material appearing in permanent form in the established journals. The present volume is somewhat unusual, of course, in its pedagogic intention: it has evidently been carefully produced—which takes time—and, in places, supplemented since the meeting. One is left wondering, however, whether the re-publication, practically word for word, of papers already in the literature is defensible in this context. Portions of no less than six chapters are reproduced from journals, and one chapter appears to be almost entirely composed in this way. It is to be hoped that this practice will not catch on: both the student and the library deserve a better deal. R. BONNETT

STUDENT MICROBIOLOGY

The Biology of Fungi, Bacteria and Viruses

By Greta Stevenson. (Contemporary Biology.) Pp. xiii+202. (London: Edward Arnold (Publishers), Ltd., 1967.) 42s. net, boards; 21s. net, paper.

This comparatively short book, intended for first year students, covers a wide range, though perhaps rather less wide than the title suggests. By far the greatest emphasis is on fungi, with bacteria given much less space and the viruses being little more than mentioned. The book is divided into three main sections: "Cell Organization and Physiology", "Special Habitats and Economic Aspects", and "Diversity of Fungi in Relation to Environment".

The first section is perhaps the least satisfactory. So far as the fungi are concerned the account of cell organization is not very clearly distinct from the themes of the last section, and the account of the organization of bacterial cells is brief and superficial. The chapter on metabolism outlines a number of metabolic pathways and says something about macromolecules, not always accurately. For example, on page 31 acetaldehyde is said to combine with oxaloacetate to form citrate in the tricarboxylic acid cycle, on page 43 ribosomal RNA is not mentioned when the main classes of RNA are being listed, while, on the following page, a relationship between amino-acid and tRNA anti-codon is implied which is, to say the least, not generally accepted as true. This part

of the book may be useful for quick reference but cannot be recommended for students who have no biochemistry text against which to check their facts.

The second section is somewhat discursive, dealing with such varied topics as soils, the biology of water supplies and plant and animal pathology. In the available space there is room for only a few examples, and others would no doubt have placed the emphasis differently. I felt that the section on soils was given more weight than was warranted by its microbiological content. However, this attempt to place micro-organisms in their proper biological contexts is refreshing and should catch the interest of students.

The most solid and conventional but at the same time probably the most valuable section is the last. Here is a brief but comprehensive guide to the main fungal groups with a summary of their system of classification. For those who do not need to pursue mycology to the extent of buying a specialized text, this section provides a brief but adequate account of the fungi. Another feature which will be very useful for students is the table showing comparative sizes of various microbiological and molecular objects.

In summary, Dr Stevenson has written an original book which, although not outstandingly successful in all respects, should prove useful to many of the students at whom it was aimed.

J. R. S. FINCHAM

WORK WITH TISSUES

Animal Tissue Techniques

By Gretchen L. Humanson. Second edition. (A Series of Books in Biology.) Pp. xvi+569. (Sin Francisco and London: W. H. Freeman and Company, 1967.) \$9.00; 72s. net.

It would not be fair to describe this book as just another manual of histological techniques; it aims to explain the basis and application of the techniques rather than simply to present a list of favourite recipes. It contains sections on histochemistry and autoradiography, on exfoliative cytology, on the preparation of invertebrates for whole mounts and sections; and the student is introduced, briefly, to electron microscopy and immunofluorescence techniques. But it certainly does not contain anything which tissue culturists, cell biologists or experimental pathologists might expect to find in a book bearing this title, nor is the author attempting to cater for them.

The opening section, on fixation, dehydration, embedding, sectioning and staining of tissues, contains well illustrated accounts of the cutting of paraffin sections and the sharpening of microtome knives, but the chapter on the use of the microscope is not illustrated and the descriptions are barely adequate. The following section on the staining of tissues, with sub-sections on the staining of intracellular organelles and micro-organisms and on vital staining, contains a wealth of detail and references. There is a useful closing section on solution preparation and general laboratory aids. Some of the materials are referred to by their trade names, although the composition of the product is usually indicated. Readers outside the United States, when they are not able to purchase similar products locally, will wish that the addresses of the suppliers of apparatus and materials referred to in the footnotes were given more fully.

In her incidental comments the author occasionally seems out of her depth. We are informed, in the opening sentences of the book, that during autolysis intracellular enzymes "appear to reverse their action; instead of synthesizing amino acids into cell proteins, they begin to split proteins into amino acids", and later (page 231) that lymphoblasts produce monocytes, plasma cells and lymphocytes. The remarks in the section on antigenantibodies which introduces the Coon's fluorescent

antibody technique are equally naive: "...If the fluid portion of blood plasma is allowed to clot (fibrinogen precipitates out), or a relatively stable serum remains. This is made up of two protein fractions: albumin and globulin. The globulins consist of two alpha globulins, one beta globulin and one gamma globulin..." The author seems to have included these comments as a matter of policy without giving them much thought; her real interest appears to be in the methods themselves and it is easy to believe that she has had personal experience of most of them.

N. R. Ling Norman Williamson

TEACHING PLANT PATHOLOGY

Sourcebook of Laboratory Exercises in Plant Pathology (Sourcebook Committee of the American Phytopathological Society.) Pp. xxvii+388. (San Francisco and London: W. H. Freeman and Company, 1967.) 68s.

THE material in this book has been assembled in its present form by members of the American Phytopathological Society with the intention of encouraging a more experimental approach to the teaching of plant pathology. The method chosen to achieve this aim has been to set down in detail a series of laboratory exercises in the hope that individual teachers may find at least some of the ideas pertinent to their particular courses.

The experiments cover all the major activities of the fungi, bacteria, viruses and nematodes in relation to plant pathology, while the standard of the exercises ranges from the straightforward isolation of fungi from the soil through to advanced research procedures only suitable for demonstrations. Some of the experiments will no doubt make interesting and worthwhile additions to specialist courses in this subject, and the descriptions of the techniques employed are, on the whole, lucid and informative. The very clarity of the text, however, is sometimes deceptive and many of the apparently "simple" exercises may well prove more difficult than indicated when executed by students not too familiar with the handling of micro-organisms. This possible drawback is enhanced by the fact that no real indication is given as to how skilled an experimenter needs to be in order to obtain the suggested results, and because many of the techniques are intended as innovations to general laboratory work, this lack of guidance may well lead to some disillusionment among those tempted to abandon familiar pathways.

The overall approach adopted in this book is, in many ways, rather disappointing, for the editors seem to have ignored the basic problem that they set out to solve, namely, "how the descriptive and taxonomic approach to the teaching of plant pathology may be integrated with the more dynamic aspects of the science". Thus, in compiling these exercises, those involved seem to have been thinking solely in terms of what should theoretically be included in a plant pathology course in ideal conditions, rather than concentrating on how the basic facets of the subject could be brought to life. For this reason it seems doubtful whether this book will go very far towards achieving its aims, whereas had the committee responsible for its production been content to influence a smaller audience, with the emphasis on enlivening and expanding the type of experiments suited to a particular group, then the book would probably have more impact. As it stands, with an apparent potential readership from high school children through to postgraduate research workers, it has simply become a collection of experimental techniques, and a potentially exciting project appears to have completely lost steerage through a failure to adhere to better defined R. K. Robinson parameters.

INSIDE PLANTS

Plant Anatomy

By A. Fahn. Translated from the Hebrew by Sybil Broido-Altman. -Pp. viii+534. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 75s. net.

This book by the professor of botany at the Hebrew University of Jerusalem is an English translation of the Hebrew edition. It is intended as a general and comprehensive introduction to plant anatomy and as such it is conventional in plan. The book begins with a consideration of the cell; has chapters on meristems, tissues, the primary and the secondary plant body, and concludes with three chapters on reproductive organs.

Any text book on plant anatomy must deal with a vast quantity of facts and, perhaps inevitably, mistakes will occur. This book is no exception. For example, parenchyma is not always composed of living cells as is implied on page 73, neither is all collenchyma formed in the way described on page 80. The statement on page 273 "that in woody . . . [plants] . . . the primary tissues of the stem and root exist for a relatively short period before they become destroyed or obliterated by the development of the secondary vascular tissue . . ." is not only wrong and misleading but suggests some misunderstanding of

the process of secondary thickening.

On page 292 it is stated that resin ducts occur in the secondary xylem of Abies and Cedrus, two genera in which resin ducts do not normally occur although occasionally traumatic ducts may be found, more particularly in Cedrus, and it is these abnormal ducts which are illustrated in Fig. 128.2.

There is in many places a lack of precision in the writing and in many instances statements on the same topic in different places are not consistent; for example, xylem as defined on page 102 and subsequent descriptions of this tissue. There are other examples and it may be that these are, at least in part, the results of the problems of translation.

An undergraduate student using this book will learn many facts about plant anatomy, but I doubt whether such a student will obtain an integrated picture of the structure of a living plant. Perhaps the author has tried to include too many facts so that it has become difficult to see the wood for the trees.

The book is copiously illustrated, but the illustrations vary in quality. The line diagrams are generally clear but the same cannot be said for all the micrographs. The student is likely to find Fig. 117 or Fig. 120.2 difficult to interpret.

Most of the genera, if not all the species, of plants referred to will be familiar to students in this country. The book has a glossary, and is provided with both an author and a subject index.

D. J. B. White

EXPERIMENTAL ANIMAL ECOLOGY

Introduction to Experimental Ecology

By T. Lewis and L. R. Taylor. Pp. xi+401. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 37s. 6d.; \$6.50.

As a university teacher who is continually faced with first year students completely lacking any previous practical ecological experience at the school level, it was with considerable pleasure that I read *Introduction to Experimental Ecology*.

It is obvious a considerable amount of work and careful selection of material has gone into the preparation of this book. The exercises are chosen from a wide range of habitats, which makes the book equally valuable to schools situated in towns as well as the more fortunate rural ones. The statistical treatment of the data has been

explained in a clear and lucid fashion and the maximum information extracted by use of simple techniques.

Both the authors and publishers are to be congratulated on an excellent book—the only serious criticism I make is the title, which should read "Introduction to Experimental Animal Ecology".

K. A. Kershaw

PEEPHOLES INTO COGNITION

Concepts and the Structure of Memory

Edited by Benjamin Kleinmuntz. (Carnegie Series on Cognition.) Pp. xiv+286. (New York and London: John Wiley and Sons, 1967.) 64s.

The psychology department at the Carnegie Institute of Technology has collected funds from a variety of sources in order to promote annual symposia on various aspects of cognition. The proceedings of the brief meetings together with prepared comments on the papers are published in hardback book form. The present volume results from the second symposium of the series. It consists of seven original contributions, four on concept attainment, three on memory, comments on these taken in pairs (one escapes) and an "overview" by Newell and Simon.

Whereas the title, by including the archaic term "structure of memory", carries overtones of mentalistic psychology of the nineteenth century, the book actually provides glimpses of current research projects. With the exception of Hunt's paper on the computer simulation of concept formation, all deal with the molar behaviour of normal human subjects in the tradition of modern experimental psychology.

The papers on concept learning are notable for the breadth of the interpretation the topic is given. Bourne shows how he is extending the development of the analysis of traditional concept attainment tasks and procedures which was initiated by Bruner, Goodnow and Austin. While, by contrast, Martin adopts the position of an extremist S-R theorist: "...all behavior is caused, that is ... every response has a stimulus". His employment of S-R theory is novel, however, for he stresses the significance of responses which are inhibited in any particular situation. His field of application is also novel, for he argues that multiple choice reaction time experiments can properly be regarded as concept learning tasks.

The contributions on memory are unexciting by comparison. The main theme is the retrieval process. Peterson's thesis is that it can be separated into search and decision components, while Cofer predictably examines clustering as an example of conceptual organization. Surprisingly, he finds that it does not generally result in augmented recall.

These comments on the content should make it clear that this book is intended for the initiated. It is primarily of interest to researchers in these two fields for whom the informal presentation of work which is in its preliminary stages will be especially fascinating. As an attempt to bring two research areas together the exercise has largely failed. There are obvious points of contact between concept learning and memory, and many contributors have mentioned them. In the main, however, after briefly pointing to the relevance of their work to those in the other field, they have then proceeded with an exposition of their own favourite topic. If the conference was aimed at cross fertilization, then the progeny are yet awaited. And this admixture reduces the appeal of the book. It would be more useful if it had provided a wider sample of contributions on a single topic.

A general point is that books of this kind can serve a useful purpose. Their publication lag is less than that of many journals and the preparation lag is obviously considerably shorter. They therefore speed up communication in science. But they are only of interest to the

specialist and are only of passing interest at that. Why then are they not published as paperbacks at a fraction of the price?

H. C. A. Dale

OBITUARIES

Dr J. C. Trevor

J. C. Trevor, who died in July, aged 59, had a considerable reputation as a physical anthropologist in Great Britain and abroad. He had a wide knowledge and was particularly interested in the history of the subject. His special interests were race mixture, the physical history of man in Africa and the British Isles, and the anthropology of New World Negroes. He was something of a perfectionist, both concerning the collection of his data and in the way in which he wrote about them, and this probably accounts for the relatively small number of his publications. He could not bear to publish what he considered to be a shoddy piece of work and was untiring in his efforts to encourage his pupils to be accurate in their work and elegant in their writing.

Almost all of his work was based on biometric methods

Almost all of his work was based on biometric methods and his articles in *Chambers's Encyclopaedia* and the *Encyclopaedia Hebraica* on anthropometry and race express concisely Trevor's view of his special interests in

physical anthropology.

Before the war he published several papers, the most interesting being "Some Anthropological Characteristics of Hybrid Populations", which was followed in 1953 by "Race Crossing in Man: the Analysis of Metrical Characters". Professor L. S. Penrose, writing in the introduction to the latter, says: "It summarizes information which can never be out of date. As a contribution to both human genetics and physical anthropology it falls naturally into place as a Eugenics Laboratory Memoir. This series . . . was started by Karl Pearson in 1907 and has been continued ever since". These comments seem to be as valid today as they were in 1953.

Trevor's interest in the history of man in Britain ("The Rough Island Story", as he often called it) is reflected in papers in *Biometrika* and in his Monroc Lectures to the University of Edinburgh in 1952 on "The

Racial History of Britain".

His childhood and war service in Africa certainly gave Trevor an interest in the racial history of that continent. His 1949 paper on "The Physical Characteristics of the Sandawe" in the Journal of the Royal Anthropological Institute is a good example of his thorough anthropometric and anthroposcopic work and careful statistical analysis of the results. The Ancient Inhabitants of Jebel Moya (with R. Mukherjee and C. R. Rao) is another massive contribution to the racial history of the northeastern part of the African continent.

Lastly, there remain a group of publications like his contributions to Simpson's Modern Trends in Forensic Medicine, "The Fontechevade Skulls and the Origin of Homo sapiens" (Nature, 1959), "Oreopithecus bambolii" (Antiquity, 1959), which reflect Trevor's wide interest and very considerable knowledge of physical anthropology.

He used to say that neither biometric methods nor the "trained-eye" methods were valuable by themselves. He always maintained that when studying primate osteological material one should remove subjectivity as much as possible, but that nevertheless a trained eye could see much that measurements might overlook. In consequence, he advised the use of both techniques.

Some may feel that Jack Trevor's kind of anthropology has become a little outmoded, but certainly his professional integrity and high standards are manifest in his publications and could well be emulated by any beginner in the field.

MARIE LAWRENCE

CORRESPONDENCE

Special Relativity

Sir,—I refer to Professor Dingle's paper on "The Case

Against Special Relativity" (Nature, 216, 119; 1967). It is, I think, implicit in Professor Dingle's argument that equation (3) is derived by an observer at rest in the AH frame who initially synchronizes A, B and H to zero but not N. He later, again at rest at H in the AHframe, reads B and H when they are adjacent. He thus determines the ratio of the rates of clock A to clock B from the point of view of an observer at rest in the AH

It is similarly implicit that equation (4) is derived by an observer at rest in the NB frame who initially synchronizes NB and A to zero but not H. Later at rest at N in the NB frame he reads N and A when they are adjacent. He thus again determines the ratio of the rates of clock A to clock B but this time from the point of view of an observer at rest in the NB frame.

It is entirely in accord with the principle of relativity that one of these ratios is the reciprocal of the other, for the clock which moves relative to the observer always goes slower. It does not alter the argument if one observer sets all the clocks and makes all the observations by accelerating back and forward between the two frames.

Yours faithfully,

J. H. FULLERTON

11 Purley Bury Avenue, Purley, Surrey.

SIR,—Without detracting from the completeness of Professor McCrea's reply to Professor Dingle, may I suggest a simple way of pointing the fallacy in the latter's argument?

Professor Dingle's rate-ratios (3) and (4) both purport to refer to the same observer A; (3) is correct, but (4) is false and is actually the rate-ratio as observed by B, for the following reasons. The following arguments can be levelled against it.

(i) The time-interval $(0, t_2) = (0, at_2)$ is an interval on A's clock-for any observer.

(ii) The time-interval $(0, t_2')$ is the difference between the reading "zero" on B's clock, and the reading t_2 on N's clock. For A, this difference is physically meaningless, since for A these two clocks are not synchronized. According to the theory, A observes a constant difference between their readings.

However, if the readings are regarded as observations by B, the intervals are valid, so that as stated before, formula (4) is the rate-ratio as observed by B and is, naturally, the reciprocal of that observed by A.

Yours faithfully,

W. BARRETT

Department of Mathematics, University of Leeds.

"Snags in Space"

SR,—Your conference report of October 21 (Nature, 216, 215; 1967) refers to certain difficulties which have arisen in decoding a fraction of the data from the scientific experiments on board the Ariel III satellite. The statements in your report arose from a discussion of these difficulties at a meeting which was concerned with the

apparatus rather than the scientific results, and which may therefore have given an unbalanced impression of the performance of the experiments as a whole. We wish to offer some corrections.

The first point is the reference, in the conference and in your report, to "interference" from the Birmingham experiments. This might be thought to be some type of spurious signal generated in the apparatus of the Birmingham electron density or electron temperature experiments, but in fact no such signals are present in the intended frequency bands of any of the seven high gain radio receivers associated with other experiments on board the satellite. What is observed instead is an unusual form of cross modulation, by way of the plasma sheaths surrounding the satellite and Birmingham experiment sensors. which appears to occur between the audio-frequency signals of the Birmingham experiments and the input stage of the Jodrell Bank galactic radio noise experiment.

If it had been anticipated in advance that such a cross modulation were likely to occur, it might have been avoided either by modifying the switching sequence programme of the Birmingham experiments or by changes to the input stages of the Jodrell instrument; but it is not profitable to speculate, after the event, on the possibility that the problems with which we are faced could have been avoided by more detailed consideration at the development stage. The cross modulation is a form not previously known and the knowledge we stand to gain relating to its exact mechanism will offset to some small extent any loss of expected data.

There are considerable periods when the effects of the ionospheric cross modulation are negligible. At these times we have been able to demonstrate the anticipated effect of the ionosphere on the cosmic radio background showing as a progressive reduction in radiation resistance of the loop antenna as the electron density increases. At the upper end of the frequency sweep, at 4.2 MHz, where the effects of the geomagnetic field are small, it is easy to show that the values of electron density measured by the Birmingham experiment are in close agreement with values obtained from measurements of the radiation resistance which must refer to ionosphere propagation conditions over a large volume in the vicinity of the This encourages us to believe that both spacecraft. experiments are working exactly as planned and that the radio astronomical experiment will fulfil at least part of its intended purpose.

Some misunderstanding must also have arisen concerning the Meteorological Office experiment. You state that "The Birmingham experiments have also upset the attempt by the Meteorological Office to measure the concentration of molecular oxygen in space". This is simply not true. There is a quite marginal amount of cross modulation present in the Meteorological Office data, but this can readily be allowed for in the analysis and does not significantly affect the quality of the measurements of molecular oxygen concentration.

J. SAYERS

University of Birmingham.

F. G. SMITH

Nuffield Radio Astronomy Laboratories, Jodrell Bank.

K. H. STEWART

Meteorological Office, Bracknell.

This account is substantially in agreement with that published in Nature two weeks ago. The term "interference" is that used in the abstracts circulated at the meeting. The report also described the interference with the Meteorological Office experiment as "not too severe", but it is good to know that even that was an exaggeration. Editor, Nature.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 6

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 1.15 p.m.-Professor Ronald King: "Faraday—Father of Electrical Engineering".

FARADAY SOCIETY (in the Department of Chemistry, The University Edinburgh), at 4.30 p.m.—Professor Dudley Herschbach (Harvard University): "Molecular Beam Kinetics" (Bourke Lecture).*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Lecture on "AC and DC Current Comparators".

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "What Can We Do Without in a Nuclear Power Station?".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, WC2), at 6.30 p.m.—Mr F. F. Mazda: "Thyristor Control".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, SW1), at 6.30 p.m.—Dr M. W. Davies: "The Chemistry of Iron and Steel Making".

Tuesday, November 7

FARADAY SOCIETY (in Lecture Theatre No. 4, School of Chemistry, The University, Bristol), at 4.30 p.m.—Professor Dudley Herschbach (Harvard University): "Molecular Beam Kinetics" (Bourke Lecture).*

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP—PHYSICOCHEMICAL AND BIOPHYSICAL PANEL (at 14 Belgrave Square, London, SW1), at 5 p.m.—Dr W. P. Anderson: "Free Space in the Plant Leaf".

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, NW1), at 5 p.m.—Dr C. R. W. Spedding: "Intensive Sheep Production from Pasture"."

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5.30 p.m.—Dr B. DeLisle: "Functional Studies of the Cortex Using Microelectrodes".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Eccleston Hotel, Victoria, London, SW1), 5.30 p.m.—Mr T. Plant: "Role of Specifications in the Rubber Industry". 7 p.m.—Mr J. A. Stephens: "Design and Application of Seals".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5.30 p.m.—Sir Lawrence Bragg, FRS: "Waves and Vibrations". (Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on November 8, 14 and 15.)

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Professor D. W. Smithers: "Clinical Research in Hodgkin's Disease". (Seventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)"

University of London Institute of Education (in the Beveridge Hall, Senate House, Malet Street, London, WC1), at 5.30 p.m.—Professor Doris M. Lee: "Perspectives in the Education of Teachers".*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at West Ham College of Technology, Romford Road, London, E15), at 6.30 p.m.—Mr P. Cooke: "Microelectronics".

Wednesday, November 8

ROYAL INSTITUTION, HISTORY OF SCIENCE DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Professor A. R. Hall: "Galileo and the Science of Ballistics".

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, London, SW7), at 2.30 p.m.—Meeting on "Phosphors". Dr P. W. Ranby: "Phosphors in Lamps and Cathode-ray Tubes"; Mr R. C. Marshman: "Phosphors in Postal Sorting".

PLASTICS INSTITUTE, POLYMER PROPERTIES DISCUSSION CIRCLE (at 11 Hobart Place, London, SW1), at 2.30 p.m.—Meeting on "Stress Cracking".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 4.30 p.m.—Meeting on "Exhaust and Inlet Silencing".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, SW1), at 5 p.m.—Professor J. F. C. Conn and Mr A. M. Ferguson: "Results Obtained with a Series of Geometrically Similar Models"; Dr R. L. Towsin: "Viscous Drag from a Wake Survey—Measurements in the Wake of Lucy Ashton Model".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, WC2), at 6 p.m.—Mr J. N. White: "Farm Buildings Design and the Landscape".

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, WC1), at 6 p.m.—Dr J. Marshall and Dr J. R. A. Mitchell: "Cerebrovascular Disease—Clinical and Biochemical Aspects".*

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (joint meeting with IFST, at 14 Belgrave Square, London, SW1), at 6.15 p.m.—Mr J. H. V. Davies: "Codex Alimentarius".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (at the Euston Tavern, 73 Euston Road, London, NW1), at 6.30 p.m.—Mr B. G. Toms: "The Road Transport Environment".

Wednesday, November 8-Friday, November 10

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS AND THE INSTITUTION of Electrical Engineers (at the Institution of Electrical Engineers, Savoy Place, London, WC2)—Conference on "MF, LF, and VLF Propagation".

Thursday, November 9

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, SW1)—All-day Meeting on "Management of Quality".

University of London (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 1.20 p.m.—Dr W. A. Smeaton "Science in the Capital of Burgundy: The Dijon Academy, 1740-1793".*

GEOLOGICAL SOCIETY, VOLCANIC STUDIES GROUP (at Burlington House. Piccadilly, London, W1), at 2 p.m.—Meeting on "Major Volcanic Structures".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m. Professor E. R. Laithwaite: "Analogy—The Art of the Engineer" (Ci Service Lecture).

FARADAY SOCIETY (in the Chemistry Department (Faculty of Science), The University, Manchester), at 4 p.m.—Professor Dudley Herschbach (Harvard University): "Molecular Beam Kinetics" (Bourke Lecture).*

ROYAL SOCIETY (at 6 Carlton House Terrace, London, SW1), at 4.30 p.m.— Professor J. Baddiley, FRS: "Teichoic Acids and the Molecular Structure of Bacterial Walls" (The Leeuwenhoek Lecture).

UNIVERSITY COLLEGE LONDON (in the Galton Theatre, Gower Street, London, WC1), at 5 p.m.—Mr E. D. Brown: "The Lessons of the Torrey Canyon".*

UNIVERSITY OF LONDON (in the Physics Lecture Theatre, Westfield College, Kidderpore Avenue, London, NW3), at 5.15 p.m.—Professor D. R. Hughes: "The Uses of Mathematics".*

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W1), at 5.30 p.m.—Mr J. Lutoslawski: "The Polish Oil Industry".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr H. Goldenberg: "Transient Heating of Buried Power Cables".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr J. L. Turk: "The Cell Mediated Immunological Response". (Eighth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

Friday, November 10

ASSOCIATION OF APPLIED BIOLOGISTS (at the Royal Society of Arts, John Adam Street, Adelphi, London, WC2), at 11 a.m.—Symposium on "Wild Mammals and Their Control".

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemaric Street, London, W1), at 1 p.m.—Dr B. Ward: "The Reactions of Hydrogen Atoms with Benzene and Toluene".

ROYAL INSTITUTE OF CHEMISTRY (at Birkbeck College, Malet Street, London, WC1), at 5.30 p.m.—Dr D. H. Williams: "Some Aspects of the Mass Spectra of Organic Compounds" (Meldola Medal Lecture).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 9 p.m.—Dr C. A. Wright: "The Island of Aldabra".

Monday, November 13

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Electron Microscopy Group of the Institute of Physics and the Physical Society, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 10 a.m.—Colloquium on "Electron Probe Instrumentation".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, SW1), at 5 p.m.—Dr M. Elliott: "Structural Requirements for Pyrethrin-like Activity".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr A. R. W. Broadway and Mr G. Thomas: "Single-Unit P.A.M. Induction Frequency Convertors".

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "The Transport of Solids by Gases".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the

dates mentioned:
SENIOR LECTURER (with qualifications in a branch of science or technology SENIOR LECTURER (with qualifications in a branch of science at postdoctoral SENIOR LECTURER (with qualifications in a branch of science or technology together with relevant academic or industrial experience at postdoctoral level) in the DIVISION OF MATERIALS AND MODECULAR SCIENCE—Clerk to the Governing Body, Woolwich Polytechnic, Wellington Street, London. SE18 (November 14).

RESEARCH ASSISTANT IN CANCER ENZYMOLOGY—The Registrar, The University, Liverpool, quoting Ref. RV/218/N (November 15).

TUTORIAL RESEARCH STUDENT (graduate) IN PHYSIOLOGY—The Secretary, Bedford College (University of London), Regent's Park, London, NW1 (November 16).

(November 16).

EXPERIMENTAL OFFICER (with experience of instrument design or construction involving electronics or optics, and preferably experience in spc ctroscopy) in the DEPARTMENT OF CHEMISTRY—The Registrar, University of Manchester, Manchester, 132, quoting Ref. 162/67/Na (November 18).

LECTURER IN PHYSICS—The Principal, Bede College, Durham (November 18).

Manchester, Manchester, 13, quoting Ref. 162/67/Na (November 18).

Lecturer in Physics—The Principal, Bede College, Durham (November 18).

Chair of Anatomy at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW7 (Hong Kong and London, November 24).

Regus Chair of Botany in the University of Glasgow—The Private Secretary, Secretary of State for Scotland, Room 507, St. Andrew's House, Ediburgh, 1 (November 24).

Temporary Lecturer (with a medical degree registrable in the State of Queensland, and preferably postgraduate experience of some five years' duration in a clinical and pathological discipline) in Pathology at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Brisbane and London, November 24).

Lecturer/Assistant Lecturer in Physiology at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Hong Kong and London, November 25).

Lecturer in Theoretical Chemistry—The Registrar, University Senate House, Bristol, 2 (November 30).

CHAIR OF PRYSIOLOGY at St. Mary's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, WC1 (December 1).

(December 1).

LECTURER (graduate in agricultural, veterinary or rural science, with a higher degree in a field of animal husbandry) in ANIMAL MANAGEMENT in the DEPARTMENT OF ANIMAL HUSBANDRY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, December 8)

The Association of Commonwealth Universities (Branch Office), altriborough House, Pall Mail, London, SW1 (Australia and London, December 8).

SENIOR DEMONSTRATOR (with a B.Sc. honours degree, or equivalent, in geology) in the Department of Geology, University of Melbourne—The Registrar (Appointment & 57), University of Melbourne, Parkville, Victoria, 3052, Australia (December 15).

University Lecturer in Physical Anthropology in the Department of Archaeology and Anthropology, The University, Downing Street, Cambridge (December 15).

Lecturer (Sexior and Anthropology—Dr S. J. Tambiah, Secretary of the Appointments Committee of the Faculty of Archaeology and Anthropology, The University, Downing Street, Cambridge (December 15).

Lecturer (Sexior and Anthropology), The University, Downing Street, Cambridge (December 15).

Lecturer (Sexior and Anthropology), University, Clayton, Victoria 3168, Australia; or The Secretary-General, Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (December 22).

Principal Lecturer in Polymer Physics; and an Instructor in Polymer Physics—Clerk to the Governors, National College of Rubber Technology, Northern Polytechnic, Holloway, London, Nr.

Research Assistant (with a degree in biochemistry and preferably experience with proteins or nucleic acids) to participate in work on mode of action of vitamin D—The Director, University of Cambridge and Medical Research Assistant (with a degree in microbiology or bacteriology, or in biology with training in microbiology, and preferably some training in bacterial genetics to carry out research into the genetics of resistance to antibiotics in bacteria—The Deputy Director, A.R.C. Unit of Animal Genetics, Institute of Animal Genetics, West Mains Road, Edinburgh, 9.

Scientific Assistant—The Deputy Director, A.R.C. Unit of Plant Physiology, Impertal College of Science and Technology, London, SW7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Great Britain and Ireland

Overseas Development Institute. Annual Report 1967. Pp. 33. (London: Overseas Development Institute, 1967.)

Wye College (University of London). Annual Report of the Department of Hop Research from 1st April, 1966, to 31st March, 1967. Pp. iii + 77. (Ashford, Kent: Wye College, 1967.) 6s.

Department of Education and Science. Building Bulletin No. 36: Evoline Lowe Primary School, London. Pp. 93. (London: H.M. Stationery Office, 1967.) 10s. 6d.

Direct Correlation of Physical Constants through Transcendental Equations. By Frederick Crook. Pp. 16. (Grange Place, Guernsey, C.I.: Frederick Crook, 1967.) 21s.

The Ministry of Technology 1967. Pp. i+30. (London: Ministry of Technology, 1967.)

Ministry of Overseas Development. Report of the Tropical Products Institute 1968. Pp. iv+43+10 plates. (London: H.M. Stationery Office, 1967.) 5s. net.

Bullding Research Station Digest No. 85 (Second Series): Joints Between Concrete Vall Panels: Open Drained Joints. Pp. 6. (London: H.M. Stationery Office, 1987.) 4d.

University of Newcastle upon Tyne. Report of the Dove Marine Laboratory, Third Series, No. 16: The Marine Fauna of the Culercoats District 4.

a. Arthropoda 2b: Insecta; Pterygota. By H. O. Bull. b. Arthropoda 3a: Crustacea; Branchiopoda. By J. Bossanyi. c. Arthropoda 3b: Crustacea; Ostracoda. By J. Bossanyi. Pp. 67. (Cullercoats, North Shields: University of Newcastle upon Tyne, Dove Marine Laboratory, 1967.) 7s. 6d. [19

Other Countries

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United States Department of the Interior: Geological Survey. Techniques of Water-Resources Investigations of the United States Geological Survey, Book 3, Chapter A2: Measurement of Peak Discharge by the Slope-Area Method. By Tate Dalrympile and M. A. Bason. Pp. vi + 12. So. Delivery, Book 3, Chapter A2: Measurement of Peak Discharge by the Slope-Area Method. By Tate Dalrympile and M. A. Bason. Pp. vi + 12. So. Delivery, Book 3, Chapter A2: Measurement of Peak Discharge by the Slope-Area 1218: General Geology of the Mammeth General Geology of the Julympile and M. A. Bason. Pp. vi + 12. All County, Arizona, by S. C. Creasey. Pp. vi + 94 + plate 1. Buetic 120-1: Mineral Resources of the High Uintas Primitive Area, Utah. By the 120-1: Mineral Resources of the High Uintas Primitive Area, Utah. By the 120-1: Mineral Resources of the High Uintas Primitive Area, Utah. By the 120-1: Mineral Resources of the High Uintas Primitive Area, Utah. By the 120-1: Mineral Resources of the High Uintas Primitive Area, Utah. By the 120-1: Mineral Resources of the California Academy of Steinees. No. 63 (June 30, 1967): Miocene and Pliocene Marline Diatoms from California. By Walter W. Wornardt, Jr. Pp. 108. (San Francisco: California Academy of Sciences, 1967.)

The Rockefeller Foundation, 1967.)

The Rockefeller Foundation, 1967.)

Applied Science and Technological Progress—a Report to the Committee on Science and Astronautics, US House of Representatives, by the National Academy of Sciences—National Research Council, 1967. Available from US Government Printing Office.)

Transactions of the American Philosophical Society, New Series, Vol. 57. Part 5: The Architecture of the Sanctuary of Apolle Hylates at Kourion. By Robert Scranton. Pp. 85. (Philadelphia: The American Philosophical Society, 1967.) S3.

World Health Organization. Technical Report Series. No. 361: WHO Expert Committee on Biological Standardization—Ninetenth Report of the WHO Expert Committee on Mental Health. Pp. 45. No. 364: Principles for the Testin

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APPOINTMENTS VACANT

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Applications are invited for the following ap-pointments, applications for which close on the dates shown in brackets:

LECTURESHIP IN BOTANY: The teaching will be primarily in Cytology and Genetics, but may include some participation in first year teaching in general Botany, and in other areas appropriate to the qualifications of the appointee. (November 30.)

(November 30.)

LECTURESHIP IN PHYSICAL GEO-GRAPHY: Applicants should have qualifications in either geomorphology with special interests in statistical methods, or bio-geography with special interest in ecology. Asian field experience would be an added advantage. (November 30.)

The salary scale for a Lecturer is from SNZ3,000 rising to SNZ4,400, with a bar at this point, then on recommendation to SNZ4,800. The initial salary will be determined according to the qualifications and experience of an appointee. Superannuation is available on the F.S.S.U. pattern. Approved fares to Wellington will be allowed for an appointee and his dependent family together with actual removal expenses within specified limits.

Further particulars and information as to the

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, S.W.1. (1492)

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UNIVERSITY OF SIERRA LEONE
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UNIVERSITY OF SURREY EXPERIMENTAL OFFICER DEPARTMENT OF MECHANICAL ENGINEERING

ENGINERING

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The Department is scheduled to move to the new University site at Guildford in 1968. Substantial assistance will be given towards travelling expenses to staff travelling from the Guildford area whilst the University is in London.

Please apply to the Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11, for an application form. (1490)



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Further information and application forms may be obtained by writing to the Atomic Energy Adviser, Office of the High Commissioner for Australia, Canberra House, 10-16 Maltravers Street, Strand, London, W.C.2. Please quote L.01.



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DEPARTMENT OF ELECTRICAL ENGINEERING AND ELECTRONICS

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The salary will be within the scale £1,105 to £2,630 per annum according to qualifications and experience.

Applications, stating age, qualifications

Applications, stating age, qualifications and experience, together with the names of three referees, should be received not later than November 11, 1967, by the Registrar, from whom further particulars may be obtained. Please quote Ref. RV/221/N.

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At least ten posts for men and women aged at least 23 as Assistant Veterinary Investigation Officers, Grade II. Candidates must be members of the Royal College of Veterinary Surgeons. Post-graduate experience in laboratory research work desirable. The vacancies are at Leeds, Norwich (2 posts), Penrith, Reading, Sutton Bonnington (2 posts), Thirsk, Truro, and Weybridge, STARTING SALARY: £1,326 (at age 23) to £1,779 (at age 32 or over), rising to £2,031. Promotion to Grade I (salary £2,031 to £2,464) in six or seven years (or possibly earlier) for able officers with prospects of further advancement. Non-contributory pension.

Opportunities may arise for short-term service overseas.

overseas.

WRITE to Civil Service Commission, 23 Savile Row, London, W.1, for application form, quoting 266/67. Closing date November 22, 1967.

(1480)

MACAULAY INSTITUTE FOR SOIL RESEARCH

CRAIGIEBUCKLER, ABERDEEN, AB9 201 DIRECTORSHIP

DIRECTORSHIP

The Council of Management of the Macaulay Institute for Soil Research invites applications for the post of Director which becomes vacant when the present Director, Dr. A. B. Stewart, C.B.E., retires on June 30, 1968. The Institute is concerned with the problems of soils and soil-plant relationships and the work is organized in seven major Departments: Soil Survey, Pedology, Spectrochemistry, Biochemistry, Plant Physiology, Microbiology and Soil Fertility, together with a Section on Statistics. For purposes of postgraduate research leading to higher degrees, the Institute is recognized by the University of Aberdeen. Candidates must have high scientific attainment in a field relevant to the work of the Institute. The appointment will be in the grade of Chief Scientific Officer—Lower Band, salary £5,000 per annum, with superannuation under F.S.S.U.

Applications (20 copies), with the names of

F.S.S.U. Applications (20 copies), with the names of three referees, should be sent not later than December 18, 1967, to the Secretary of the Institute, from whom further particulars may be obtained. Overseas, candidates may submit one copy of application. (1488)

UNIVERSITY OF NEWCASTLE UPON TYNE

The University invites applications for the post of LECTURER IN STATISTICS in the Department of Statistics, School of Mathematics. The post is tenable from October 1, 1968, or such other date as may be arranged.

The salary will be at an appropriate point on the scale £1,470 to £2,630 according to age, qualifications and experience. Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne 2, with whom applications (three copies), together with the names and addresses of three referees, should be lodged not later than November 27, 1967.

(1463)

COCOA RESEARCH INSTITUTE (GHANA ACADEMY OF SCIENCES)

Applications are invited from suitably qualified persons for the following posts in the Cocoa Research Institute, Tafo:

AGRONOMY DIVISION

Senior Research Officer/Research Officer (Statistician) Qualifications

Post-graduate diploma in agricultural statistics with some experience in the design of field experiments on horticultural crops. Tropical experience an advantage though not essential.

(b) Principal Research Officer (Agronomist)

Qualifications*

Post-graduate degree or diploma in agriculture. Training and experience in field experimental studies, preferably with perennial tree crops. Capable of supervising the work of subordinate staff. Good record of published work required.

Ħ CHEMISTRY DIVISION

Research Officer (Soil Chemist)

Qualifications*

Post-graduate degree or qualification in the range of Agricultural Chemist/Plant Biochemist/
Plant and Soil Analyst, with some experience of routine plant and soil analysis.

m ENTOMOLOGY DIVISION

(a) Principal Research Officer (Insecticides Chemist)

Qualifications*

Post-graduate degree in entomology or organic chemistry, with experience in the insecticidal control of crops pests. Good record of published work required and must be capable of supervising subordinate staff.

Senior Research Officer/Research Officer (Entomologists) for Plant Protection

Onalifications*

Post-graduate degree in entomology or organic chemistry, with experience in the insecticidal control of crops pests. Good record of published work required and must be capable of supervising subordinate staff.

Senior Research Officer/Research Officer (Entomologist) for Insect Ecology

Onalifications*

Post-graduate degree in entomology, with experience and a bias towards the dynamics of insect ecology under conditions of insecticide application to field crops. Good record of published work required and must be capable of supervising subordinate staff.

IV

PLANT BREEDING DIVISION

Principal Research Officer (Plant Breeder) to head the Division

Post-graduate degree or equivalent qualification in Plant Breeding. Experience with perennial horticultural crops and good record of published work desirable.

PLANT PATHOLOGY DIVISION

Research Officer (Mycologist)

Post-graduate degree or equivalent qualification in Mycology or Plant Pathology.

PLANT PHYSIOLOGY DIVISION

Principal Research Officer/Senior Research Officer (Plant Physiologist) to head the Division

Qualifications*

Post-graduate degree in Plant Physiology or Plant Biochemistry and interest in environmental effects on plant growth. A reasonable record of published work is necessary.

The Institute, which is entirely residential, is located only 67 miles from Accra and offers attractive living conditions and surroundings.

*Post-Qualification Experience

Principal Research Officer: minimum of 7 years Senior Research Officer; minimum of 5 years Research Officer: minimum of 3 years Salaries (under review) are superannuated.

Principal Research Officer

Ghanaian NC3,800 by NC150 to NC4,400 (£G1,900 by £G75 to £G2,200). Non-Ghanaian NC4,560 by NC180 to NC5,280 (£G2,280 by £G90 to £G2,640).

Senior Research Officer

Ghanalan NC3,500 by NC150 to NC4,100 (£G1,750 by £G75 to £G2,050). Non-Ghanalan NC4,200 by NC180 to NC4,920 (£G2,100 by £G90 to £G2,460).

Research Officer

Ghanaian NC2,100 by NC100 to NC2,600 by NC150 to NC3,350 (Bar) to NC3,500 to NC150 to NC3,800 (£G1,050 by £G50 to £G1,300 by £G75 to £G1,675 (Bar) to £G1,750 by £G75 to £G1,900).

Non-Ghanaian NC2,520 by NC120 to NC3,120 by NC180 to NC4,560 (£G1,260 by £G60 to £G1,560 by £G90 to £G2,280).

Point of entry in the scale depends on qualifications and experience.

Applicants should write for application forms, to be completed and returned not later than November 30, 1967, to the Acting General Secretary, Ghana Academy of Sciences, P.O. Box M.32, Accra.

(A stamped addressed envelope should accompany requests for applications.) Documents

(A stamped addressed envelope should accompany requests for applications.) Documents attached to applications are not returnable.

For overseas candidates application forms are obtainable from the Recruitment Officer, Ghana High Commission, 38 Queensgate, South Kensington, London, S.W.7, or Ghana Embassy, 2460-16th Street, N.W., Washington, D.C., U.S.A., or Ghana High Commission, 75 Albert Street, Ottawa, Canada. (1502)

SCOTTISH PLANT BREEDING STATION

Applications are invited (male or female) for three

SCIENTIFIC OFFICER

posts as follows: (1) a cereal breeder to join the team working on the production of new varieties of feeding barleys and oats; (2) a geneticist to join the cereals team to work on population-genetic problems related to cereal breeding; (3) a geneticist to work in the Potato Department either on the Commonwealth Potato Collection or on economic-genetic problems posed by potato breeding. Applicants for post (1) should have a background in agriculture or agricultural botany; for (2) and (3), a sound training in genetics, preferably with some biometrical experience. Younger applicants will be preferred.

Applications are also invited for an Assistant Experimental/Experimental Officer post, as Junior Field Officer (male applicants only). This Officer will assist in the conduct of field trials of various crops in many different localities in Scotland; he will be based at Pentlandfield but will have to travel widely in due season.

Candidates for S.O. posts should hold a First or Upper Second Class Honours Degree in Science; for A.E.O./E.O. post, a degree or diploma in Agriculture or Agricultural Botany.

Salary for S.O. posts £926 to £1,574 per annum according to experience. Superannuation under F.S.S.U. Salary for A.E.O./E.O. post £744 at age 21 rising to £1,243 per annum (A.E.O.) or £1,365 rising to £1,734 (E.O.) per annum with placing according to age and experience.

Applications giving full personal particulars and naming two referees to Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, by November 17, 1967.

biochemistry research

Are you capable of doing a wide range of biochemical measurements? Or could you be taught this?

Do you feel bored or restricted in your present job? Have you a B.Sc. or equivalent (H.N.C. considered) in biochemistry, chemistry,

s xxx s

-5

If your answer is "yes" to all these you should consider applying for a job in the Biochemistry Department at Chesterford Park Research Station, where you could join a team of young scientists who are trying to make better pesticides biochemically. This job is not routine, and your advancement would depend solely on your performance. Interested?

Write for further information and application form quoting serial number 139 to:



Personnel Officer (10), Chesterford Park Research Station, Fisons Pest Control Limited, Nr. Saffron Walden, Essex. Telephone: Saffron Walden 3542

DEPARTMENT OF GEOPHYSICS UNIVERSITY OF WESTERN ONTARIO LONDON, CANADA

Applications are invited for the following positions:

(a) Visiting Professor; minimum salary £5,000 er annum; a senior appointment for one year

only;
(b) Assistant Professor; minimum salary £3,200
per annum; minimum qualifications, Ph.D.;
(c) Technical Officer of Professional Assistant;
minimum salary £2,2000 per annum; minimum
qualifications, H.N.C. with experience in electro-

qualifications, H.N.C. with experience in electro-nics but preference given to Honours B.Sc. with experience.

Some Assistantships and Demonstratorships will all be available for graduate students who are accepted for M.Sc. and Ph.D. programmes. For further information, write to Professor A. E. Beck, Head, Department of Geophysics.

(1445)

PORTSMOUTH COLLEGE OF TECHNOLOGY

CHIEF LABORATORY TECHNICIAN

Applications are invited for the above post in the ZOOLOGY section of the department of BIOLOGICAL SCIENCES. Administrative or managerial experience together with knowledge of some zoological techniques is desirable. Salary scale within: £1,020 to £1,220 per annum. Excellent conditions of service.

Application forms are obtainable from: The Staff Officer, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hants. Closing date: November 17, 1967. (1448)

GRADUATE CHEMIST WITH AN Honours degree is required for research work on wood preservation. Research Laboratories are in a country district at Frome, Somerset.—Apply Personnel Manager, Cuprinol Limited, Adderwell, Frome, Somerset.

UNIVERSITY OF LONDON KING'S COLLEGE

SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF BIOPHYSICS

The College requires a Lecturer with research interests in Cell Biology, especially its more molecular aspects. Teaching duties will be within the School of Biological Sciences in undergraduate and postgraduate courses on Molecular and Cell Biology. Salary scale: £1,470 to £2,270, then, subject to review, to £2,630. A London Allowance of £60 plus F.S.S.U. benefits will also be payable. be payable.

Conditions of appointment and application forms may be obtained from the Registrar, King's College London, Strand, W.C.2, and completed forms should reach him by December 31, 1967. (1483) uid reach (1483)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF NATURAL PHILOSOPHY

LECTURESHIP

Applications are invited from physicists preferably with theoretical or experimental research interests in lasers or plasma physics for appointment as LECTURBR in the Department of Natural Philosophy.

The successful candidate will be required to take part in undergraduate teaching and to participate in the programme of research in the Department.

Salary scale: £1.470 to £2,630 per annum plus F.S.S.U., with placing according to experience and qualifications.

Application forms and further particulars (quoting \$9/67) can be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.I., with whom applications should be lodged by November 30, 1067 (1485)

CORRECTED ADVERTISEMENT

UNIVERSITY OF LEEDS

DEPARTMENT OF CHEMICAL PATHOLOGY

Applications are invited from either medically or scientifically qualified candidates for appointment as Lecturer at a salary on the scale £1,745 to £3,340 (efficiency bar £2,525) for a medically qualified Lecturer or £1,470 to £2,630 (efficiency bar £2,270) for a scientifically qualified Lecturer. Initiat appointment at any point on the scale. Duties involve work in the hospital biochemistry laboratory which the department runs for the General Infirmary at Leeds. The Lecturer will be expected to spend half his time in research, and may work for a higher qualification. The department may be visited by arrangement with Professor G. H. Lathe. Previous applicants need not re-apply.

Applications (three copies), stating age, qualifications and experience, and naming three referees, should reach the Registrar and Secretary, the University, Leeds, 2 (from whom further particulars may be obtained) not later than November 18, 1967.

UNIVERSITY OF GLASGOW

UNIVERSITY OF GLASGUVY
DEPARTMENT OF GENETICS
Applications are invited for the post of Graduate Research Assistant for research project on the chromosomes of self healing skin tumours.
Salary scale: £850 to £1,250 per annum, according to experience.
Applications should be sent to Department of Genetics, University of Glasgow, Glasgow, W.2.
(1497)

Metropolitan Problems

THE first report of the South-East Economic Planning Council is called A Strategy for the South-East (HMSO, 15s.) and the title is the best thing about it. Much of the report is worthy, but most of it is dull. Although there is some stirring talk about the scale on which problems must be tackled, the underlying spirit is one of inaction. For all that the south-east planning council knows, the 1980s will be the same as the 1960s but more so. Faced with the demand to predict the future, the council has summoned up the natural conservatism of the English which makes it possible to embrace the notion of reform without the necessity for actual change. A part of the trouble is that the council seems to have been overawed by the solemnity of its problem. It is true that the south-east of England, which includes London, is the biggest planning problem in England, but the council seems sadly innocent of the plain truth that exactly the same problem has to be tackled by all those required to plan metropolitan areas, from Moscow to Paris. (In the United States, there are at least four such areas to be battled with.) But the council has also been handicapped by its awareness that if it did not say something-even anything-quite soon, there would be a serious danger of being overtaken by events or even of being forgotten altogether.

The nature of the metropolitan problem is now familiar. Metropolitan areas suffer from too many people, too much congestion, problems of pollution and a scarcity of chlorophyll and sunlight. In most places, people—even planners—have only just woken up to the scale of the problem. Less than five years ago, for example, the central problem of the metropolitan area around London may have seemed to be the need to preserve inviolate, or mostly inviolate, the area of administratively imposed greenery which surrounds the city roughly twenty miles from the centre. But then, quite suddenly, people woke up to the fact that the metropolitan region includes 17 million people and 10,000 square miles. Three years ago, this phenomenon was the chief marvel in the South-East Study-one of the then spate of public documents which helped the regional planning councils into being. And now there is at least a suspicion that the scale of the English metropolitan problem has outstripped the geographical terms of reference of the council which has now reported. It may be an accident that much of the natural growth in southern England in the past few years has happened to fall just outside the boundaries of the council's parish—in places such as Swindon, for example—but that is at least one reason for scrutinizing the council's present generalizations with the greatest care.

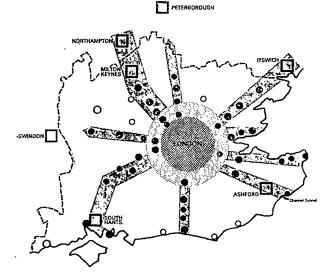
Many readers of the council's proposals will not, however, get that far. Page one of the report is so

littered with begged questions that only the most trusting will penetrate beyond it without a sense of having been cheated. There is, for example, a brief passage about the virtues of London as "a unique international centre for commerce and finance, as a world-wide tourist attraction and as a centre for the arts, education, religion and science" followed by the declaration that the council will try to "enable London to work as efficiently as possible". Those who enjoy the place may be charitable enough to overlook the order in which these virtues have been spelled out, but they will find it hard to see how the council leaps from that to the doctrine that "To this end, the growth of London must be contained. Firm controls must be exercised to relieve traffic congestion, to reduce the difficulties and excessive costs of business firms and to make life as pleasant as possible for the individual Londoner . . . Continual efforts must be made to prevent unnecessary concentration of activities in London, particularly in the central area . . . We fully endorse the concept of holding the resident population of Greater London at or under 8 million . . . " And this, says the council, is why it is essential to develop "city regions around the periphery of the region". This, the council says, is "the only means we see of creating effective counter-magnets that will attract population and industry away from London". It so happens that many of the detailed proposals which the council endorses—the new city on the Solent, for example—are eminently worthy causes on any view of the character of metropolitan planning, but this is plainly an accident and not the consequence of wise design. The council seems to have based its plan on platitude.

How, for example, does the council arrive at the conclusion that the only way to deal with the problems of a metropolitan city is to persuade people to go away by creating "counter-magnets" elsewhere? For one thing, of course, this policy has been tried and found to fail in a host of different ways. For another, there is no earthly reason why people should be denied the pleasures of living in London—if they want themsimply because the council decides that the jobs they do can "reasonably and efficiently be carried out elsewhere". Are the affairs of England to be managed in such a way that the population of London consists almost exclusively of theatre-going politicians and those who assist at the Changing of the Guard? And will it be necessary for those who like sailing at the weekend to train themselves for the kind of jobs which the council has in mind for the Solent city ten or twenty years from now? These exaggerations are absurdities, as even the council would be quick to point out. It is unfortunately less obviously a falsehood to pretend that it is possible to preserve the character of a city whichlike New York or Paris to name only two others—depends for its strength and its creativeness on the unpredictable inventiveness of unsorted people. In other words, the doctrine of counter-magnets is either a dead duck or a death knell.

But what can be done about the traffic jams? These and similar questions provide the plaintive undertone for the council's report. The council's response is to attempt to solve its problems by extrapolating the present into the future, and by embracing the doctrine of counter-magnets to the metropolis. Implicitly the council seems to make the assumption that there will be no change in the character of urban life in the years ahead but only in the scale and extent of it.

This is where technology comes in. With the improvements now in prospect for the decades ahead, there is good reason to re-examine the assumptions on which the doctrine of counter-magnet cities has been based. Specifically, it would be good to know how far it may be possible to give people who live and work in satellite cities a sense that they are nevertheless a part of the metropolis. Why, for example, should not the city now certain to emerge on the Solent be deliberately linked with London by fast train services and cheap telephone circuits in such a way that it would seem no farther away than the outer suburbs of the metropolitan sprawl? The objective should be a physical communications link taking half an hour or so between the two cities—which are only 80 miles apart—and a return fare which is com-



Britain's Small Strong Voice

British politicians are fond of saying that Britain speaks with an authoritative and influential voice at the world's council tables, and there is indeed one set of deliberations in which a British voice, were it loud and clear, would be entirely welcome. In 1969 it is intended that the future shape of an international satellite network should be settled. What is the British line to be? There is a real danger that it will be hammered out on too narrow an anvil between the men from the

parable with but smaller than the price of a theatre ticket. With telecommunications, the prices should and could be such—given the capacity of modern microwave links—that the two cities could cherish the illusion of contiguity. The result could be an exciting extension of the scope of urban living. It would also help to make the south-east of England "work as efficiently as possible".

It is hard to see how the council's strategy will lead in that direction. The principal feature of the council's proposals is a pattern of radial development along ribbons stretching out in several directions around London. The intention is to confine development within the region to these narrow sectors. Even though the council insists that it does not want to see each of these sectors jammed tightly with new developments, the effect of these proposals would be to create a series of linear cities lying along the lines of the new motorways out of London. The trouble, of course, is that the success of a linear city must stand or fall by the efficiency of the transport system which serves as a backbone, and it is entirely ludicrous to expect that the roads now being built will be able to serve the purposes which the council will clearly be expecting of them twenty years or so from now. If, indeed, the council is wedded to the construction of linear cities—and there is a good case to be made for them this is another reason why it should begin by paying some attention to the modern technology of trans-

The council should also do much more than is apparent from its report to translate its problems into the language of what is called systems engineering. To begin with, it should try to create some kinds of criteria which can be used for telling whether one pattern of urban living is more or less efficient than another. As things are, the only criterion on which everybody is agreed is that there should be a vigorous attempt to preserve as much open space as possible around London. It should then set out to define a pattern of urban life-cities, transport and telecommunications—which can give the fullest expression to objectives commonly held to be desirable. In other words, if the committee chose to do so, it could produce a design for the south-east of England that would be worth living in, and could at the same time set a new pattern in regional planning. The glitter of the prizes to be won adds to the disappointment in the council's first report.

General Post Office and the Foreign Office and then trimmed and tempered to suit the demands from the United States. That would be a great opportunity lost.

The manoeuvres in preparation for 1969 have already begun. European interests are talking about the consolidation of their ideas on the outcome they seek from the negotiations, and President Johnson has laid the first American cards on the table. His administration would be willing to see Comsat, the American satellite corporation, relinquish its dominant position as holder of 53.8 per cent of the vote in the international consortium (Intelsat) which runs the network. He would also like to see the Soviet Union and Eastern European countries as partners in the network and, failing that, as co-operating rivals. But he has said nothing about changing the formula that effectively keeps the Soviet Union out of Intelsat—the allocation of voting power on a basis of the amount of traffic sent through the system. (That gives Britain roughly eight per cent of the voting strength, although smaller countries have to share a single vote and the Soviet Union would be down among the one percenters.)

So should the permanent agreement be no more than a hardened version of the present one? There might be nothing so terribly wrong with that. Under Comsat's lead and a strictly commercial philosophy, Intelsat has performed near miracles since the interim agreement was signed in 1964. A good part of the globe is linked by four synchronous satellites. The gap over the Indian Ocean will be filled next year and capacity added all around as the new generation of satellites, labelled Intelsat III, is launched. So far, satellites have begun to pick up transatlantic customers that the cables cannot handle (Spain and Italy have just begun operating receiving stations) and the first over the Pacific was an instant sell-out.

What is wrong with the present international committee is that it leaves too much power in the hands of the somewhat stodgy, traditional communications carriers like the GPO and the American Telephone and Telegraph Company (biggest shareholder and biggest bogy of Comsat). According to the GPO's thinking, satellites are now being cosseted slightly; there is a holding back on extra cables until satellites have had a

Icarus in Parliament

THE asteroid Icarus has made an unexpected impact in the British House of Commons where Mr Keith Stainton, Member of Parliament for Sudbury and Woodbridge, has been asking the Government for assurances that there will be no collision with the Earth next June, when the two objects are predicted to pass within four million miles of each other. Stainton's anxiety is that the predictions of the path of the asteroid may be seriously in error and he has in particular chided the British Government for what seems to him to be an over-trusting reliance on the calculations of the Institute of Theoretical Astronomy in Leningrad. The Government, in the person of Mrs Shirley Williams, Secretary of State at the Department of Education and Science, has replied to Mr Stainton's enquiries with a statement of the Government's confidence that Icarus will indeed miss the Earth.

According to Dr Samuel Herrick, of the University of California at Los Angeles, the uncertainty in the prediction of Icarus next year amounts to about 1,000 miles in the distance of the nearest passage, but this should be reduced to about 150 miles when the ephemeris is corrected to take account of an observation this summer by Dr Elizabeth Roemer at Tucson, Arizona. Dr Herrick has taken a continuing interest in

chance to prove what they can do. But in the last analysis, the Post Office is simply concerned with selling communications at the best possible price. If anybody in Britain is going to give the naive idealistic speech about what satellites can do to break down barriers of geography and politics and to educate the masses, he is not going to come from the GPO. President Johnson has done it in America, but he cannot be expected to suggest that Comsat take a back seat in Intelsat (where it could be outvoted by something less than the combined weight of all the other members).

There are other imbalances to be put right in the permanent agreement. More of the contracts should be placed outside the United States. There should be guarantees that communications networks in other countries should not find themselves, like the neighbour's children, restricted by the Federal Communications Commission's rules for Comsat. There should be some general assembly kind of body to give all of the 59 or so member countries of Intelsat a chance to speak out, even if commercial considerations keep their actual voting power in proportion to their investment in the network.

A lot of guidance could come from Britain on these matters, intricate to the point of tedium. As second in power only to Comsat, Britain is probably the only country which can tactfully suggest Comsat's graceful retreat while asking France and Germany not to launch an independent satellite of their own. The papers in Britain gave considerable space to Professor Fred Friendly's warning from America that lack of planning about satellite communications could create an electronic slum in the sky. The powers that be in British communications have as much and probably more chance than their counterparts in any other country to see that this does not happen.

the motion of Icarus since its discovery by Dr W. Baade at Mount Palomar in 1947. The most recent circular of the International Astronomical Union contains an ephemeris for the asteroid calculated in the United States and collated by Dr Herrick. The asteroid should first become visible in the early days of June next year as an object of the eighteenth magnitude low on the horizon. The closest passage on June 14–15 should be visible from both hemispheres, and the movement of the asteroid towards the Sun should be observable throughout the second half of June. Dr Herrick is planning to circulate more detailed instructions for the observation of the asteroid nearer the time of its closest passage.

The particular interest of the orbit of Icarus next year is that the asteroid will pass close to Mercury as well as to the Earth. This should make it possible to obtain a refined estimate of the ratio of the masses of the two planets. Because of the eccentricity of the orbit of Icarus and the fact that the perihelion lies 0.18 astronomical units from the Sun, precession should amount to about 11 sec of are a century. This implies that study of the orbit may provide a further test of predictions of general relativity, if not on this passage then on some future occasion.

Two Cheers for Nuclear Physics

CRITICISM of the way in which the UK has supported big science was given by Sir Harry Melville, the retired chairman of the Council for Scientific Policy, in the Appleton Memorial Lecture to the Institution of Electrical Engineers. In nuclear physics, Sir Harry said, the home effort was certainly too large if the powerful international facilities to which the UK contributes were taken into account. "By arranging better collaboration in Europe, considerable economy could have been achieved in the construction and operation of machines of moderate size." It was argued, Sir Harry said, that nuclear physicists needed to be trained on medium-sized machines if they were to use the "Yet many countries bigger international machines. in Europe do not have national machines and they still work effectively with the international facility.' national prestige were to be an index of success, the accelerators in the UK had been badly timed.

Big projects well timed, on the other hand, could give the country a lead for many years over other countries and add to its scientific prestige. telescopes, Sir Harry thought, had been well timed and, although the capital cost of the equipment was high, the costs of running telescopes were relatively low. Radio astronomers were also more useful in industry than others, because of their varied experience. But Sir Harry had nothing kind to say about big optical "In optical astronomy the telescopes in Britain. utilization of telescopes is very low indeed because of the atmospheric conditions and it is questionable whether any large telescope should ever have been installed in this country. The natural trend is to erect telescopes in countries overseas where observing conditions can be relied on for long periods of the year."

The next few years would be of great importance to the universities. The Government had announced the financial provision for the next five years, and the research councils knew how much they would be getting in the next three years. Sir Harry was unimpressed. "The increase in moneys year by year is extremely modest, having regard to the job which has to be done, especially in science. A good deal of re-thinking and pruning will have to be done if quality is to be maintained . . . there can be a very real danger of stagnation if vigilance is not maintained and management is not made effective."

ELDO Hopeful

WITHIN the next fortnight, all being well, the next launching in the ELDO programme will take place from Woomera, Australia. ELDO staff are hoping that the launching, called F6/2, will make up for the disappointment of the last launching, which went perfectly until after the separation of the first and second stages. The second stage engines failed to ignite. ELDO is confident, however, that the failure did not reflect on the design of the second stage, but was caused by an electronic failure. For the next firing, simple modifications have been introduced to prevent the same thing happening again.

For the F6/2 launching, the second stage will again be live, and the upper stages will carry the basic elements of systems intended for full test in later flights.

It will be the first flight for testing all the separation systems for all three stages and for satellite injection. After separation, the second and third stage plus satellite will continue in ballistic flight a small distance from each other. The impact zone, in the Pacific some 2,300 miles from Woomera, will be reached in about 18 minutes.

Robbing Peter, Paying Paul

from our Oxford Correspondent

OXFORD colleges vary greatly in wealth, from the older men's colleges—St John's, Christ Church and All Souls—with their munificent endowments, to the penury of the newer men's colleges and most of the women's. Shortage of money has put the members of poorer colleges at a disadvantage in many ways; it is difficult for them to appoint tutors in recondite subjects, so that many students have to be taught outside their colleges. Stipends at some women's colleges have been very considerably lower than those at men's colleges, and there have been far fewer tutors for the same number of undergraduates.

Congregation has now, however, passed a statute which, it is hoped, will eliminate these disparities. Colleges are to contribute to a university fund on a sliding scale so that the poorer colleges will be paying nothing and the richer ones up to a quarter of their income above £150,000. The fund will then be distributed among the poorer colleges so that eventually the endowments of all colleges will exceed £600,000, £200,000 more than the figure recommended by the Franks Commission. The contribution of colleges to the fund will be assessed according to capital rather than income. The Franks Commission had originally recommended an assessment on the basis of capital as being ideal, for certain colleges have large amounts of capital yielding low interest rates, but the commission concluded that this method of assessment would be too difficult to undertake, and decided that contributions should be evaluated on the basis of college incomes. The problem of valuation now lies with the University Chest and the College Contributions Committee, which will be administering the scheme from July 31 next year.

Transitional arrangements have been made: the two graduate colleges, St Anthony's and Nuffield, which specialize in the social sciences, will be taxed by the university at a lower rate than undergraduate colleges. For some time, grants to poorer colleges have been made by the Common University Fund, but the last payments of this kind have been made in the form of £80,000 to be divided between three colleges, and all the work of making the colleges financially equal will now be carried out by the Contributions Committee.

Hydrofoils or Hovercraft?

THE British interest in hovercraft has tended to overshadow hydrofoils, another interesting development. Mr R. Gresham Cook, Conservative MP for Twickenham, is alarmed about this, and opened an adjournment debate on the subject in the House of Commons last week. While Britain has been building 40 hovercraft, he said, nearly 1,000 hydrofoils—craft the bows of which are raised from the water by the action of wing-

like foils—were in operation all over the world. Even in rough water, the ride in a hydrofoil is comparatively smooth-better, it seems, than in a craft of the same weight without hydrofoils. Mr Cook said that hydrofoils are cheaper to manufacture than hovercraft, although more expensive than conventional passenger

In April this year, the Ministry of Technology established a working party to examine the design, construction and operation of hydrofoils. Its chairman is Mr A. Silverleaf, deputy director of the National Physical Laboratory, and among its members are Mr Peter Dory, who operates a hydrofoil service between Jersey and Guernsey and the French coast, and Mr M. N. Parker, of the British Ship Research Association. So far the committee has been involved with a survey of the possible markets for hydrofoils; when this is finished it will turn to the research side.

Research on hydrofoils has in fact been undertaken at the Ship Division of the National Physical Labora-This has been work of a fairly fundamental nature, including studies of the lift produced by the hydrofoils and the forces experienced by them. There has also been some work on propeller design and on the behaviour of the hydrofoils in waves. Experience at the NPL confirms that hydrofoils ought to be able to manage in conditions at sea. Costs depend greatly on the degree of sophistication. At the NPL, work has been carried out on surface piercing foils, which are likely to need less sophisticated control than other types. With surface piercing foils, changes of speed are automatically taken care of by the shape of the foils. If, for example, the craft slows down and sinks into the water, a greater area of foil becomes an effective lift surface, and the necessary lift is maintained. If speed is increased the effective area of foil is reduced, and the craft retains roughly the same attitude. Fully submerged foils, on the other hand, call for more complicated control systems, more like those of aircraft.

Many of those involved deny that there is any conflict between hovercraft and hydrofoils, and say that there is room for both. In a recent paper presented to a Canadian symposium (and reprinted in Flight International on October 19) Mr Christopher Cockerell, inventor of the hovercraft, suggested that hydrofoils would be more suitable at speeds below 40 knots and sizes of less than 100 tons. Mr Cockerell's paper suggests that hovercraft and hydrofoils would be similar in cost, but hovercraft pay a heavy penalty by the lack of suitable engines. With better engines, fuel costs, which amount to 25 per cent of total operating costs, could be reduced by a factor of two or three.

Regulated Surpluses

NATIONALIZED industries in Britain may from time to time complain that they are starved of capital, but there is no doubt that they are generously supplied with decrees by the Government about the conduct of their affairs. The most recent of these is the White Paper on the economic and financial objectives laid down for the various industries (HMSO, 1s. 9d.). For practical purposes, this document is a revised version of that which appeared in 1961 and which first laid it down that nationalized industries should earn a surplus on the capital employed in their businesses which would be comparable in many ways with the profits earned by commercial industries. issue is important because of its influence on the prices charged by the several industries and because of the way in which it affects decisions about large items of capital expenditure.

The new document is more a statement of general principles than a set of detailed instructions—the ministers responsible for individual industries will issue them. The Treasury, which determines general policy, suggests that a revision or at least a restatement of policy has been made necessary by the "important technological changes and discoveries of natural resources" since 1961, but it is also clear that the anomalous differences which have sprung up between different industries have contributed to the need for rationalization. No doubt the Government has in mind the way in which the electricity industry, now wishing to invest in nuclear power stations, should aim at a higher rate of return than the gas industry, now unexpectedly prosperous.

Differences like these should be comfortably ironed out by the doctrine, now promulgated, that decisions about alternative forms of capital investment should be based on the same kind of calculation—one which has the effect of making the effective rate of interest a cool 8 per cent. This is the rate the Central Electricity Generating Board will have to use in calculating the economic balance between nuclear reactors and conventional power stations. This, too, is how the Gas Council will have to make decisions about alternative ways of using gas from the North Sea. This is a good start, and it is also sensible that the new statement of policy should emphasize the importance of linking the prices charged for the products and services of the nationalized industries with the marginal costs of providing them. If this means that the new commercial Post Office will charge less for trunk telephone calls, everybody will be delighted. But it is also important that the new policy statement emphasizes that industries need not slavishly aim to earn the surpluses laid down for them, for this may help in future to avoid some of the absurdities about electricity prices which there have been this summer. In other words, the new White Paper is full of good sense, although it does not go so far as to suggest that ministers should in future refrain from nullifying the benefits of sound policy by pointless interference.

More Money for France

A SUBSTANTIAL increase of the spending of the French Government on scientific research and development is allowed for in the budget for 1968, now adopted. There will be an increase of 17 per cent in the total cost of supporting what is described as scientific and technical research through the public purse, so that the amount spent in the year ahead will be approximately 1,591 million francs. The programme for assisting the development of computers, and the programme of space research, will each receive an extra 20 per cent. One of the greatest of the various increases in the year ahead is that for the support of space research, which will be absorbing an extra 68 million francs. Support for science and technology through the educational budget is substantial, and will take an extra 96 million francs in 1968.

Insuring Nuclear Power

VERY soon, the Paris convention on third party liability in nuclear energy will come into force. The convention, which provides the principles on which international agreements on nuclear liability are based, was signed in Paris on July 20, 1960, but does not come into force until it and ar additional protocol are ratified by five governments. The convention has now been ratified by Turkey, Spain, the United Kingdom, France and Belgium; the additional protocol, which is designed to avoid conflict with another international conventionthe convention on civil liability for nuclear damage, defined by a conference in 1963 convened by the International Atomic Energy Agency—has been signed by the same group, with the exception of Turkey. Turkey is now on the verge of ratifying the protocol, whereupon the whole convention will come into force.

The subject is complicated. Briefly, the convention provides five principles on which legislation should be based. The operator of the nuclear station is absolutely and exclusively liable—without proof that it is his fault —for any accident, but only if claims are made within ten years of the incident. The convention limits the amount of liability, in principle, to \$15 million, and obliges the operator to cover his liability by insurance or otherwise. All claims arising from the same incident should be dealt with in the same court, in the place where the incident took place, and all countries who are party to the convention are obliged to accept the court's rulings. In 1963, the provisions of the convention were extended to provide additional compensation, up to a value of \$120 million, through State intervention.

The convention falls under the jurisdiction of the European Nuclear Energy Agency, which has now published a review of the legislation which obtains in fourteen OECD countries and of how this meets the needs of the Paris convention. Most of the countries have legislation which incorporates the principles of the convention, and some go beyond it. There are, however, some differences—in Japan, for example, accidents during transport are the liability of the consignee. In Germany, there is no provision to limit liability in the event of force majeure. Most countries incorporate clauses which free the operator from liability in such cases, or when the claimant was injured as a result of his own negligence or a wilful act. In the United Kingdom, when the victim has been guilty of committing an act with the intention of causing harm or with reckless disregard for the consequences, the amount of compensation may be reduced. In the United States, the cost of an accident can be even greater—the law provides for liability of up to \$560 million.

Books to Borrow

"NEITHER a borrower nor a lender be" may have been good advice for Hamlet, but luckily for the British general public there are people who do not take it too seriously. The National Central Library borrows and lends more each year from its stock of volumes, which has doubled over the past five years to the present total of 364,000. 1,037 current periodicals are taken, 68 of them being in Slavonic languages. The library, which is largely financed by the Department of Educa-

tion and Science—£141,000 last year—has just produced its annual report for 1966-67.

The British library system works on a two-tier basis, with the central library available to answer requests from both parts. Most libraries belong to regional systems, which are as far as possible selfsufficient. Unsatisfied queries are passed on to the central library. Other libraries which, for one reason or another, are not included in any regional system form the second tier and have direct contact with the central library. As overall centre of the national interloan system, the central library handled 136,660 requests during 1966-67, with 77.5 per cent success, compared with 128,567, 75.2 per cent, in the previous year. Through the co-operating libraries the central library has access to some 20 million volumes. 7,969 overseas requests were received, of which 83·1 per cent were satisfied, as were 72 per cent of the 9,954 requests made by the central library to those abroad. The total number of applications received by the library rose by 6.3 per cent and were handled despite staff shortages. A successful year was recorded by the library, but it ended with the resignation of its chairman because of ill health. Lord Elgin, associated with the library since its reconstruction in 1930 and chairman of the trustees since 1955, had presided over many important developments in the library's affairs.

Another library publication appeared this week, a list of the current serials received by the National Lending Library for Science and Technology at Boston Spa, Yorkshire. (HMSO, £2.) The alphabetical list has been produced as a by-product of the recording system at the library, and, it is stated, may not include a certain number of titles which are in fact available. Only those titles which were current and at the library in March 1967 are included, but not those which were on order. The list is not intended to deter those who would like to borrow periodicals that are not mentioned, as the library relies on such requests for titles of serials that should be obtained. A large number of cyrillic titles are included in the total of 26,000, and for the first time the social sciences are covered.

Fission of Physics Journals

THE Institute of Physics and the Physical Society (which is the name for a single learned society) is embarking on an important change in its publications policy. The Proceedings of the Physical Society is to be split into three sections to be known as Journal of Physics A, B and C dealing respectively with general physics, atomic and molecular physics and solid state physics. The intention is that contributions to the three sections will appear six times a year in alternate months. The price of subscriptions to the new journals is to be £40 (for 18 issues a year) which is more than a proportionate increase on the cost of £24 for one subscription to 12 issues of the *Proceedings*. It will, however, be possible for non-members of the society to take out separate subscriptions to the three parts at a cost of £15 each.

By making this change, the British society is clearly following in the steps of the American Physical Society, which publishes *The Physical Review* and which split this into separate sections at the beginning of 1967. Only time will tell whether the obvious advantages of

these changes will outweigh the disadvantages. No doubt readers will appreciate the preselection of articles which separate publication makes possible. To set against this may be a slight increase of the time-lag before publication and the possibility that important articles will not reach as wide a readership as they deserve. Although it is known that the British society would like to see a wider integration of scientific journals on a European basis, the splitting of the journals is not directly related to developments of that kind.



The society has also in the past few weeks lost through retirement Dr A. C. Stickland, editor and deputy secretary. Dr Stickland joined the staff of the Physical Society twenty years ago. She has been appointed managing editor of the *Annals of the International Years of the Quiet Sun* under the auspices of the International Council of Scientific Unions.

Making the Most of Manpower

A POLICY conference on highly qualified manpower held in September 1966 is agreed to have raised more problems than it solved, but the report just published by the Organization for Economic Co-operation and Development (OECD), which ran the conference, includes an impressive list of recommendations.

Means of adapting educational systems to meet economic needs were discussed, together with the use of manpower by employers. In an age when science and technology are expanding quickly, education and training are vital and must be continued throughout the working life of each individual if accumulated knowledge is not to become obsolete—this is a familiar theme of OECD. The report lists five areas in which research must be done if the problems involved are to be pinned down. These cover improvement and standardization of statistics of personnel and the reasons why different people chose various academic subjects, training and jobs. Only by following individuals through various stages in their careers will the important influences be discovered.

The conference recommends to the member countries that they should develop systematic policies for the training and efficient employment of highly qualified manpower to meet their economic needs. The aim should be to provide education at school, university and

in the job which adds up to a coherent whole for each individual, as well as providing a complete information service on employment situations and possibilities so that the best use is made of qualified workers. National bodies would have to be set up to organize the required research and put results into effect. On an international level, OECD members were asked to continue the present work by referring back to the governments concerned. A further conference is proposed.

Foot and Mouth Disease

More than 21,000 animals have so far been slaughtered during the fastest moving epidemic of foot and mouth disease for nine years in Britain. The number slaughtered includes 7,614 cattle, 6,709 sheep and 7,335 pigs. There have been 87 separate outbreaks.

The causative agent is a spherical RNA virus, 22 millimicrons in diameter and designated sub-type O1. Of the seven immunological types so far characterized, foot and mouth disease in Britain is usually attributable to the three types O, A and C. There is no reason to believe that type O1 is particularly virulent, and it has indeed occurred in several European countries during the past few years. The most recent alarming outbreak of the disease in Northumberland and Southampton in 1966 was also caused by type O1.

The present outbreak was confirmed on October 25 at a farm near Oswestry in Shropshire, at a time when a large animal market was being held in the vicinity. There seems, however, to be no link between these cases and outbreaks in other counties. The veterinary service of the Ministry of Agriculture fears that there may well be an undisclosed source of infection in the area. It is possible, but unproved, that this epidemic is the result of infected meat imported from abroad. The virus can survive for days outside the animal body. It can be transmitted mechanically by direct contact from one animal to another, on cars or on the beaks and feathers of birds; transmission can also be airborne. As long as trade continues with infected countries, sporadic outbreaks in Britain will occur.

Another feature of the virus is its tendency to undergo antigenic variation. A vaccine incorporating the three types O, A and C is already being used on cattle in France, Belgium and Holland with encouraging results. The cost of such a vaccination scheme in Britain is said to be prohibitive—far greater than the cost of slaughter—and would, in any case, be impracticable. This is why there is a tendency to believe that charity begins abroad. Vaccine produced at the Animal Virus Research Institute, Pirbright, is exported to Turkey, Bulgaria and Greece. The institute also serves as the world reference laboratory for typing foot and mouth virus and for collecting information about epidemics.

How to Deal with Oil

"The greatest peace-time operation ever mounted in the United Kingdom." This is how the recent report compiled by L. R. Beynon, British Petroleum Company, describes the efforts to fight the oil pollution after the Torrey Canyon incident. Far from being a mere account of events, the report includes the action which was taken and its effectiveness, and possible alternatives and considerations for future treatment of similar disasters.

The beginning of the operation was actually a series of failures. The rough weather did not make salvaging operations easy and pollution on this scale had never occurred before. The floating boom which had been intended to encircle the ship and reduce the spread of oil had not arrived. Furthermore, it was not until ten days after the ship had run aground that the air attacks were begun to fire some of the oil slicks and to destroy the remaining cargo. In all, 160,000 lb. of high explosives, 10,000 gallons of aviation kerosene, 3,000 gallons of napalm and several rockets were used to burn the oil in the ship and the surrounding area of sea.

Early attempts on the Cornish coast to fight the oil slicks which were building up on over a hundred miles of beaches are described as largely uncoordinated and haphazard. "Detergent was being sprayed or slopped from small boats with little effect and its application to polluted beaches and rocks was, in general, remarkably inefficient and wasteful." Detergent treatment without subsequent water washing added to the distressing situation instead of abating it, and booms which had been installed at Porthleven Harbour proved useless. It was not until troops had been drafted into the area and vast supplies of detergent were introduced that any encouraging results were seen.

One possible method for fighting oil pollution on the sea involves the use of vessels designed to skim the oil from the surface. Such vessels have been used in sheltered harbours but cannot be considered as sea going vessels. The report suggests that the problems of skimming oil off the sea might be reduced by the application of coagulants and floating absorbents; but the problem still remains of collecting the coagulated contaminant in open sea conditions. Another alternative is to sink the oil using granulated materials of high density. The sunken oil, however, could damage marine life and there is a danger that it would not be rendered permanently immobile and could repollute the water. The destruction of oil by burning on a damaged tanker is impracticable, the report says, and attempts to burn oil floating on the sea are inefficient. The only practical means of dispersing the oil is by the application of detergent: in all, about one million gallons were used to emulsify oil at sea in the case of the Torrey Canyon.

Several lessons were learned about operations on and close to shore. Booms were found to be effective in preventing the passage of oil only if exposed to sea conditions with currents of under two knots or waves less than a foot high. Where oil reaches appreciable thickness in harbours, sewage vehicles can be used to skim off the oil, or absorbent substances such as straw, ash and plastic materials can be used to absorb the oil. Oil stained harbours and estuary walls can be cleaned effectively by spraying with detergent and then hosing down with jets of water. The quickest method of cleaning a sandy beach involves the use of an agricultural rotary cultivator. This breaks up the oil and mixes it with sand, and detergent is then sprayed on.

For the future, the report recommends the building of tankers with high speed pumps to transfer the oil from a damaged tanker, some efficient means of mixing the sea water, oil and detergent after spraying the oil slicks and the development of cheaper, less toxic but effective detergents.

Step Nearer to Space Stations

from a Correspondent

Ir is curiously appropriate that one of the space "spectaculars" marking the fiftieth anniversary of the Russian Revolution should have been a demonstration that a technique fundamental to building orbiting space stations has been mastered—that is, the automatic rendezvous and docking of two spacecraft. It is to that astonishingly practical analyst of the laws of space exploration, Tsiolkovsky-whose principal work was done in isolation and hardship in the later days of the Tsars—that is due the main credit for the space station concept—a combination of springboard to the stars and the most economic means of obtaining an extra-terrestrial observing platform. After the revolution, Tsiolkovsky received the recognition and applause he no longer sought, but his message is remembered. As the Russian space strategy emerges, it sometimes seems that Tsiolkovsky's ideas are the conceptual blueprint for it.

It may be months or longer before a space station exists in orbit, but this is probably now the Russian intention. Such a contraption could be of great value in its own right, both as a manned observatory for a wide range of surveys and for astronomy freed from the encumbrance of atmospheric instability. Several senior Russian scientists—at the Belgrade Astronautical Congress and elsewhere—have recently expressed unqualified enthusiasm for the observational possibilities. Equally, a space station could be used as a staging post for assembling the components of the substantial "spaceship" required for the manned leap to the Moon. The basic techniques are the same, and the smooth encounter, joining and separation of Cosmos 186 and 188 show these no longer pose insuperable problems. Similar manoeuvres will have to become routine and a considerably higher orbit achieved before a multi-component space station can be assembled.

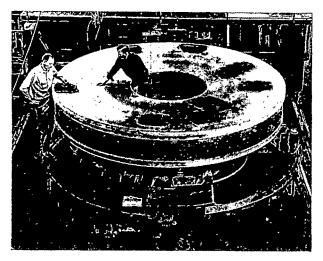
But the Russian claim that the automatic link-up is the key technological advance in the present phase of space development seems justified.

In last week's encounter, Cosmos 186 was the "active" partner, and was subsequently recovered. Independent evidence indicates that Cosmos 186 and 188 were of Soyuz dimensions (3×9 m) and can therefore carry a man, or even men. The active Cosmos 186 seen on television pictures was equipped with brief curved "wings", which suggests a certain degree of built-in aerodynamic control. Hints of such a development for the more complex manoeuvres of the future emerged in the remarks of an influential Soviet engineer at one of the technical sessions of the Belgrade meeting. It would obviously be more convenient if a returning cosmonaut could arrange to touch down near home rather than abroad, even if in future he will also be equipped to land in the sea.

Quartz Mirror for Kitt Peak

The fusion of fifteen tons of quartz into a mirror blank takes the astronomical telescope at Kitt Peak National Observatory, Arizona, one step nearer completion, but there is still more than two years of optical work to be done on the mirror before it is ready for use. The General Electric Company of the USA made several attempts at fusion before successful treatment of the 158 inch mirror blank at 3,300° F in a specially

designed furnace. The mirror will now be transported from the works in Ohio to Kitt Peak where the grinding and figuring of the surface to an accuracy of one millionth of an inch will be carried out.



The world's largest quartz mirror blank, now ready for shaping and polishing for the Kitt Peak telescope.

When completed, the Kitt Peak telescope will be the largest telescope to use a quartz mirror instead of conventional glass. Quartz has the advantage that its expansion with temperature is one fifth that of ordinary glass, as well as being very hard and rigid. According to Mr R. A. Popp of GE(USA) it can be polished and finished faster than borosilicate glass. The Kitt Peak installation will be the first big telescope to use the Ritchey-Chrétien optical system. This form is not new, but is only now being generally adopted for astronomical telescopes. The Newtonian-Cassegraine system, used for almost all existing large telescopes, gives perfect axial imagery, but its inherent coma limits the useful field size, particularly at the prime focus. The Ritchey-Chrétien form gives a much larger coma-free secondary focus but the prime focus image has quite heavy spherical aberration. In either form the field of the prime focus can be extended by the use of afocal lens systems, but rather unexpectedly these give considerably useful fields on the Ritchey-Chrétien telescope.

The Optical Design Group at Imperial College under Dr C. G. Wynne has investigated field corrector systems for such telescopes as the 200 inch at Mount Palomar (Newtonian-Cassegraine), Macdonald (Ritchey-Chrétien) and Kitt Peak. The 108 inch Macdonald telescope, like that at Kitt Peak, is to use a quartz mirror and is at present under construction.

Research into Blindness

Wanted—one million pounds by the "Fight for Sight" campaign to provide an annual income of at least £50,000 for work undertaken at the Institute of Ophthalmology in London. Government financial aid to the institute is insufficient to cover the cost of the work; consequently, the institute must augment its income by private benefaction. The president and emeritus director of research, Sir Stewart Duke-Elder, was also the prime mover in establishing the institute in 1946, in association with Moorfields Medical School

and as part of the University of London. All the teaching and part of the research costs are met by grants from the University of London and the institute has so far received £419,345 from other sources. This has enabled the setting up of two research fellowships for the study of glaucoma and a fellowship has also been initiated for the study of eye diseases and related problems, particularly in children.

The research work in progress is extremely varied, but with more than five million blind children alive in the world today there can never be too many investigations into the causes and cures of blindness. Some types of blindness are caused by hereditary factors-retinitis pigmentosa or degeneration of the retina, for example. Work in this direction is conducted at the molecular level. Other types of retinal abnormalities are caused by environmental factors, as seen in premature babies who become blind when supplied with oxygen shortly after birth. Increased blood pressure or hypertension is another important factor determining defective sight. Under the direction of Professor N. Ashton, this condition is being induced experimentally in animals with a view to determining the cause of the defect. The use of lasers for bloodless surgery on the anterior part of the eye (iridectomy) is also being investigated at the institute and a laser slit lamp—really an ophthalmoscope incorporating a laser has been developed there. Other projects include an investigation of visual pigments, laboratory diagnosis of eye diseases, electron microscopy and serial reconstruction of the retina as well as efforts to improve underwater vision.

The research teams are, however, hindered by lack of space. The construction of another floor at the institute is being debated but, until something definite is arranged, the number of staff will be limited and research programmes will take longer to complete.

Generator for Surrey

THE rewards of collaboration between universities and industry would appear to outweigh any possible loss of academic freedom. This is true, at least, for the University of Surrey. The Standard Telecommunication Laboratory has just presented the Electrical Engineering Department with a 2 million volt Van de Graaf generator. The generator is not new; it is, in fact, eleven years old and STL had no further use for it, but it seems admirably suited to the intentions of the department.

Professor D. R. Chick, of the Electrical Engineering Department, has a research team working in collaboration with the UKAEA on the implantation of heavy positively charged ions into semiconductors and metals. The generator will be used to implant impurities directly into silicon and germanium. It will also be used for X-ray and neutron studies of interest to the physics department and the radiation unit of the university. The machine will also be an invaluable teaching tool in the department's new M.Sc course on nuclear radiation and semiconductors which is to be run in collaboration with other departments of the university and the UKAEA, and which starts in October 1968.

This M.Sc course will have its emphasis on the experimental and engineering techniques of radiation production and detection, and of diagnostic methods

of investigating the behaviour of semiconductor materials. The student will also gain a sound background in nuclear and atomic physics and solid state theory. The object of the course is to produce nuclear and solid state technologists able to apply themselves to problems which do not fall into the individual category of nuclear physics, solid state physics or electrical materials.

Money for Caltech

A CAMPAIGN to raise \$85.4 million in capital funds has been launched by the California Institute of Technology. This includes \$20 million for the endowment of faculty salaries, \$30 million for new buildings and renovations and \$35 million for the increased operating costs of its academic programmes and physical plant. The president, Dr L. A. Dubridge, suggests that the most urgently required buildings are a geophysics and planetary sciences laboratory (\$2.45 million), a cyclotron building to house a particle accelerator (\$1.7 million), an applied mathematics building (\$1.625 million) and an engineering laboratory to provide room for an increasing variety of projects in the fields of electronics, materials, aeronautics and hydraulics. Four halls of residence are also required for unmarried students (\$2.425 million) and a new physical education building is also needed (\$1.15 million). Last but not least, a building is needed to house the Buildings Operations department, already the largest business activity in the city of Pasadena (\$2.425 million).

Caltech already owns the land necessary for the new buildings, according to Dr Dubridge, and some of the buildings will be located on the main campus.

Scant Recognition

The discovery of radium by Pierre and Marie Curie is being marked by an exhibit in the Atomic Physics Gallery of the Science Museum, London. Visitors should go up to the second floor and proceed to section 44 where, if they look carefully, they will see an unobtrusive picture of Marie Curie bearing the following inscription: "Becquerel discovered the radioactivity of uranium in 1896 and in 1898 Madame Curie showed that thorium was also radioactive. In the same year, Pierre and Madame Curie, working together, succeeded in separating from uranium ores two much more strongly radioactive elements which they named polonium and radium. In her later life, Madame Curie played a greater part in the application of radium and other radioactive substances in medicine."

It is to be hoped that the museum will do better when, next month, it celebrates the 25th anniversary of the first controlled nuclear pile, built by Enrico Fermi at Chicago.

'Nature' Appointment

DR JOHN TOOZE, a lecturer in the Department of Biophysics at King's College, London, will join the staff of Nature as Assistant Editor early in the New Year. Dr Tooze was educated at Jesus College, Cambridge, and has spent some time at the biology laboratories at Harvard University on a Wellcome Trust post-doctoral fellowship. In the next few months he will be helping with the production of the journal.

Parliament in Britain

Education

THE Government should keep an open mind on the question of loans and grants for postgraduate education, according to Sir Edward Boyle, Opposition spokesman on education. He was speaking in a debate on the Queen's Address, in which Conservative members took the opportunity of criticizing Mr Patrick Gordon Walker's handling of comprehensive schooling and the British Museum. Nothing should be done to deter working class children from going to university, he said, but if we wanted a large expansion in postgraduate education in the seventies, the possibility of a loan element should not be forgotten. Sir Edward also said that the Government's new target figure of 220,000 to 225,000 students by 1971 was too low, although it was well above the Robbins predictions. The vice-chancellors' committee, he said, had put the figure at 245,000. Why had the Government not accepted this?

Mr Gordon Walker first explained his position on the British Museum. He had been charged, he said, with reversing a firm decision to go ahead with the Bloomsbury scheme. This was simply not true. "There was no clear decision by the previous government, nor any firmness of action or clarity of purpose. On the contrary, it was a sorry tale of indecision." Several members were reluctant to accept this version of events, but Mrs Lena Jeger leapt to the defence of Mr Gordon Walker. It was, she said, an unbearable hypocrisy for Conservative members to appear as the sudden friends of the British Museum and sudden lovers of books. The recent report of the British Museum Trustees was an indictment of successive governments. It was certainly no comfort to any of them.

Finally, Mrs Shirley Williams was able to answer Sir Edward's question. The vice-chancellors' estimate of 245,000 students by 1971 was simply the adding together of the reasonable ambitions of all vice-chancellors, and was not put forward as a collective view. The target of 225,000 would be met without damaging other parts of higher education by trying to shift resources even further towards university expansion. (Debate, November 3.)

Child Care

LORD STONHAM, Minister of State, Home Office, said that the Home Secretary had decided to establish a development group within the Children's Department The group would work in close and Inspectorate. co-operation with the universities, the professional associations and organizations such as the National Bureau for Co-operation in Child Care, seeking to supplement and support the research and development work which these bodies already undertook. The Home Secretary had also proposed to the local authority associations that the present Advisory Council on Child Care and the Central Training Council should be reconstituted and amalgamated to become the central forum for the co-operative discussions and steering of research, development and training in child care. The reconstituted council would be supported in its work by the new development group and by the Home Office Research Unit, both of which would undertake projects within programmes drawn up by the council and approved by the Home Secretary and the local authority associations. (Debate, October 25.)

NEWS AND VIEWS

Why Physics is Fun

Physics at its best is nothing if not elegant. memorable experiments are somehow distinguished by their simplicity and even by their cheekiness. With the benefit of hindsight, for example, it is plain that Rutherford and his associates in the 1900s were asking altogether too much of the simple apparatus with which they sought to understand the processes of radio-This means that their success seems even more remarkable. But the same is also true of the way in which J. J. Thomson first measured the ratio of charge and mass for an electron and—for that matter of the way in which Joule established the quantitative relationships between different kinds of energy. Simple apparatus and clever design can do more than merely establish a proposition: by their simplicity they carry extra conviction. And to make the process even more absorbing, the very best of the experiments in physics contain an element of paradox. Something that might be expected to happen fails to happen.

But is there not a danger that these qualities will be lost by the coming of the new style of working in physics? This is a reasonable question to ask. It is, however, pleasing that the way in which it is now necessary for large groups of people to work closely together on the design and the conduct of a single experiment has not meant a sacrifice of elegance but, if anything, the opposite. A great many of the experiments carried out in connexion with the large particle accelerators, for example, have benefited from the close and detailed study of a group of men under pressure to make the best possible use of expensive equipment and restricted access to it. The result is that experiments like those on neutrinos and muons designed at CERN and Brookhaven in the past few years are in the best traditions of elegance in physics. It is true, of course, that string and sealing wax have no place in circumstances like these, but these were never essential ingredients of Rutherford's experiments either. What matters is the directness of the design.

But elegant experiments are to be found elsewhere than in attendance on the big machines. An intriguing illustration of this has now appeared in *Physical*

Gould's Belt Defined

EARLIER calculations of the relationship between the local cluster of stars and the rest of the galaxy have been confirmed and refined by an article by Dr F. V. M. Clube of the Royal Greenwich Observatory (Mon. Not. Roy. Astron. Soc., 137, 189; 1967). Dr Clube has made use of several recent measurements of the velocity and position of the stars in the local cluster as well as information about the distribution of neutral hydrogen in the nearby parts of the galaxy obtained by radio astronomers in recent years. His general con-

Review Letters (19, 1049; 1967). Drs F. C. Witteborn and W. M. Fairbank there describe an experiment to measure the free fall of electrons in a vacuum enclosed by a copper tube. The immediate object is to test the predictions of Professor L. I. Schiff and M. V. Barnhill a year ago that in circumstances like these the gravitational force on an electron is exactly cancelled out by electrical forces. These, in turn, are supposed to arise because of the way in which the balance between positive and negative electrical charges within the metal is distorted by the presence of a gravitational field. In the experiment which Drs Witteborn and Fairbank have designed, electrons from the cathode are tracked upwards through a metre long copper tube by a moderate electric field. To make sure that they travel along the axis of the tube, the whole apparatus is placed within a superconducting solenoid which provides a magnetic field of 7,000 Gauss or more. The technique is to measure the time taken by the slowest electrons to travel the length of the drift tube and to calculate from this the upper limit to the effect of gravitational force on these same electrons. The first result of this experiment is that the effective force is less than nine per cent of that appropriate to the inertial mass of an electron. The next step will be to repeat the experiment with positrons, for there it is expected that the effective force on the electrified particle will not be cancelled out but, rather, doubled. At this stage there is no way of knowing whether the equipment will provide a means of testing the suggestion that particles such as electrons and anti-particles such as positrons may be found to repel each other, but this is at least an intriguing possibility.

It is true, of course, that this experiment is only possible because of the way in which large superconducting magnets can now be designed. It is also true that the phenomenon which is being studied has been drawn to public attention in the most indirect way. But the way in which the experiment has been designed and carried out is a proof that the traditions of elegance in physics continue. That is something to be grateful for.

clusion is that the local cluster, as defined by the presence of B type stars, occupies a strip 200 parsecs wide and 500 parsecs long with the Sun at the leading edge of the strip and roughly 75 parsecs from the central axis.

In his analysis, Dr Clube has used a total of 113 B stars with accurate radial velocities and with proper motions which have been measured accurately. Following earlier investigators, he has sought to represent the motions of these stars by a model in

which the stars as a whole are streaming and at the same time dispersing as if from some common origin. On this basis, Dr Clube is able to calculate the most probable direction and velocity of streaming and also to infer that the stars of the local cluster would have been at a common origin roughly 37 million years ago. He points out that this estimate is uncertain but that it is also comparable with the time scale for oscillations about the galactic plane, estimated to be about 80 million years. From this point of view, it is inferred that the stars of the local cluster can have had very little influence on the dynamical behaviour of other stars in the neighbourhood.

The local cluster, defined by the B stars, is also known as Gould's Belt, after the English astronomer Gould who worked in the Argentine in the late nineteenth century. The significance of the concentration of bright B stars towards the direction of galactic longitude 160 was first appreciated by Herschel early in the nineteenth century.

Hallucinogens and Psychosis

from a Pharmacology Correspondent

HALLUCINATION provoked by drugs may not be quite the same as the perceptual distortion of schizophrenia, but the two mental disturbances are close enough to lead us to seek a link between their mechanisms. One link proposed is that, in schizophrenia, an abnormal endogenous substance (or excess of a normal one) with hallucinogenic activity is present in the brain. As the recently published symposium Amines and Schizophrenia (Pergamon, Oxford, 1967) bears witness, the likeliest place for such a substance still seems to be among the amines where the quest was started some 15 years ago by Osmond and Smythies. Hitherto, amines that are hallucinogenic-such as mescaline, bufotenin and N,N-dimethyltryptamine—have not been indubitably detected in the human body, whether schizophrenic or normal, whereas the amines which are present—such as dopamine, 5-hydroxytryptamine and 3,4-dimethoxyphenylethylamine—have not certainly been found to induce hallucinations. If the report of Tanimukai et al. (Nature, 216, 490; 1967) is confirmed, however, this impasse may now have been ended by the detection of bufotenin in the urine of all of six schizophrenic patients (three paranoid and three catatonic) and in one of four mental defectives.

In the search for an abnormal amine in schizophrenia, much interest and controversy has centred on the pink spot, first observed in paper chromatograms of the urine of schizophrenics by Friedhoff and Van Winkle (Nature, 194, 897; 1962). That this spot contains 3,4-dimethoxyphenylethylamine has just been confirmed by Creveling and Daly (Nature, 216, 190; 1967), although its chief constituent is probably p-tyramine (Boulton et al., Nature, 215, 132; 1967). The further finding of Creveling and Daly that the pink spot in the urine from two schizophrenic patients contained at least seven different compounds shows how far we are still from fully understanding the spot. The scrutiny of amines in the urine may now be expected to intensify.

Progress in this field might be quicker if a completely reliable means of detecting and measuring an hallucinatory effect in laboratory animals were available. The difficulty of knowing whether a measurable response of animals corresponds to a psychic effect in

man was illustrated in the recent identification by Isbell and his co-workers (*Psychopharmacologia*, 11, 184; 1967) of $(-)\Delta^9$ -trans-tetrahydrocannabinol (Δ' 3,4-trans-tetrahydrocannabinol by another notation) as an active principle of cannabis. In dogs, this compound and cannabichromene both caused ataxia, which was thought to be a good indication of cannabis-like activity; but, in human volunteers at Lexington, only the tetrahydrocannabinol induced sensory distortion and euphoria resembling those of cannabis.

One wonders whether any of the animal tests proposed to predict hallucinogenic activity in mansuch as those of Jacob et al. (Med. Exp., 7, 296; 1962), of Lipman et al. (Arch. int. Pharmacodyn., 146, 174; 1963), of Knoll et al. (Arch. int. Pharmacodyn., 159, 442; 1966), of Smythies et al. (Psychopharmacologia, 10, 379; 1967) or of Corne and Pickering (Psychopharmacologia, 11, 65; 1967)—would have given the correct answer with the two substances from cannabis. Although this question is unanswered, there is no doubt that, at least within a group of compounds, good correlations can be found between an animal response and hallucinogenicity in man, although no single test so far devised has been shown to predict hallucinogenicity in every group of compounds known to possess that property. The mouse head-twitch test of Corne and Pickering, for example, though usually correlating well with the human findings, fails to predict the hallucinogenicity of drugs of the nalorphine type.

Such tests are therefore likely to be most useful when applied to a single group of compounds, several of which are of known hallucinogenic activity in man. Even so, proof of hallucinogenicity in new compounds must rest on experiments in man himself. These experiments should be strictly controlled and supervised by a clinical pharmacologist; but a portion of the youth of today is so hungry for hallucinations that it may provide, unasked, a rough guide to the effects of some of the new compounds in man.

Since the fashionable hallucinogen STP is a methoxy-amphetamine derivative (Snyder et al., Science, 158, 669; 1967), we can take the finding of Smythies and his colleagues (Nature, 216, 128; 1967)—that 3,4-dimethoxyamphetamine and 4-methoxyamphetamine disrupt the behaviour of rats as mescaline does—to mean that two further potent drugs have now been added to the list of hallucinogenic amines. Unless evidence can be got that schizophrenics are able to synthesize one of these methoxyamphetamines, neither compound is likely to prove the missing piece of the jigsaw; but either may well provide the true explanation of how the psychosis of amphetamine addicts develops.

Strength of Ordered Alloys

How does the crystallographic order in alloys affect their mechanical properties? Professor R. W. Cahn, of the Department of Materials Science at the University of Sussex, considered the problem in a lecture to the London Metallurgical Society on November 2.

Professor Cahn began by drawing a contrast between alloys, such as Cu₃Au and Ni₃Fe, in which ordering of the constituent atoms is not associated with any change of symmetry, and alloys such as CuAu and CuPt, in which ordering leads to a change of symmetry and also to a substantial change of shape and dimensions of the unit cell. These two categories behave quite

differently, but share the unique property that the influence of atomic configuration on physical and mechanical properties can be separated from the influence of variables such as composition, grain size, etc.; and the configuration is changed when an alloy is heat-treated to order it. By quenching out different states of order and testing at ambient temperature, even the influence of temperature can be separated out.

A substantial body of research on several alloys of the first type had established that the flow stress is a function of degree of order, reaching a maximum at intermediate degrees of order. Numerous interpretations had been advanced to account for this maximum; perhaps the most attractive, Professor Cahn thought, invoked the observed pairing of dislocations to form "super-dislocations" linked by a ribbon of imperfectly ordered alloy. The ribbon broadens as order diminishes and eventually the dislocation pairs disperse; this stage is held to correspond to maximum strength. Other features discussed included the work-hardening capacity of ordered alloys, their strain-ageing behaviour (which revealed a negative correlation between order and rate of recrystallization of a cold-worked alloy), their creep (revealing a pronounced anomaly at the temperature at which order finally disappears) and their fatigue and fracture properties. These last were rationalized in terms of the influence of order on the morphology of plastic slip in alloys. Order totally inhibits cross-slip, which has a crucial influence on both fatigue and

The second category of alloys—those which change symmetry on ordering—have been much less investigated. Professor Cahn described a recent study by V. S. Arunachalam and himself on the mechanical properties of CuAu, which transforms from cubic (disordered) to tetragonal (ordered). A single cubic grain acquires a system of microstrains because of the shape changes associated with ordering in the various microdomains into which the crystal decomposes. These microstrains produce hardening; if they become too large, spontaneous fracture may also ensue. Strainageing behaviour is quite different from the first category of alloys. A strange consequence of the change of shape of the unit cell is that a very small elastic stress applied during ordering can completely change the morphology of ordered domains and alter both the dimensions of the entire sample and also its mechanical properties. In Professor Cahn's view, this novel form of 'superplasticity', and indeed the behaviour of this class of alloys generally, deserves more attention than it has had in the past.

Bacteria and Plastics

from a Correspondent

THE Microbiology Group of the Society of Chemical Industry held a meeting on Thursday, November 2, on the Biodeterioration of Rubbers and Plastics. First Dr G. H. Booth and Mr J. A. Robb of the National Physical Laboratory read a joint paper on the bacterial degradation of plasticized PVC, describing their experiments with five PVC polymers, 13 plasticizers and two stabilizers. Films were buried at 22°-25° C in neutral soil which had been enriched with bacterial cultures from a variety of sources. When diiso-octyl phthalate was used as the plasticizer and white lead as

stabilizer, the change in properties after burial was relatively small for all fine polymers. One of the homopolymers was then combined with each plasticizer in turn, using white lead as a stabilizer, and finally the series was repeated with a cadmium stabilizer. When di-ammonium phosphate or a chlorinated hydrocarbon was used as plasticizer, the change in properties was less for the cadmium-stabilized film than for the lead-stabilized film, suggesting that the cadmium compound was more toxic than the lead compound towards the bacteria. The fact that the degree of attack depended on the plasticizer suggested that it was this which was being consumed in all cases.

Dr S. H. Morrell of the Rubber and Plastics Research Association, Shawbury, Shropshire, gave a general paper reviewing the effects of micro-organisms, principally fungi, on rubbers and plastics. He reminded his audience that rubbers and plastics were often complex materials containing inorganic as well as organic additives, and suggested that reported attacks might well be confined to consumption of these additives rather than destruction of the polymer. In some cases the attack had been exaggerated, incidents of "failure" being quoted when the material was well able to give good service for some considerable time.

There was a detailed description of the various rubbers and plastics, together with comments on the chemical nature of the material and possible reasons for its attack or otherwise by micro-organisms. It was concluded that evidence of attack was sometimes confused and contradictory, and in some cases it was necessary to distinguish between actual attack and growth on the surface of the material. It could not be said that only naturally occurring polymers were attacked, nor was it possible at this stage to say which chemical groups in each polymer were susceptible. The meeting concluded with a vigorous discussion on both papers.

Simulated Growth and Morphogenesis

from our Microbiology Correspondent

THE behaviour of complex systems, most prominently illustrated by those of air and road traffic control, can be analysed effectively by simulation techniques. The usefulness of such studies is determined by the number of parameters of the system which can be programmed. In biological systems the parameters of growth and morphogenesis are inadequately defined and complete simulations are not feasible. Biological patterns can, however, be studied with computers and two recent papers are sound advocates of this approach.

În this journal Cohen has reported attempts to simulate two-dimensional branching patterns of the type produced by surface cultures of fungi and the vascular systems in leaves (Nature, 216, 246; 1967). Programmes were written for each class of pattern to be simulated, the rules being determined from acceptable hypotheses of the natural system concerned. Cohen limited his analyses to patterns based upon apical growth and sub-apical branching and examined the introduction of four types of effect: (1) varied growth parameters in branches of different rank, an effect producing hierarchical organization; (2) changing parameters during the course of growth; (3) influence of branch length on the extent of growth and branching; and (4) local disturbances in the growth domain. The

latter effect induced the most complex patterns which were analogous to phenomena such as antibiosis and local concentration of nutrients in the growth of fungal colonies.

The second report comes from Dr George Ware of the University of Bristol (J. Theoret. Biol., 17, 91; 1967), who has simulated bacterial growth in submerged culture on a digital computer. Here a programme was compiled which allowed the simulation of mixed cultures and which could accommodate the appearance of spontaneous mutants and contaminants. programme was written assuming that a proportion of the cells were non-viable and that the rate of death was dependent upon the half-life of the organism, the total population and the M-concentration (a strain constant defined as the yield per unit volume of medium). Information of the following kinds was required in the programme: (1) strain parameters such as doubling time (t_d) , yield constant and half-life; (2) culture parameters such as substrate levels and incubation times; (3) specific growth rate and factors modifying it; and (4) the probabilities of forward and backward mutation rates. The killing of cells by antibacterial substances was assumed to be controlled by the strain sensitivity and the concentration and activity of the substance. Ware has used his programme to study the lag phase, simple and complex cultures and the production of mutants. Duration of the lag phase was varied by manipulating the length of the delay array, that is, the delay response to the substrate. With a delay array length of one location, lag was absent, but tenfold increases extended the lag phase to 26, 202 and 1,920 min respectively. In the latter case (1,000 delay locations) the population had nearly attained its maximum size before the theoretical growth rate was reached. The behaviour of mixed cultures was tested by co-inoculation of a bacteriocide-resistant strain $(t_d=45 \text{ min})$ and a bacteriocide-sensitive strain $(t_d=$ 20 min). Following the lag period, both strains grew exponentially for 8 h until growth limiting conditions became established. Subsequent addition of an antibacterial agent produced a decline in the growth of the former strain while the growth of the resistant strain recommenced and continued until the substrate was exhausted. The aptness of assumptions made in programme compilation is criticized by Ware and, despite certain invalidations, well defined growth patterns can be simulated by considering relatively few parameters. That we may predict bacterial behaviour in mixed systems and responses to quantitative and qualitative changes in the growth medium and the population per se is likely to stimulate great interest in both academic and commercially orientated laboratories. At this time, when growth parameters are only partly documented, there is no thought of the computer replacing the pilot fermenter. Eventually, however, we might anticipate wide use of culture simulators to test theoretical schemes before taking up the commitments of laboratory trials.

Myosin Revealed

from our Molecular Biology Correspondent

THERE has for some time been conflict in the field of muscle biochemistry about the nature of the myosin

The thick filaments in striated muscle are molecule. known to consist essentially of this protein, which is responsible for the hydrolysis of ATP, and its interaction with actin is central to the process of muscular contraction. It has been known for some time that myosin consists of a long shaft (some 1400 Å by 20 Å thick), with a globular head at one end, which is the seat of the ATPase activity and of the actin-combining sites. The dispute has centred around the number of long polypeptide chains contained in the myosin molecule—two on one view and three on another. To decide between these is important, for it must be supposed also to define the number of active sites, and whether the shaft (known to be fully a-helical) is a two or three stranded supercoil.

The question seems now to have been resolved by new observations with the electron microscope, and the protagonists of the three-chain model would seem to have been vanquished. Slayter and Lowey (Proc. US Nat. Acad. Sci., 58, 1611; 1967) show electron micrographs of rabbit myosin molecules in which it is for the first time apparent that there is not one head at the end of the molecule, but two. This result was achieved by the technique of rotary shadowing—that is by rotating the specimen during the shadowing process: in earlier work, the outline of one head was always obscured by, or merged into, the shadow of the other. The conclusion is thus almost inescapable that there are two similar chains in the molecule, and this symmetry must be regarded as an important clue to the mechanics of muscular contraction.

Slayter and Lowey have also clarified the relation between the major proteolytic fragments of myosin: tryptic digestion of the native molecule leads to the well known light meromyosin (shaft) and heavy meromyosin (head and some shaft) fragments. In this cleavage, about one-third of the shaft is present in the heavy fragment. The products of further cleavage, subfragments 2 and 1, of heavy meromyosin are now recognized as the portion of shaft and separated single heads respectively. In the electron microscope, heavy meromyosin is indeed seen as a truncated myosin, and subfragment 1 as isolated globules. (It is to be noted that these retain their ATPase activity.)

Although this work settles the gross structure of the myosin molecule, there remains the problem of the mysterious fragments of low molecular weight which are liberated on denaturation and without proteolysis. Several workers have reported that these components constitute perhaps 15 per cent of the total myosin In the most recent study, Locker and Hagyard (Arch. Biochem., 120, 454; 1967) have found that on disruption of the myosin structure by chemical modification, three separate protein components of molecular weight about 17,000, 19,000 and 20,000 are released. Two of these species originate solely from the "heavy" end of the myosin—that is to say they must be present in the heads. Because it has so far been possible to release the fragments only under conditions leading also to loss of activity of the myosin, it is not possible to assert whether they are functional parts of the whole. It has not yet been properly established whether these fragments of low molecular weight in fact form an integral part of the myosiu molecule, or are merely strongly bound extraneous components.

Self Assembly of R17

from our Cell Biology Correspondent

Earlier this year (see Nature, 214, 1074; 1967) Sugiyama et al. reported that incubating MS2 phage coat protein with MS2 RNA produces phage-like particles. Although these self assembled particles cannot be distinguished from authentic phage in the electron microscope, they are not infectious and sediment at 70S instead of 80S. Since then Hohn (Europ. J. Biochem., 2, 152; 1967) has done similar experiments with the closely related phage fr and obtained essentially identical results. These noninfectious particles, assembled in vitro, closely resemble defective non-infectious particles produced in vivo during replication of certain amber mutants (A cistron or Sul mutants) of fr and the closely related phage f₂ and R17. Apparently, both types of non-infectious particle are defective because they lack a minor protein component, the A protein specified by the A cistron, which is present in intact infectious RNA phage (Nathans et al., 1966; Argetsinger Steitz, 1967) and somehow ensures either the correct encapsulation of the phage RNA or the absorption of the phage to E. coli hosts or both.

The obvious experiment was to add this missing A protein to the coat protein/phage RNA mixture and see if infectious phage are reconstituted. The great difficulty, of course, was isolating enough A protein; each intact phage probably contains only one molecule of A protein and this has to be separated from the 180 coat protein molecules. Argetsinger Steitz has done this; the A protein apparently has a molecular weight of about 35,000, and although it is highly insoluble in aqueous buffers enough has been obtained to do the self assembly experiment, and Roberts and Argetsinger Steitz now report the successful in vitro assembly of infectious R17 phage (Proc. US Nat. Acad. Sci., 58, 1416; 1967).

When A protein is added and dialysed with a mixture of R17 coat protein and RNA, the yield of infectious phage increases by a factor of several hundred. The titres of infectious phage obtained are impressively high, up to 1.3×10^7 phage/ml., although the efficiency of reconstitution is only 2×10^{-6} . The maximum yield of infectious phage is obtained when one A protein molecule per phage RNA molecule is added: more A protein does not increase the yield. Two factors probably account for the low efficiency of assembly of infectious phage. First, RNase almost certainly contaminates the isolated components, and this would degrade the RI7 RNA before it is incorporated into particles. Second, the protein and RNA must necessarily be subjected to harsh treatments during isolation and are probably partially inactivated as a result. When the complete assembly mixture was analysed on a sucrose gradient, the bulk of the reconstituted particles were 70S defectives, but the majority of the infectious particles sedimented at 80S like wild-type phage. Mild RNase treatment of the reaction mixture completely removed the 708 defectives leaving only the 80S infectious particles, but even some of the infectious particles are sensitive to RNase whereas normal phage is completely resistant. Clearly some of the reconstituted phage, though infectious, are not quite normal: it seems probable that these arise because some of the protein incorporated is partially damaged

and does not protect the RNA from degradation by RNase.

Despite these qualifications, this experiment is the first in which a phage has been reconstituted in vitro, albeit at low efficiency, from two species of protein and a nucleic acid, and this has considerable implications for ideas of organelle self assembly. Furthermore, the experiment provides definite proof that the A protein is a necessary constituent of infectious RNA.

No Life on Life Origins

from our Special Correspondent

THE first public meeting on November 2 at the opulent new premises of the Royal Society was a disappointing affair. As Sir Robert Robinson said when introducing the morning session, the subject, "Anomalous Aspects of Biochemistry of Possible Significance in Discussing the Origins of and Distribution of Life", was particularly fitting for the occasion because it provided an opportunity for the sort of speculation and intellectual curiosity which had excited the founders of the Royal Society. In the event, the speakers and the audience rarely responded to this challenge. Instead, there was a series of thirty minute reviews—inevitably superficial for lack of time—of the occurrence in some biological systems of carbon, halogen, silicon and vanadium compounds and of why ATP is the universal mediation of biosynthesis. But speculation about either the terrestrial origin of life or the possibilities of extraterrestrial life was really not noticeable. The speakers, many of whom no doubt seldom view their work in this light, may have done their best, but the general discussion never came to life and was very much restricted to the here and now. The smell of the lunch being cooked in the kitchens below was, perhaps, too distracting.

In the afternoon, Professor H. E. Hinton (University of Bristol) described his experiments on suspended animation in insects which prove that living systems can survive alternate cycles of hydration and dehydration-some species apparently survive exposure to temperatures varying from below the boiling point of helium to +104° C after total dehydration. He then gave several cogent reasons for believing that life originated on the surface of the Earth rather than in a continuously wet environment, the chief one being that only in niches of rocks or dust particles, or in small pools subjected to drying, would concentrations of chemicals have become sufficiently high for macromolecules to be formed. Dr M. V. Tracey (New South Wales) then described fascinating experiments on the way stimulants of the central nervous system and anaesthetics induce ordered clusters of water molecules in bread dough, of all unlikely things. He suggested that plant alkaloids originated because of their ability to regulate cell water content and postulated a closed circulation plant. Then there was a description of an anaerobic ecosystem based on the sulphate/sulphide transformations and the possibility that such systems could well be similar to an early stage in the evolution of life after its origin. The last paper reviewed the great diversity of compromising environments which support microbial life, including a brief mention of experiments with simulated Martian conditions. The applause woke the sleepers, and after a few questions the meeting closed.

Diversification in a Government Laboratory

A year has gone since the Ministry of Aviation and the Ministry of Technology were amalgamated. What follows is an account of how one laboratory has responded.

THE Royal Radar Establishment at Malvern, the largest centre for electronics research in the United Kingdom, is still a defence research establishment. But, like other laboratories run by the Ministry of Technology, it is showing an increasing interest in industrial research. Perhaps a tenth of its effort is now directed in this way, and the defence work is also closely scrutinized to see if it could yield commercial dividends. Is this a sensible policy? The question is worth asking, because the tendency to turn military research establishments into industrial laboratories is being actively encouraged by the ministry.

The move towards civil applications at Malvern has had the official seal of approval for only a year, when it was announced that the Ministry of Aviation was to be merged with the Ministry of Technology. In February of this year, the RRE set up a special industrial unit at Malvern to speed the process—the Industrial Applications Unit, directed by Mr B. W. Oakley. This new unit is the channel through which industrialists can gain access to the establishment, which offers them a consultative service with experimental and research backing. The service stops short of engineering development for production, which is left entirely to industry. So far the developments of the unit show more promise than commercial success, but it is early days yet, and a number of the products may well have a future.

In the radar field, use is being made of the Gunn oscillator, which enables transmitters of radar sets to be reduced in size. The oscillator consists of a slice of the semiconductor gallium arsenide, deposited as an epitaxial layer on a substrate of the same substance. Because of the electrical properties of the material, it acts as a source of microwaves when a potential is applied between the epitaxial layer, which acts as a cathode, and the substrate, which acts as an anode. This dispenses with the need for klystron tubes and bulky power packs, since the gallium arsenide slice is very tiny, and the device operates at a voltage of 6-12 volts. The oscillator, invented in the United States in 1963 by J. B. Gunn, has been used at RRE in a variety of small radar sets. These can be used as burglar alarms, portable speedometers, or very accurate radars for short range work. The Gunn effect can be used to produce very short bursts of microwaves, which enables the small radar sets to distinguish distances as short as three feet at a range of fifty yards. This may be of importance in harbour navigation in dense fog.

Other radar developments look far more sophisticated and more distant than this. One group at Malvern is working on the integration of radar with computers, and is developing systems which can function both as general surveillance or as horizon scanning radars. Another military technique, the use of infrared detectors, is also expected to have civil applications. The detectors can be used in a variety of ways—as flaw detectors in such things as brake linings, or for the detection of breast cancers or circulation deficiencies such as varicose veins. Alternatively the detectors can be used as a form of aerial photography, and provide information about sewage outfalls, or the flows of heated water from power stations. Since the possibilities of the technique were first made public six months ago, more than 100 requests for information have come in from industry.

Malvern also boasts a powerful computer group, entirely devoted to development of software. Two main projects are in progress—the development of a simple language for simple computer applications, and the computer-aided

design of micro-electronic circuits. Another development of computers is in the supply of information in the form of displays on a cathode ray tube. This can be particularly valuable to air traffic controllers, who have to handle and correlate large quantities of data, but there is no reason why displays should not be applied to more mundane computer applications such as stock control. By the use of touch wires—essentially switches set into the face of the CRT—the operator can achieve some sort of interaction with the machine, and call up from the data stores such information as he requires.

All these developments are interesting, and possibly profitable. It seems clear that industry has been sniffing round Malvern with increased enthusiasm in recent months. What is not clear yet is whether this inversion of normal industrial policy, with market research following rather than preceding research, can ever work. For one thing, it is likely to prove an expensive way of doing research, both in time and in manpower. Unless industry can tell the RRE exactly what it wants, the laboratory is likely to produce ten developments for every one it manages to sell. An industrial laboratory with this sort of record would soon be shut down, but Malvern will never be subject to the full rigour of industrial competition.

This is really the fundamental problem. It is agreed that one of the problems of British industry is the long gap between discovery and commercial application. As Dr Glazier, Director at Malvern, puts it—"It is well known that the development cycle (research to in-service) for modern systems for defence can extend over many years, and civil systems of any magnitude are not likely to be much different". But they must be different if British industry is to shake off its unhappy reputation for getting to the answer too late. On the face of it, it seems unlikely that a division of responsibility between research and production will help. It may well be a positive hindrance. Without in any way maligning government establishments, it is certainly true that they lack the commercial drive which is a characteristic of the best industrial laboratories.

At Malvern itself, there is marked enthusiasm for the new approach. Dr Glazier draws a parallel between Britain's wartime position and her industrial position now, and hopes that RRE has as significant a part to play in the second as it did in the first. Mr Oakley says that enthusiasm is such that it has become difficult to find the men and the money to do all the things industry would like him to do. "But the important effect," he says, "has been to the defence work. The attitude is now completely different." Dr Glazier makes the same point—"We're trying hard to ensure that defence work useful to the economy is not overlooked." This is fair enough—as long as there is a need for defence research, it should be supported, and any industrial benefits can be considered as happy but unplanned accidents. Real industrial research is a very different problem.

Much will depend on the detailed arrangements. Although in the short term the industrial applications unit will be handling projects begun under defence contracts. ultimately it will have to initiate new projects intended solely for commercial development. When this happens it will be important to insist that industry is involved right from the start, and preferably on a sponsorship basis, so that it has a financial interest in seeing the project through quickly. And it may be better if Malvern restricts itself to research of a fairly basic kind—the supply of critical data, for instance—if it is to avoid problems of commercial secrecy.

Promise and Problems of Modern Science

by C. F. POWELL In his concluding address at the symposium on "Perspectives of Nuclear Physics, Elementary Particle Physics, Radio-chemistry and Nuclear Chemistry", held in Warsaw in Cctober to mark the centenary of the birth of Marie-Curie, Professor Powell discusses some of the problems of the advancement of science in the future.

When I was invited to give this concluding address, it was suggested that it would be appropriate if I spoke briefly on the perspectives of the subjects to which Maria Sklodowska-Curie gave such a great stimulus and the problems which their rapid development raises for the future; on the influence of fundamental research. particularly in particle physics and the manifold applications of radioactivity, on the general development of science and human culture; on the importance of the international co-operation which it engenders; the promise of science for economic development; and the problems raised by the increasing resources in men and money necessary to carry it out. These subjects have been widely discussed during the past few years, and Professor Amaldi and I contributed to them at the meeting of the Council of the European Centre for Nuclear Research at Geneva held to mark the tenth anniversary of the foundation of that organization. Much of what I have to say today is complementary to what was said then. Let me begin by summarizing only briefly the main arguments which we developed on that occasion.

Developments in Particle Physics

I suppose that most people would now agree that one of the outstanding features of our times is the headlong advance of science and technology and that it is in these fields that the human creative intelligence today finds one of its chief means of expression. A country not involved in some aspects at least of advanced science tends to be outside the main stream of human development with the most serious consequences for its intellectual life and its productive power.

Nuclear and particle physics, and the associated subjects which have been reviewed at this symposium, are among the main growing points of science and are concerned with our deepest penetration into the structure of the material universe. From the time of classical antiquity it has commonly been assumed that there would one day be an end to the process of delving deeper into the nature of matter. But such a position can no longer be asserted and it is now not unreasonable to suppose that there are no "atoms" in the o'd Greek sense of the word—"that which cannot be cut". The contributions to this conference have brilliantly illuminated the immense promise and vitality of the subject.

The developments of the past decade, the discovery of large numbers of particles, less stable but not less significant than the electrons, protons and neutrons of our familiar world, and their arrangement into ordered families in a way so reminiscent of the Mendeleef Table of a hundred years ago, demonstrate conclusively that we are entering fundamentally new domains. I recently recalled the astonishing remark made by Lenin in Empirio-Criticism in 1912, when the electron was the only known elementary

particle. At a time when the whole scientific world tended to think of fixed unchanging particles he said: "The electron is inexhaustible."

The great generality of these advances and their profound implications give us confidence that the subject will continue to be one of the principal areas of advance in fundamental science for many years to come; and that the new picture of the constitution of matter which will be established will have resounding effects on the whole of natural philosophy.

In response to the challenge of the subject, and its great promise, large resources in men and money are now being devoted to the national and international institutions housing the great accelerators and associated equipment indispensable for present studies in particle physics. The most powerful states are still able to build great machines from their own resources and there are substantial advantages to the physicists of any country in having their own accelerator. But for smaller states it is difficult to find the means in men and money for the construction and effective exploitation of machines of this magnitude and a widely ranging collaboration has been established in Europe at CERN, Geneva, which has been eminently successful in constructing and running the CERN 28 GeV proton-synchrotron; and this institution serves as the active centre for the consideration of the 300 GeV machine which would meet the needs of western Europe during the last quarter of this century, and provide complementary facilities to the 70 GeV machine at Serpukhov and the 200 GeV accelerator in the United States.

Changes in the Style of Work

It is difficult for a scientist brought up in the style and traditions of thirty or forty years ago to visualize, without seeing them in action, the immense change in the method of work which these institutions represent. They involve thousands of people, and are the embodiment of the most sophisticated technology, of the most beautiful precision engineering, of the most advanced science, and they pose most stringent problems in planning and management.

Sixty years ago, when the Curies were at the height of their powers, and even thirty or forty years later, research in particle physics still had all the charms of individual creation. A man or woman might still have the idea for an experiment, construct the apparatus, with the assistance perhaps of a good mechanic, make the observations, and write an account of the work. It was still even possible to conceive of an experiment and carry it out, with materials immediately to hand, in the space of a few weeks. An artist enjoys similar advantages, and scientists are very reluctant to abandon that close and satisfying method of work until the growing complexity of the subject and the inescapable sophistication of the

methods indispensable for significant investigation force them to do so.

During the 1930s, we began to see a different pattern emerging with the introduction of the particle accelerators. The change was greatly stimulated by the development of nuclear energy for peace and war, the experience gained by a whole generation of physicists of operations on an industrial scale, and the necessity of working together in large teams.

So it was in particle physics that we first saw something of the tone of the science of the future, of the style of work which we may expect to prevail in more and more branches of science as the techniques develop. But what is the justification for such enterprises which make great demands on money and scarce manpower?

Promise of Modern Science

It has always been difficult to assess the implications of fundamental advances in science in their early stages, for we fail to see beyond the horizons of our own times. There is a remarkable passage from a lecture made by Clerk-Maxwell a hundred years ago: "For us who know only the spirit of our own age, and the characteristics of contemporary thought, it is as impossible to anticipate the general tone of the science of the future as it is to predict the particular discoveries it will make. Experimental science is continually revealing to us new features of natural processes and we are thus compelled to search for radically new forms of thought for their description."

It has often been remarked that it took 50 years for Faraday's experiments in electro-magnetism to reach practical fruition. Another example is provided by the developments of the 1920s and early 1930s which saw the birth of quantum mechanics and the incorporation within its framework of the theory of relativity. The concepts which were then introduced seemed strange and esoteric at the time, and of little practical importance; they have now pervaded the whole of science and are of fundamental importance for whole industries. It is similarly difficult for us to assess the consequences which will flow from the developments of recent years. They will surely be very profound, but all our experience suggests they will far exceed our most daring expectations.

It is sometimes said that, for the practice of the future, the deeper penetration on which we are now engaged is unlikely to have great implications since the processes are remote from those which are the most significant for our ordinary experience. This seems to me to be too narrow a view. Even if they do not contribute greatly to developments of industry as we know it, and I think they will and do, it is one of the functions of the most sophisticated science to give rise to radically new industries, undreamt of within the framework of our present perspectives.

Taking into account the recently discovered astronomical objects such as "quasars" and "exploding galaxies" in which there are prodigious sources of energy, estimated to be sometimes as great as 1062 ergs, which cannot be accounted for in terms of conventional nuclear processes, and the most suggestive regularities among the newly discovered particles, who would assert that in a hundred years time, if we do not destroy our whole civilization, we shall not have understood and mastered new sources of energy immensely more productive than nuclear sources of power?

Or who would set a limit to the perspectives which are emerging from the tremendous advances coming from the application of radioactivity to chemistry, medicine and biology? If we fail to think imaginatively about the possibilities arising from the advancement of science, and the means to realize them, who will? Who can?

But our present knowledge in many of these new fields is still rudimentary, like that in the early days of discoveries about electricity when the main facts were the twitching of a frog's leg under electrical stimulus, or the lightning discharge. Who could then have foreseen that such phenomena, so completely remote, so it seemed, from practical application, would one day provide an indispensable element for the whole of our civilization?

Of course it is in the nature of fundamental discoveries that they cannot be foreseen and that we can have no assurance of all the consequences which will flow from them. The new feature of our situation is that the resources required by science are substantial both in men and treasure. We should be careful to distinguish what is assured and what can be reasonably anticipated in making a case for great new scientific enterprises and be prepared continually to assess their significance and to run them down if our hopes seem unlikely to be realized. But if we fail to act with imagination and boldness, very grave consequences will certainly ensue.

At the present time the great states devote about three parts in one thousand of their gross national product to fundamental science, and this fraction is increasing. But how dominant a part will science play in our culture in 100 years time? It has sometimes been remarked that, starting at present levels, and, if the proportion of our resources going into fundamental science doubles as at present every 8 or 10 years, then in 100 years time there would be nothing left for anything else but fundamental science. Some scientists have suggested that the proportion spent on fundamental science should level off at about six parts in one thousand of the GNP; others, that in 100 years we may devote 50 per cent of our resources to it in a situation where, as in the institutions supporting the accelerator, the distinction between science and technology has largely disappeared. It is difficult to find a firm basis for distinguishing between these very divergent predictions, but I would think it most unlikely that in 25 years time we shall keep expenditure down to 1 per cent of the GNP.

But the fruits of profound scientific advances are not confined to the material benefits which arise from them more or less directly in the form of radically new industries. The whole of science and technology, theory and practice, constitutes a most complex organism with innumerable concatenations and we shall need all the wisdom we can muster to ensure a balanced development. But, in our era, the history of science demonstrates the indispensable role, in the advancement of science as a whole, of our basic understanding of the constitution and interaction of the elements of matter at different levels according to the sophistication of our understanding. It seems most unlikely that a real understanding of the new realm in the hierarchy of "elementary particles" which we now seem to be entering can fail to have a similar significance for the general body of the science.

Again, it is an essential feature of the institutions supporting the great national and international accelerators that they work in the most intimate collaboration with the scientists in our universities and other institutions of higher learning. This is of great value to the international institution, but it also has the consequence that the tone of the university departments of science involved, and the quality of the thinking and teaching within them, are stimulated by the fact that they are peopled by men and women engaged in work at the frontiers of knowledge whose imagination is, in a phrase of Bacon's, "being stretched and enlarged to take in the image of the universe as it is discovered". This stretching and enlarging process produces confident and lively minds, capable of inspiring the youth with their own enthusiasm for science and technology.

So I would say that the justification for great expenditures on fundamental science has three aspects. First, because of its effect on the general body of science and on our scientific world outlook; secondly, for the practical consequences which flow directly and indirectly from the advances in science generally in the form of radically new industries and improvements in current practice;

and, thirdly, from the fact that the pursuit of knowledge is an essential element in contributing to a healthy tone in our universities and institutions of higher learning; that this can only be ensured if the people in them are engaged in exacting investigations on the frontiers of knowledge, and is of crucial importance for our whole culture.

Difficulties arising from the Changing Methods of Science

It is sometimes said that the science of 50 years ago was the science of the "pre-historic" scientific epoch. It is a phrase which makes me very uneasy. It implies too little acknowledgment that we see farther, because we, too, stand on the shoulders of giants. It is of course true that there are great advantages in having sufficient resources. Madame Sklodowska-Curie, in a discourse at the Sorbonne in 1924, remarked: "Il est vrai que la découverte du radium a été faite dans des conditions précaires, et le hangar qui l'a abritée apparaît revetu du charme de la légende. Mais cet élément romanesque n'a pas été un avantage: il a usé nos forces et retardé les réalisations. Avec les moyens meilleurs, on eût put réduire a deux ans les cinq premières années de notre travail et en atténuer la tension—l'experience du passé ne doit pas être perdue pour l'avenir."

But while it is a great advantage to have the tremendous resources which are now available, it is not a virtue, and we shall be judged by what we achieve with them—by the quality and tone of our inspiration. We are in a situation where we can afford to be neither complacent nor arrogant.

I see the science of the past 70 years as a kind of golden age and our principal task to ensure its continuation. In the past, periods of the highest achievement have been short and precarious. The principal figures are themselves unique; and so also is the complex of situations and influences which make up the historico-social background. In our times, if science and technology have a tremendous impact on our societies, it is no less true that they are dependent on the general tone of the society in which they exist, on prevailing attitudes towards science, on the esteem in which it is held. And there are a number of disturbing signs of the time which should teach us not to take for granted an automatic progression. The provision of adequate resources is not the only thing necessary for distinguished scientific work. It has long been recognized that it also requires great determination, passion and imagination. There is an illuminating passage from Erasistratus: "Those who are altogether unaccustomed to investigation are, at the first exercise of their intelligence, befogged and blinded and quickly desist owing to fatigue and failure of intellectual power, like one who without training attempts a race. But he who is experienced in making experiments, twisting and turning and worming his way through, does not give up the search, I will not say day or night, but rather his whole life long. He will not rest but will turn his attention to one thing after another which seems relevant to his problem, until he arrives at the solution.'

This passage characterizes the kind of intense enthusiasm which has always been the spur to imaginative scientific work, and it is an attitude we should be concerned to preserve in the greatly changing circumstances of our times. It is not only essential for the advancement of science in the era of great scientific enterprises but the best receipt for a humane, productive and satisfying life. It ought to be an aim for the industry of the future. But great science can never prosper without it and there are some dangers that we shall lose it. I see a great danger that science is tending to become dehumanized.

There are, in the first place, though they are not the most important factors, the difficulties arising from the inescapable transformation in the scale of operations in the most sophisticated sciences and the quite new demands on scientists which follow from them. They are required to work as members of a team, for long periods away from their homes and families, and they commonly play only a modest part in a large enterprise the nature of which often imposes a severe discipline, long hours, careful and relastic planning and a strict adherence to a determined time-table.

We have already seen a similar transformation on the passage from handicraft to factory production and modern large-scale industry. There it often has the consequence that a factory operative finds no outlet for his creative imagination in his work, and his real life begins only when he is relieved of the tedium of labour. The analogy between modern science and industry should not be pressed too far, for in science we rarely do things twice in the same way, but certainly such an attitude is incompatible with penetrating scientific work.

It may be remarked that it is a problem which has been overcome in the past. The building of the Parthenon made relatively greater economic demands on Athens than do large scientific enterprises on our own society. If you make large-scale drawings of the Parthenon and try to duplicate it, you get a building, but one manifestly lacking in genius. The original is in effect a gigantic work of sculpture and the subtlety of line is lost even in geometrical drawings on the largest scale. I am told that each column in the original building was in charge of a master mason who, with his artisans, worked on it for about a year. It is clear that they understood the essential place of what they were doing for the whole enterprise; and that the work made satisfying demands on their taste and skill. L'experience du passe ne doit pas être We must maintain sufficiently perdue pour l'avenir. interesting and challenging conditions of work in our scientific enterprises to produce really creative results.

In the great institutions for particle physics we seem at present to be succeeding, for the subject continues to attract a growing number of the best of the young people who devote themselves to science and this is living testimony to the promise and vitality of the subject. The Parthenon was built before that fatal division between the architects and builders had come about, and it is an important fact about our great scientific institutions that the planning and the execution are all in the hands of scientists. As with the Parthenon, we seem at present to be able to organize the work into groups in a way which does indeed give satisfying scope for skill and originality, and we must make sure we continue to do so.

Dangers for the Advancement of Science

But there are more serious features in our situation. First, the benevolent role of science as the instrument for human advancement, which was clearly enunciated by many of the early protagonists of science in our era, is now seriously called into question. This carries great dangers in a situation where the development of science may be limited more by the supply of gifted people attracted into it rather than by financial limitations on the available resources. There is in some countries a turning away of young people from science to what they feel to be more innocent pursuits. They cannot fail to see that, in spite of the great material benefits which have followed in the rich countries from the development and application of science, and its potentialities for good on a world-scale in the future, there is little indication that these possibilities are being realized. On the contrary, the rich countries are becoming richer and the poor poorer: not only in nutrition and technology, but in science itself.

Far from becoming the great creative element in a new world culture, science is tending to be more and more confined to the scientifically advanced states and this tendency is reinforced by the migration of substantial numbers of the most gifted of the youth, from their own countries, where they are indispensable for its advancement, towards the richer countries where alone can be found the means for significant investigations in the subjects of their choice. And whereas the developments in the poor countries are slow and halting—indeed, we hardly understand how to give aid effectively—a very large fraction of the body of science in the advanced countries, backed by immense material resources, is engaged in the production of armaments and an increasing development of more and more lethal weapons of mass destruction.

These tendencies apply unequally in different countries, but unless effective steps are taken to counteract them they will be very damaging for the advance of science in the future. In the interest of science itself, therefore, not to speak of wider and even graver implications, it is important that some scientists at least, and the more the better, should show themselves to be more than narrow specialists indifferent to the consequences of their discoveries, and should actively contribute towards resolving some of the grave and profoundly difficult problems raised by the headlong advancement of science, which hang like a thundercloud over everything we think and do, by giving some of their time and energy to their resolution. If we do not secure the peace of the world our whole societies are in jeopardy, but it is possible for science to lose its inspiration even without a general war with nuclear weapons.

It is unnecessary for me here to labour another point; that, in the most general sense, fundamental science and technology are indispensable elements in our culture and that in our times it is not sufficient for an educated man who aspires to be an administrator, or to occupy a position of power, to be well acquainted with the humanities and the fine arts only. But in some countries science is in fact held in little regard and such a point of view is tacitly assumed and sometimes even explicitly stated. It is of crucial importance that all over the world science shall be cultivated as a great instrument for human advancement; that its place in our educational systems and in our societies generally shall be strengthened. In many countries, including my own, the great majority of the population gain little acquaintance with science in the schools and it is almost outside the common culture.

There is a final point. I have spoken of the losses associated with the migration of young students from poorer to richer centres. Even more serious is the fact that, in an era where we need all the intelligence we can find, many potentially bright intelligences are being destroyed, especially in the early years of life, even in relatively well-to-do states. In Britain, for example, it is estimated that the intelligences of something like 30 per cent of the children will never be cultivated because of the social conditions in which they live. A school inspector recently stated that a large fraction of the children under 5 entering some schools are already conditioned not to listen because in their homes most of the communication with their parents is in the form of admonition or invective. In many countries where poverty prevails and parents are under greater stress, the proportion must be much larger.

Role of International Scientific Institutions

The establishment of international scientific centres can make a contribution to the resolution of some of these problems. Experience shows that when they are well organized and sufficiently independent the fact of nationality, far from raising difficulties, adds greatly to the strength of such institutions. Nothing binds men together like effective collaboration in attacking difficult and worthy tasks; and the joint effort is strengthened by the various qualities in which different nations excel.

When I was invited to make this address I was curious to see the tone of the scientific world not 100 but 300 years ago, and I found the following passage in the *History of the Royal Society of London* by Thomas Spratt, Bishop of Rochester, published in 1667.

"If I could fetch Materials whence I pleased to fashion the Idea of a perfect Philosopher, he should not be all of one Clime, but should have the different Excellences of several Countries. First, he should have the Industry, Activity, and inquisitive Humour of the Dutch, French, Scotch and English, in laying the Ground Work, the Heap of Experiments; and then he should have added the cold, circumspect and wary disposition of the Italians and Spaniards, in meditating upon them, before he brings them fully into Speculation. All this is scarce ever to be found in one single Man; seldom in the same Countrymen. It must then be supplied, as well it may, by a publick Council, wherein the various dispositions of all these Nations may be blended together..."

Let me add in parentheses that this passage seems to me to give a clear demonstration that, not to particularize, what we call national characteristics, which we tend to think of as relatively permanent, can in fact be dramatically changed in as short a time as 300 years.

Spratt might have widened his list of countries even in his own time. But the presence of many distinguished scientists at a symposium in Warsaw, coming from countries that he could hardly have included, shows that, in spite of lamentable gaps, we have indeed made some progress towards a public council which is really world wide.

Great international scientific undertakings, when well conceived and organized, can help to promote mutual understanding and sympathy among nations. They can help small states to provide stimulating conditions of work for some of their most gifted young people without promoting their emigration; and thus allow the most advanced science to be more closely integrated with their own culture. They can even arrest and reverse what we call the "brain-drain" and help in replacing it by a mutually advantageous two-way exchange.

There are encouraging signs that a wider co-operation is being established around both the national and the international enterprises. There is a concern to co-ordinate our efforts so that we match our machines to the talent available internationally, and do not waste money and man-power. In twenty years time, perhaps less, we may embark on truly world-wide scientific enterprises.

To further steps in these directions, I would like to suggest that this conference might be followed, in due course, by others of a similar kind. This symposium has been remarkably successful, and different from most others, in bringing together scientists from a very considerable range of subjects; and these wider exchanges have been of great advantage. Should it not be repeated and the scope of the subjects be made even wider?

I suppose it is impossible to see how a truly world society will be brought into being, but it is sure that it will be established, if at all, only after long experience of successful collaboration in many fields. I would like to think that it is in the sciences, where we work together so effectively and profitably, that some of the first steps in this direction are being taken and that when the two hundredth anniversary of Maria Sklodowska-Curie is celebrated in Warsaw we shall have gone a long way further. Perhaps we may hope that the institutions for particle physics, and similar enterprises in other disciplines, may lead to the creation of a great international academy devoted not only to the advancement of science on a world scale but also contributing to the establishment of a peaceful world in which the aims of science to be the instrument for promoting human welfare may be realized.

If you find this too fanciful, let me remind you that, in dreaming, we are in good company. In the same discourse to which I have already referred, Maria Sklodowska-Curie, speaking of her husband, said: "Nous savons quelle a été la règle de sa vie: C'est de continuer, quoiqu'il arrive, la tâche commencée: et, selon sa belle expression, 'faire de la vie un rêve, et faire d'un rêve une réalité'." Received October 27. 1967.

Origin of Indigo of Woad

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Although the ancient Briton's woad, from Isatis tinctoria L., has been differentiated from the indigo dye indoxyl-β-D-glucoside, from the tropical legume genus Indigofera, it has never been identified. It has now been shown to be indoxyl-5-ketogluconate and its structure is completely specified.

SINCE antiquity, in temperate climates, woad (Isatis tinctoria L.) has served as a source of the blue dye indigo. The use of this plant of the crucifer family by ancient Britons to tint themselves blue has captured the popular imagination. Less well recognized is the fact that the cultivation of the plant and the production of the dye were principal features of Renaissance agriculture and commerce1. The woad industry declined because of competition from indigo produced from the tropical legume genus Indigofera and has disappeared with the advent of cheaper synthetic

indigo.

Indigofera plants contain colourless plant indican (indoxyl-β-D-glucoside), which on hydrolysis liberates indoxyl, which in turn oxidizes to the dye indigo. It is commonly supposed that the indigo of woad is also derived from the same indican and many modern references state this as fact²⁻⁵. Although the name indican was first coined in 1855 by Schunck⁶ to describe the indigo precursor in woad, it was, however, later used by him, and others for the indigo precursor of other plants, in particular those of Indigofera sp. and Polygonum tinctorium Ait. Its chemical nature in them was established⁸ as indoxyl-β-D-glucoside, to which the trivial name plant indican is now firmly

At the age of eighty, Schunck belatedly recognized that the properties of pure indoxyl-β-D-glucoside were incompatible with those of his woad substance, particularly with regard to the non-fermentable "peculiar sugar" the latter possessed. He proposed that the woad material be called α -indican, and indoxyl- β -D-glucoside be renamed β -indican. but this terminology has never been used.

The most convincing differentiation of the two indigo precursors was presented by Beijerinck¹⁰ at the turn of the century. He clearly demonstrated that woad indigo cannot come from indoxyl-β-D-glucoside. Briefly, the most compelling items in Beijerinck's argument were the facts that enzyme-free woad extracts reliably produce indigo only when treated with weak alkalis, while indoxylβ-D-glucoside is stable even to strong bases. Furthermore, plant and bacterial enzymes which hydrolyse indoxyl-ß-D-glucoside fail to act on woad preparations. Woad tissues contain an enzyme which Beijerinck named "isatase". Isatase action leads to the formation of indigo when it is incubated with woad extracts, but the enzyme fails to attack indoxyl-β-D-glucoside. Accordingly, Beijerinck concluded that a β -D-glucosidic indigo precursor could not be present in woad, but some other substance which liberates indoxyl for which he proposed the name "isatan". Perkin¹¹ confirmed the principal conclusions of Beijerinck's work, but his announced intention to isolate the woad colouring principle himself does not appear to have been

Unfortunately, Beijerinck's choice of the name isatan further confused the already far from clear nomenclatural situation. Isatane already had been employed in 1842 by Laurent12 to designate quite another substance for which a structure was not proposed until 1916 by Lefèvre¹³.

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Hansen¹⁴ confirmed by synthesis 8 yr later Lefèvre's suggestion that Laurent's isatane was 3-hydroxyl-3,3'bioxindole. It has been suggested that this substance, too, may not be assigned the correct name15 or correct discoverer18

None of these workers seem to have been aware of all of the terminology in use, and thus ambiguity still persists in reference works. For example, *Chemical* For example, Chemical Abstracts uses isatan to refer to Laurent's substance, while the isatan in the Merck Index is Beijerinck's compound. Laurent's name clearly has priority in time, but in view of their long co-existence, we suggest Beijerinck's name for the indigo precursor in woad be slightly amended to "isatan B" to avoid introducing entirely new terminology.

This situation was uncovered during the preparation of a review on plant indoles17, and has taken on new urgency with the reports that other indoles isolated from crucifers may be artefacts. For instance, both the plant hormonally active indole-3-acetonitrile and ascorbigen, which is of concern to animal nutrition, now seem to arise by enzyme action during isolation and are not natural products^{18,19}. Thus, study of another crucifer which produces relatively large quantities of an apparently indolic substance may east light on these other problems. Moreover, being employed by a university which bears the Celtic name of an indigo-trader who was its first benefactor²⁰, and whose principal educational efforts are devoted to the production of old Blues, a study of the origin of one of them seemed singularly appropriate.

Woad was grown in Yale University's Marsh Botanic Garden from seed either collected in the herb garden of Sissinghurst Castle, Kent, or purchased from Thomson and Morgan (Ipswich) Ltd. Fresh leaves were placed in boiling water, and the cooled solution extracted with ethyl acetate. The extract was evaporated to a small volume, and samples applied to thin-layer chromatographic plates coated with silica gel G and carboxymethyl cellulose21. Hexane: 2-butanone (1:1) was the most useful of several solvents22. Its resolution was improved by saturation with 0.5 molar formic acid. conditions a blue spot with the visible absorption spectrum of indigo developed at R_F 0.5 on spraying with 1 per cent sodium bicarbonate or with other mild alkalis, in agreement with the reported base lability of isatan B. Indole chromogenic sprays like the Salkowski and Ehrlich reagents²³ gave a blue and a fading purple reaction respectively at this locus. On the other hand, indoxyl-β-D-glucoside had an R_F of 0·1 in this system and did not react to bicarbonate. Indoxyl acetate and indoxyl sulphate (urinary indican) also were well separated from isatan B. Beijerinck's belief that a new substance is present in woad was thus readily confirmed by modern methods.

Accordingly, large scale isolation of isatan B was attempted. Quantitative estimations of the amount of isatan B obtained by different extraction media were made and these supported Beijerinck's report that mildly acid aqueous media are optimal and critical. We found boiling 0.2 molar formic acid: sodium formate buffer, pH 3.5, gave maximal yields of isatan B; appreciable losses

Fig. 1. Structure of isotan B, the woad from Isatis tinctoria L.

occurred at pH 2 or 5. Further studies showed ethyl acetate to be the most convenient solvent and partitioning the water extract five times with an equal volume of ethyl acetate transferred nearly all isatan B to the organic phase. The combined extracts were then evaporated to a smaller volume, dried over anhydrous sodium sulphate and evaporated to dryness. The brown powder so obtained was placed on a 100–200 mesh acid washed silica gel column and eluted with a gradient obtained from a 'Varigrad' with 50 ml. of chloroform in the first chamber and 200 ml. of ethyl acetate in the next two chambers. On a 19 mm diameter column containing 8 g silica gel, isatan B was found to elute after 130 ml. had been collected.

In large scale isolations, isatan B was accompanied by a red pigment, even after a second column chromatography on silica gel G or treatment with charcoal. Evaporation to dryness resulted in it darkening to a deep purple, very hygroscopic material. Repeated attempts to crystallize isatan B have failed, probably because of this contamination. Rapid smaller scale isolations from lyophilized leaves gave less than a milligram of material, but it was only slightly coloured and quantitative assays of both indigo production and sugar content indicated a purity of 60–70 per cent if the structure is as indicated in Fig. 1.

An ultraviolet absorption spectrum of isatan B suggested the presence of an indoxyl moiety and this was confirmed by a positive fluorindal reaction which is a specific test for indoxyls²³. Because the base lability of isatan B suggested it was an indoxyl ester, and its solubility properties indicated the likelihood that a sugar moiety was present, comparisons of the water soluble alkaline hydrolysis products were made after removal of the indigo by extraction with chloroform. Paper chromatography in various solvents indicated that a substance giving typical carbohydrate reactions, reducing 2,6dichlorophenolindophenol, and giving a yellow reaction to bromeresol green was present. It was easily separable from any of the common glyconic and glycuronic acids. No other carbohydrate was detected. Its reactivity to colour reagents seemed most compatible with 5-ketogluconic acid, a carbohydrate not previously reported in higher organisms, although it is known in bacteria. The calcium salt of this sugar was synthesized from glucose by the method of Barch²⁴. The chromatographic and colour reacting properties of the synthetic 5-ketogluconic acid were identical to the sugar acid in the isatan B hydrolysate, and could be distinguished from the otherwise very similar 2-ketogluconic acid (Table 1).

The calcium salt of the sugar isolated from the isatan B hydrolysate was crystallized from water at $p{\rm H}$ 5·0. It was first necessary to treat the hydrolysate with charcoal to remove a yellow pigment and then with 'Amberlite IR-120' to remove potassium ions. Then calcium hydroxide was added to bring the $p{\rm H}$ to 5·0, the volume reduced to 2 ml. by a flash evaporator and the solution kept in a refrigerator overnight. The salts of both the synthetic and the isolated acids lost water at 130° C but did not have a sharp melting point²4.

Final confirmation was the preparation of the semicarbazide derivative of both the plant and the synthetic sugar acids. The decomposition point of both was between 215° C and 220° C as expected²⁸.

Quantitative estimates of the keto-acid content of isatan B hydrolysate by the methyl-1-phenylhydrazine sulphate method³⁰ not only showed the characteristic spectrum of 5-ketogluconic acid, but on a molar basis were exactly twice that of indigo determinations indicating that one molecule of the acid is produced for each molecule of indoxyl. Isatan B is therefore indoxyl-5-ketogluconate. Because isatan B does not react to ketone reagents, the sugar moiety must be in the hemiacetal form. As only the furances structure is possible, the structure of isatan B can be completely specified as indicated in Fig. 1.

This identification has several points of general interest. First, it explains why assays of indican content of woad have been so low as to lead to doubts that it could have been an important Renaissance source of indigo. Analytical methods for indican which employ acid hydrolysis lead to considerable losses when applied to isatan B. Thus the results reported by Berkeley³¹ probably must be multiplied by three or four times to obtain correct indigo contents of woad leaves, and this reduces his calculations of the masses of plant material which must have been handled by the woad industry to a more reasonable value. It is probably still the case, however, that one of the principal values of woad was not as the primary dye source alone, but as a favourable medium for the bacterial fermentation which reduced indigo to leuco indigo, a necessary step in the dyeing process.

Second, the presence of relatively large quantities of a 5-ketogluconic acid derivative in a higher plant has relevance to the still unsettled question of the pathway of ascorbic acid biosynthesis. Indeed, on largely theoretical grounds Loewus and co-workers³² have proposed a 5-keto sugar acid intermediate; their suggestion has been critically discussed by Isherwood and Mapson³². Because woad, like other crucifers, has a high content of ascorbic acid³⁴, it should be good material with which to test the relationship. In this connexion, it also is noteworthy that 5-ketogluconic acid readily reduces 2,6-dichlorophenolindophenol in conditions that are usually considered specific for ascorbic acid. It may be, then, that estimates of ascorbic acid for dietary and other purposes, which have not included a chromatographic separation, have been erroneous, particularly when applied to the many edible crucifers.

Third, ascorbic acid also brings to mind the substance ascorbigen, once thought to be a natural product in many crucifers and to be of dietary importance to animals, but which is now believed to be an artefact produced by condensation of ascorbic acid with decomposition products of glucobrassicin18,19. Ascorbigen is found in woad extracts too, but the connexion with glucobrassicin has not yet been studied. The structure of ascorbigen is still uncertain, but its structure obviously has similarities to that we are proposing for isatan B. Isatan B itself is not likely to be an artefact, for we have rapidly extracted it both from fresh and from quick frozen lyophilized woad leaves with several solvents, and the highest yields are obtained when the solvents are boiling and should be inactivating enzymes. It is believed ascorbigen can be synthesized non-enzymatically from indole, formaldehyde, and ascorbic acid, as well as from the myrosinase induced hydrolytic products of glucobrassicin plus ascorbic acid18. But because ascorbigen, like isatan B, has not been conclusively purified and has an indefinite melting point, the

Descending Paincase on Whatman 3MM paper

Table 1. PAPER CHROMATOGRAPHY OF KETOGLUCONIC ACIDS AND WOAD HYDROLYSATE

Solvent	Woad	5KGA	2KGA	Woad	5KGA	2KGA	Woad	5KGA	2KGA
Butan-1-ol: propionic acid: water = 10:5: 725	1.20	1.17	0.90	1.02	1.05	0.85	1-07	1.10	0.88
Propan-1-ol : formic acid : water = 6 : 3 : 1 **		1.37	1.14	1.00	1.04	1.12	0.98	1.00	1.11
4 per cent pieric acid in 2-methyl-propan-2-ol: water = 4:1	1.53	1.53	1.10	1.58	1.58	1.15		1.57	
Iso-butyric acid (water saturated)20	1.13	1.07	0.94	1.13	1.13	1.01	1.15	1.08	0.98
Spray reagent	o-Phenyl	enediamin	e di-HCl ²⁷	Anili	ae-H-phth	alate**		ine-H-oxa	
Colour developed	Blue	Blue	Green	Brown	Brown	Red	Yellow	Yellow	\mathbf{Red}

homogeneity and identity of these condensation products are open to question. Our results suggest that the structure and potential natural role of ascorbigen deserve further examination.

Finally, because many indigo producing plants are known from widespread parts of the plant kingdom, it is possible that isatan B is not the only exception to the previously accepted generality that indican is the sole precursor of indigo. Hadders³⁵ himself notes, in his comprehensive listing of indigo producing species from twentythree genera of nine families, that it is the formation of indigo, not the presence of indican, which has been reported in all but a few instances. Some of these plants accumulate several per cent dry weight of the indigo precursor, and thus seem to be the materials of choice for investigations of indole biogenesis in higher plants, rather than those plants forming much smaller amounts of indole alkaloids which have until now received the most biochemical attention.

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Nongenetic Transmission of Information

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The handling of female rats in infancy has been shown to affect the activity and weaning weight of their grandchildren.

WE have shown that one significant determinant of the rat's behaviour is the handling experience of the mother while she was an infant¹. This experience was profound enough to modify her offspring's weaning weight and open field performance in adulthood. Thus the experience of one generation was visited on the next generation. Such a finding would appear to have broad implications for the evolution of behaviour. In this context a relevant question is: How far into the future can such effects extend? We have investigated this question by determining whether the experiences of female rats during their infancy would significantly affect the behaviour of their grandpups.

Again within an evolutionary framework, the habitat in which the animal is born and reared is known to affect profoundly his subsequent performance. We have shown that rats which are born and reared in a complex free environment between birth and weaning, or which are given free environment experience after weaning, differ along a number of behavioural dimensions from rats which are reared in standard cages during infancy and after weaning²⁻⁵. Thus for the laboratory rat, cages and free environments may be thought of as two different habitats. We investigated the effects of these habitats on the offspring's behaviour in this experiment.

The grandmothers' experience was as follows. birth, litters of Purdue-Wistar rats were reduced to eight

pups. Whole litters were randomly assigned to groups to be handled or not handled. Handling consisted of removing the pups from the maternity cage, leaving the mother in the cage, and placing each one into a tin can partially filled with shavings. The pups remained in the cans for 3 min and were then returned to their home cage. This procedure was followed once a day from day I until day 20. Non-handled controls were not disturbed between day 1 and 21, when all litters were weaned. The handled and non-handled females from these litters were the grandmothers of the animals used in this study. They were bred when about 100 days old. When pregnant, the females were assigned randomly to one of two housing conditions, to be described later.

The mothers' experiences were as follows. The females were placed either into stainless steel maternity cages (15 in. \times 10 in. \times 7.5 in.) or into free environment boxes. These boxes were triangular compartments formed by placing a diagonal insert into a 34 in.2 box. Food was scattered on the floor, water was supplied by an externally mounted bottle, and "toys" (wooden block, can, ramp, running disk) were placed into each environment. At birth, litters were cut back to eight subjects consisting of four to six females.

When weaned on day 21, the females from each litter were randomly split into two groups, one going into a stainless steel laboratory cage (11 in. $\times 8.25$ in. $\times 7.5$ in.),

Table 1. SUMMARY OF MEANS FOR ALL EXPERIMENTAL CONDITIONS

Handling experience of grandmothers of experimental subjects	Preweaning housing of mothers of experimental subjects	Postweaning housing of mothers of experimental subjects	No. of litters	No. of subjects		n-field ivity Female		g weight g) Female
Non-handled	$\left\{ \begin{array}{l} \textbf{Maternity cage} \\ \textbf{Free environment} \end{array} \right.$	{ Laboratory cage { Free environment { Laboratory cage { Free environment	17 17 11 11	123 133 82 85	17·00 23·60 13·08 15·48	15·02 20·70 9·31 11·58	50-00 48-43 51-45 45-63	47·05 46·26 50·32 44·29
Handled	$\left\{egin{array}{l} ext{Maternity cage} \ ext{Free environment} \end{array} ight.$	{ Laboratory cage { Free environment { Laboratory cage { Free environment	12 12 11 11	90 86 84 86	11·39 16·32 25·55 11·35	18·30 19·17 24·29 17·46	49·73 47·07 44·76 48·76	48·35 44·76 42·93 46·91

and the other into a free environment. The free environments were the same as previously described except that the diagonal partition was removed. Two or three females were placed in each laboratory cage, while ten to twelve pups shared each free environment. On day 50 the females from the free environment were placed in the same type of laboratory cages as those described above.

These females were the parents of the animals used in this study. When approximately 150 days old, one female from each litter was bred to a randomly chosen colony male. All pregnant animals were placed in stainless steel maternity cages. At birth, litters were reduced to eight pups consisting, when possible, of four males and four females. No litter contained less than four pups. The pups remained undisturbed until they were 21 days old. At this time they were placed into a 32 in.2 open field consisting of sixty-four squares. An activity count was recorded each time a pup made contact with a different square. Each pup was given one 3 min test, and after this was weighed.

Table 1 presents the experimental design, the mean activity score, the mean body weight, the number of pups and the number of litters for each of the eight treatment combinations. In the statistical analysis of these data the litter was used as the unit of measurement with a sub-classification for the sex of the pup. For example, the activity scores of all males within a litter were combined and a mean was obtained; the same procedure was applied to the females. These litter sex scores were subjected to a split plot unweighted means analysis of variance. All F tests were based on 1/47 degrees of freedom.

Activity

The interaction of grandmother handling \times mother preweaning housing was significant at the 0.01 level (F, 7.68): descendants of non-handled grandmothers were more active than descendants of handled grandmothers if their mothers had been reared in a maternity cage between birth and weaning. Exactly the opposite pattern was obtained if their mothers had been reared in a free environment during infancy. The grandmother handling × mother postweaning housing interaction was significant (F, 5.04; $\hat{P} < 0.05$): the pattern was just the opposite to that described for the previous interaction. In addition, the preweating housing \times postweating housing interaction was significant at the 0.05 level (F, 5.77). Offspring of mothers reared in two different environments during early life (that is, cage and free environment, or free environment and cage) were more active than the offspring of mothers which had been reared only in cages or only in free environments for the first 50 days of life.

The grandmother handling \times sex interaction was significant at the 0.01 level (F, 21.44). Male weanlings were only slightly affected by the handling experience their grandmothers had received, while the females were markedly affected, with grandpups of handled females being significantly more active than grandpups of non-handled females. Finally, the preweating housing x postweating x sex interaction was significant at the 0.05 level (F, 4.55).

Weaning Weight

The two main effects of grandmother handling and mother postweaning housing were both significant at the 0.05 level (Fs of 4.55 and 5.20, respectively), while the interaction of these two factors was significant at the 0.01 level (F, 8.49). All three of these effects were brought about by one cell: those weanlings whose grandmothers were not handled in infancy and whose mothers were reared in laboratory cages after weaning weighed significantly more than the other three groups making up this interaction. Such groups did not differ among themselves. In addition, the grandmother handling x preweaning housing x postweaning housing interaction was significant (F, 18.80; P < 0.01), and sex was significant $(\overline{F}, 87.99;$ P < 0.01) with male weanlings weighing more than females.

These data for activity and weaning weight reveal that handling females in infancy can have an effect two generations further on; that the nature of the mother's living quarters during her early life will affect her offspring, and that these variables act in a non-additive interactive manner. The interactive nature of the variables should be emphasized: if we had merely taken the female offspring of handled and non-handled grandmothers and maintained them in standard laboratory caging conditions from birth until adulthood (first and fifth groups listed in Table 1) most of the significant findings would have disappeared. Thus the occurrence of free environment experience some time during the mother's early ontogeny was necessary for the effects of the grandmother's handling experience to express itself in the grandpups.

Others have reported findings extending into the next generation. Ginsburg and Hovda⁷ reduced the incidence of death from audiogenic seizures in dba mice by transplanting fertilized dba eggs into C57Bl foster mothers shortly after fertilization, and Ressler⁸ has shown that the strain of foster grandparent rearing young mice will influence the operant response rate of the offspring of those mice. As far as we know, the present experiment is the first documentation that the experiences which an animal has in early life will influence her unborn descendants two generations away by nongenetic mechanisms.

The nature of the mechanisms underlying these effects is not known. Both handling and free environment experience has behavioural and biological effects on the stimulated organisms2-5,8-12. These effects could act through changes in grandmaternal or maternal behaviour or through physiological changes which would affect the developing foetus or modify the milk supply of the grandmother or mother.

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Amide Cotton Effects of Heparin

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Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, Maryland Heparin displays the ultraviolet Cotton effects common to amide substituted polysaccharides. These effects are useful for investigating the conformational alterations, which heparin undergoes with changes in pH, or the addition of biogenic amines.

The relationship between macromolecular conformation and biological functions in polysaccharides is largely unknown because the techniques which have been used for proteins and nucleic acids have had limited applicability or been unsuccessful. The recent finding that mucopolysaccharides as diverse as heparin and chondroitin sulphate have distinct and particular optical properties possibly related to the preferred order of their amino-sugars provides a new means of examining the conformations of these biopolymers in solution.

Heparin, a highly anionic mucopolysaccharide, is involved in two basically different types of biological phenomena: (a) receptor function for histamine and serotonin, for example, in the mast cell; and (b) inhibition of blood clotting proteins and stimulation of lipid clearance in the circulation probably based on non-polar as well as polar interactions with proteins. We have demonstrated by an optical method that heparin has several conformational forms, and this helps to separate the different parameters of its multiple biological roles. All mucopolysaccharides examined so far show amide Cotton effects. These results will be reported separately.

Optical rotation was measured in the Cary model 60 spectropolarimeter and absorption was measured in the Cary model 15 spectrophotometer. Heparin samples were described earlier²⁻⁵.

Fig. 1a shows the ultraviolet Cotton effect in the range $185-220~\mathrm{m}\mu$ for a given solution of H-IV at pH 7 and $2\cdot6$. Although there is a greater noise level for the acidic solution because of the increased absorbance of the solvent, both an increase in the magnitude and a hypsochromic shift in the wavelength of the peak of the Cotton effect were reproducibly obtained. The optical rotatory dispersion (ORD) of H-II ($2\cdot85~\mathrm{mg/ml.}$) at pH 7 and $2\cdot6$ shows the same pattern and change as that illustrated for H-IV. Fig. 1a also shows the changes in the absorption of heparin in the same spectral region. In these conditions, the absorption band of the amide transition is not resolved, but hypochromism accompanies the rotational changes when the pH becomes acid.

Systematic slight changes in rotation appeared to occur at about 230–220 m μ at different pHs using the 0.5 mm path, and so the ORD between 280–220 m μ was investigated at 5 and 10 mm path. The results in Fig. 1b show that the original ORD curve¹ from 250–190 m μ at neutral pH contained an unresolved, smaller Cotton effect. Furthermore, the absorption in this region is slightly hyperchromic. Preliminary measurement of the circular dichroism of neutral heparin showed a small negative band preceding the much larger positive band in that spectral range (that is, 250–190 m μ). Thus it seems that a first, negative Cotton effect is enhanced in the acid form. The difference curve shown in Fig. 1b shows that the trough of this Cotton effect is ~232 m μ . The family of curves intersects at ~217 m μ .

The relation of pH to the changes in optical properties was readily determined for the first Cotton effect (Fig. 1b), but a regularly varying family of curves from 220–185 m μ could not be obtained for pHs 7–2·4 because of the de-

creasing signal to noise ratios and baseline variations of the acidic solutions, especially because the serial changes in pH could not be made with the same solution in the cell. There may be variation among the separately prepared solutions. Table I presents the values of the molar rotation Φ at the various wavelengths for a number of solutions at neutral pH at different concentrations of H-IV using three different Cary model 60 instruments during its development, with successively better penetration in the ultraviolet spectral range 195-185 mμ. The average Φ value for each wavelength was made for the concentrations from 1-2.68 mg/ml. showing that peak values (200 mµ) range from $75-90 \times 10^2$ with an average value of 8,100. In general, the shapes of the neutral curves are similar (see ratios Φ_{190}/Φ_{200} and Φ_{195}/Φ_{200}). Increased relative optical density of the solvent (that is for runs at 1 mm compared with 0.52 mg/ml.) seems to cause experimental errors towards higher values at 190 mµ. Increasing the ionic strength of the solvent from no added electrolyte to 0.005 molar sodium chloride (Table 1) or raising the pH to 8.0 did not affect the ORD of H-IV at a concentration of 1.01 mg/ml.

Table 1 shows the Φ values and experimental variation at pH 2·4-2·7. The $\Phi_{190/200}$ ratio in acid becomes about 2.5 times greater at neutral pH and the peak is displaced ~ 5 mm. Inspection of the data shows a bimodal peak (~ 200 mm and ~ 195 mm) which may be the result of experimental error. The data at intermediate pHs show the same irregularities in several cases. Points were averaged to yield a smooth acid curve for Fig. 1a. The data for the intermediary pHs were also averaged within certain pH ranges to sort out changes caused by experimental variation from those caused by pH. In general, the shape of the curves shifted more regularly with pH than the individual values of the rotations at the peak. The same changes with pH were found for H-II (not shown) as were found for H-IV (Fig. 1b). The difference curve gives in addition a suggestion of a peak at 210 mu with maximum change at 190 mµ and a sharp fall towards

The strong binding of histamine to heparin in aqueous solution has been demonstrated previously^{7,8}. Fig. 1c shows the effect of histamine on the ORD of heparin in the range of the negative Cotton effect. It can be seen that the conformation of heparin in the histamine—heparin complex at $pH \sim 6.5$ differs from that of neutral heparin alone. Histamine binding enhances the negative Cotton effect to a greater extent than that found in heparin alone at pH 2.6, but the minimum is at 225 m μ . The degree of change depends on the histamine to heparin ratio as would be expected from binding studies⁷; however, the maximum change in the trough occurs at a ratio of less than one histamine to each sulphamino group under these conditions.

Serotonin also causes the appearance of the trough at 225 m μ ; however, decreased signal to noise ratio as a result of its absorbance precluded measurements below 225 m μ at ratios of serotonin to heparin sulphamino groups of 0·135 and more in these conditions. Table 1

shows the ORD in the range 185 to 220 m μ for the serotonin–heparin complex, ratio 0·125, showing the changes in the same direction as the acid form (that is, increased Φ and hypsochromic shift) but to a small extent.

The optical rotatory dispersion of heparin (and other mucopolysaccharides) contains at least two Cotton effects in the region of amide-transition bands. Previous work has shown that amino derivatives of simple sugars and polysaccharides containing these derivatives show negative Cotton effects near 200 mu while simple

polysaccharides show plain dispersion to 200 m μ . The amino derivative in heparin is sulphamino glucose

In the present study, the ORD of polygalacturonic acid was measured to assess the possible role of carboxyl groups in the ultraviolet Cotton effects because heparin

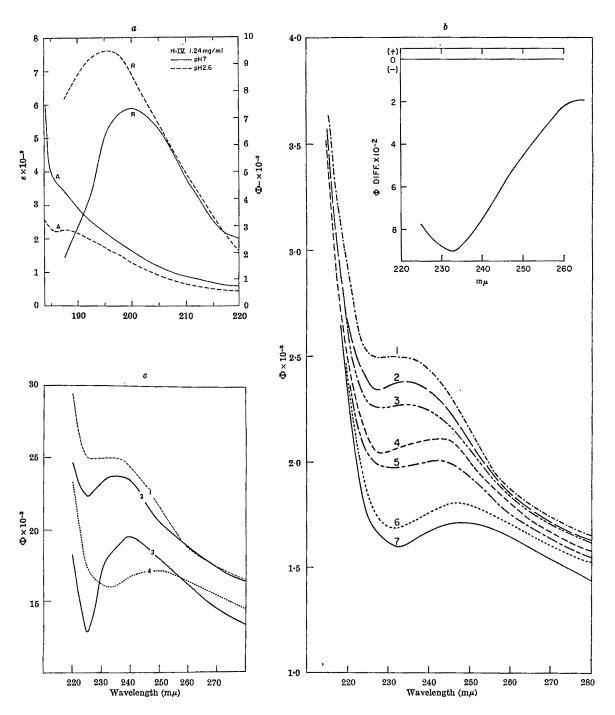


Fig. 1. a, Optical rotation (R) and absorption (A) of H-IV in the spectral range 185-220 m μ (path length 0.5 mm, 1.06 mg/ml.). Left ordinate: extinction coefficient × 10⁻⁸, based on a dimeric unit of 543 (ref. 4). Right ordinate: molar rotation, Φ × 10⁻³, same dimeric unit. Cary model 60, full scale sensitivity 40 mdegrees, 30 sec/period. b, Changes with pH of H-IV ORD in the spectral range 280-220 m μ . Curves 1-7 are those at pH 7, 6-1. 5-5, 5-0, 4-4, 3-1 and 2-7, respectively, for a given heparin solution, 1-52 mg/ml., at a path length of 0.5 cm. The inset is the difference curve between curves 1 and 7. c, Curves 1 and 4 are the neutral (pH 7) and acid (pH 2-7) ORD for H-IV. Curves 2 and 3 are for the histamine-heparin complexes, ratio of biogenic amine to sulphamino group of 0-37 and 0-79 and pH 6-9 and 6-3, respectively, at a heparin concentration of 0-88 mg/ml. These concentrations of histamine caused either no change or a shift similar to low concentrations of sodium chloride in the water baseline. Changes in ORD are attributed to changes in conformation of the heparin chromophore rather than to dispersion interactions between histamine and heparin.

							Table 1.	HEPAR	N AMIDE	corron	effect							
								Wa	velength	(Å)					_	_		
	2200	2175	2150	2125	2100	2075	2050	2025	2000	1975	1950	1925	1900	1875	D 108	Φ^{180}	λ	
H–IV (p	H ~7)	(mg/ml.)					Фх	10-1						Φ_{200}	(I): a	pe. i	
2·68 2·06 1·06 0·88 0·86	22·2 24·5 36·9 27·8	28·6 39·3	28·2 31·5 42·8 25·3	40·1 49·9 30·6	43·9 43·9 47·4 57·3 35·4	54·0 57·6 69·1 44·8	65·0 63·7 65·8 79·9 58·2	73·7 69·5 89·8 67·1	78·6 83·5 74·8 89·8 78·5	72·0 81·7 70·3 82·4 70·4	62·2 58·8 64·6 71·3 66·8	47·4 50·4 42·1 49·7 57·2	30·8 34·3 29·0 31·2 34·7	18·0 18·4 10·6	0·791 0·704 0·863 0·794 0·851	0.302 0.4.1 0.357 0.317 0.112		
Average 0.86*	28 21·0		32	40	45 36·5	56	66 60·0	75	81 83·5	75 80·0	65 70·4	49	$\frac{32}{34 \cdot 9}$	16	0·80 0·84	0·10 0·12	2000 1003	
H-IV: 1		In	50-1	50.1	56-2	73.9	86.1	97-9	104.3	98.7	89-9	87.3	78-5	54.9	0.87	0.75	_91.1	
H-IV p	H (2·4–2	.7)																
1.94 1.06 0.88 1.0	32·2 20·9 44·5	26·2 46·7	32·7 53·1	41·7 61·0	62·2 49·5 68·9 68·1	56·0 84·4 76·4	82·8 67·9 95·0 90·6	89·1 75·6 111·9 100·0	100·0 87·1 123·7 111·0	99·7 94·0 126·4	103·5 96·5 137·3 122·3	100·0 94·0 132·6 125·4	91·0 91·2 124·7 114·4	77·0 —	1·04 1·11 1·11 1·10	0·91 1·05 1·66 1·63		
Average	33	36	43	51	62	72	84	94	105	107	115	113	105		1.10	1.66	135 '	
H-IV (0	-881-0	6 mg/ml	.)‡															
pH ~7 6·1 5·4-5·7 4·3-4·6	28 24 23 26		32 31 32 37	40 39 45 39	45 52 51 56	56 57 66 67	66 70 76 79	75 75 95 93	81 76 100 104	75 74 96 106	65 68 92 108	49 57 86 96	32 52 67 90	16 24 47 80	0.80 0.90 0.92 1.04	0-40 0-68 0-68 0-87	2 67 5 266 5 1 7 3 1 7 3	
3·4-3·0 2·4-2·7	18 33	22 36	23 43	39 51	47 62	58 72	70 84	84 94	94 105	97 108	99 115	$\frac{92}{113}$	89 105	78	1·08 1·10	$\frac{0.91}{1.00}$	1995. 1955	

* Sodium chloride solvent $(5\times 10^{-8}\ molar)$. † Heparin (0.88 mg/ml.) with 0.032 ung/ml. serotonin; ratio of sulphamino to serotonin, 0.12. ‡ These curves represent averages of several curves in the pH range indicated.

is composed of dimeric units of sulphamino glucose and glucuronic acid. Polygalacturonate in the spectral region 350-185 mu had a plain dispersion curve of increasing positive rotation which was the tail of a positive Cotton effect in the low ultraviolet major absorption band of the polysaccharide. Thus it seems that the first negative Cotton effect is probably caused mainly by the sulphamino group (trough about 230 mu). In the case of neutral heparin the trailing ends of the positive rotational bands in the low ultraviolet plus the positive Cotton effect for the 185 mu amide transition mask the relatively small negative Cotton effect. On acidification, a structural change occurs in heparin which causes the enhancement of the 230 mu trough. Furthermore, there is either a shift in the position of the π - π amide band or the appearance of a new peak at about 190 mu. Changes in ionization of the carboxyl group could affect the ORD of heparin around 215 mu, and so the negative circular dichroism band should be studied further to resolve the possible mixed Cotton effects in the 220-230 mu regions. changes in pH were limited to the pH range in which the carboxyl group ionizes, avoiding lower pHs where the sulphate groups (especially the labile sulphamino group) would also be affected. The data indicate that further change would occur below pH 2.7 (Fig. 1b) because there was a significant change from pH 3·1 to pH 2·7. The greatest change in the trough per unit change in pH occurs at about pH 5.0.

The optical properties of heparin and the heparinhistamine complex support the idea that specificity of conformation explains the relative strength of histamine binding by a given heparin preparation^{3,7} and that conformation changes in heparin may accompany the release of histamine which causes the anaphylactic shock concomitant with degranulation of the mast cell in vivo. The origin of the heparin amide Cotton effects lies in the degree of order involving the sulphamino groups. It has been proposed³ from a consideration of the all 1,4-α glucosidic chain with the possibility of helical twisting of charged groups¹⁰ that a preferred orientation could be stabilized by a hydrogen bond involving the sulphamino group and the C_3 hydroxyl of the following uronic acid. This conformation also provides a molecular groove which accommodates a histamine molecule, fitting in the distance between the sulphamino and carboxyl groups with additional possibility of stabilization by nonpolar attraction between the histamine ring and the two axial C-H groups. These data show that histamine binding stabilizes a given molecular order in heparin (seen also in

acid solution) which gives relative enhancement of the negative presumably $n-\pi$ amide Cotton effect. T effect of histamine on the conformation of heparity cand relates to the π - π amide band is still undetermined because of technical limitations; however, at low rates of histamine to heparin the ORD changes in the π π region are similar to that shown for serotonin (Table 1) Quantitative comparison of the effects of the bine ag f biogenic amines and their liberator substances mist in studied further in new conditions where ORD changes can be measured more accurately. For example, cosidering the relatively high concentrations of the historian liberator 48/80 required to antagonize heparin' it may not be surprising that at the low concentrations need sitated by these measurements 48/80 has no appara conformational effect on neutral heparin; on the other hand, it may act by preventing the formation of the heparin structure which favours histamine binding 1 general, it may be considered that secondary order in mucopolysaccharides is in a dynamic state readily in fluenced by other substances in the biological system in which they operate (another example might be the chondroitin sulphate-calcium deposition system in b no formation at the epiphyseal plates).

A conformational change in heparin from pH 2.4 6.1 was suggested previously3 from the changes in incurred (extrinsic) Cotton effects in heparin-methylene blue ecoplexes. At pH 6.7 the presence of histamine caused light changes in the extrinsic effect which were similar in kine to the changes in the induced Cotton effects in herein methylene on going to acid pHs (refs. 1-3). Extrinsic Cotton effects of this magnitude in bound dyes must depend on a net helical order of dye in a given "harded ness", and so the acid-form may have greater molecular order than the neutral form as a first approach to the interpretations concerning the origin of the negative Cotton effect centred about 2175 Å.

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Nuclear Control of a Cytoplasmic Enzyme in Acetabularia

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In four different species of Dasycladaceae (Acetabularia) the isozyme pattern of malic dehydrogenase was found to be species specific. Interspecific nuclear transfers changed the isozyme patterns of the recipient species to that of the nuclear donor species.

THE giant unicellular alga Acetabularia is a good material for studies of activity of the nucleus and its regulatory influence on the cytoplasm. Hämmerling¹ has shown that: (a) the process of morphogenesis in certain species of Acetabularia is dependent on substances which are produced by the nucleus and which determine the speciesspecific morphology; (b) this process can take place in the absence of the nucleus for several weeks, indicating the stability of the morphogenetic substances; (c) the morphogenesis of one species of Acetabularia can be altered by the transplantation or implantation of a nucleus from another species. The morphological characteristics, particularly the stalk and cap formation, in the presence of a foreign nucleus are either completely or partially determined by the activity of the foreign nucleus, and resemble those of the nuclear donor species. In molecular terms these experiments prove that genetic information originating in the nucleus can be stored in a stable form in the cytoplasm.

Using the isozymes of malic dehydrogenase (MDH) as a molecular marker for the four species of the family Dasycladaceae—Acetabularia mediterranea (med), Acetabularia crenulata (cren), Acicularia schenckii (acic) and Polyphysa (Acetabularia) cliftonii (clift)—we have studied the effect of a foreign nucleus on the cytoplasm of these four species. Experiments on the influence of transplanted nuclei on the isozyme patterns of esterase2 and phosphatase³ reported previously failed to give clear evidence

of any nuclear effect.

The four species were grown under normal conditions4. Cells just prior to cap formation were used for grafting experiments. The preparation of enucleate (apical) and nucleate (basal) parts and the technique of transplantation were described by Hämmerling¹. The grafts were allowed to recover overnight in the culture medium in dim light and then observed for 28 days in normal light conditions. In other experiments, the nucleus from a donor cell of one species was isolated and implanted into the cytoplasm of a host of a different species and observed for 28 days.

After 28 days the cytoplasm was analysed for MDH isozymes by acrylamide gel disc electrophoresis. In each case, the nucleus of the cell was removed by amputating the rhizoid with stainless steel scissors and the entire cytoplasmic contents were then squeezed out and transferred into 0.4 ml. of the large pore acrylamide solution. 0.1 ml. of this solution was photopolymerized for 35 min4. We used the buffer system described by Davis⁴ at 1/5 the concentration for the electrophoresis. A current of 4.5 m.amp (300 V) was applied to each tube (6 mm × 75 mm) at 18° C for 35 min. After electrophoresis, the gels were incubated for 90 min in the dark at 18° C in 5.0 ml. of a medium made by mixing 3 ml. of sodium-p,r-malate (0.25 molar), 0.2 ml. of NAD (30 mg/ml.), 0.4 ml. of phenazine methosulphate (5 mg/ml.), 0.4 ml. of nitro BT (10 mg/ml.) (Serva) and 16 ml. of tris-HCl buffer

* Stipendiat der Alexander von Humboldt-Stiftung.

(0.05 molar), pH 8.4. After incubation the gels were washed with deionized water and stored at 4° C.

MDH activity was determined by the method of Ochoa⁵. Single cells were homogenized in 0.5 ml. of 0.25 molar tris buffer, pH 8·7, containing 50 μl. 'Triton X-100'/ml. One ml. of the test solution contained 212 mumoles NADH, 380 mµmoles of oxaloacetic acid, 100 µmoles tris-HCl at a pH of 8.7 and the enzyme. Enzyme activity was expressed as mumoles/min at 20° C. Non-enzymatic degradation of oxaloacetate to pyruvate did not interfere with the NADH oxidation because lactate dehydrogenase activity is considerably smaller than MDH in Acetabularia (unpublished results). MDH was also demonstrated histochemically in the cytoplasm of all four species using the nitro BT stain techniques.

Malic dehydrogenase isozymes in Acetabularia. preliminary investigation, the four species of Dasycladaceae were examined for differences in their specific isozyme patterns. A. mediterranea, A. crenulata, Acicularia schenckii and Polyphysa cliftonii had highly species-specific MDH isozyme patterns characterized both by the number and the positions of the bands

(Fig. 1).

These species-specific differences between the MDH isozyme patterns were confirmed by mixing the cytoplasms of the different species and then subjecting them to electrophoresis (Fig. 1). The two isozyme bands of A. mediterranea were not distinguishable from the first two of the five bands of A. crenulata. The sole isozyme band of *P. cliftonii* is distinct from the three bands of *A. schenckii*. Thus although there is no marker for *A*. mediterranea, the prominent third and fifth bands of A. crenulata, the third band of A. schenckii and the solitary band of P. cliftonii are good markers for these three species.

MDH isozyme patterns after enucleation. Acetabularia is known to form a species-specific cap and stalk even after enucleation. This indicates the morphogenetic capability of the cell even in the absence of the nucleus. Thus it was of considerable importance to see whether the species-specific MDH isozyme pattern was affected by enucleation. Cells of each of the three species, med, cren and acic, were enucleated by amputation of the rhizoids and samples were examined for the MDH isozyme patterns at different intervals up to 28 days. The MDH isozyme pattern was found to be essentially identical with that of the nucleated cells in all three species even 28 days after enucleation. In the 4 weeks following enucleation the total activity of MDH substantially increases (Table 1). This increase is, however, less pronounced than in nucleate cells. An increase in enzyme activity in enucleate cells has been reported previously1.

After enucleation the positions and relative concentrations of the different isozyme bands remain constant. It follows that in the enucleate cells the activities of all isozymes increase to more or less the same extent.

Table 1. TOTAL ACTIVITY OF MDH IN NUCLEATE AND ENUCLEATE CELLS

Day	Nucleate	Enucleate	
0	17·4	17·4	
28	52·2	33·3	

The nuclei of the nucleate cells were removed just before homogenization and enzyme determination. Enzyme activity in m μ moles/min/cell at 20° C.

Furthermore, because enucleation does not affect the isozyme pattern, any changes in this pattern produced by transplantation or implantation can be attributed to the influence of the foreign nucleus.

Transplantation. Transplantation experiments, in which the rhizoid of one species was grafted to the stalk of another, were performed for all possible combinations among med, cren and acic and between med and clift. In each case 4 weeks after the transplantation the cytoplasm was subjected to gel electrophoresis and the gels were stained for MDH activity.

In some of the combinations between med and cren (med₁ cren₀ and cren₁ med₀; Fig. 2), the MDH isozyme pattern was changed to that of the nucleus donor species. These experiments demonstrate that, under the influence of the heterologous nucleus, some sort of transformation takes place. In some experiments, however, the transformation was either incomplete or failed to occur. We refer to such results as "unsuccessful". The percentages of successful and unsuccessful transplantations depended on the particular combination. In all cases, successful transformation was accompanied by definite morphogenetic effects. In the case of cren, med, combinations, all ten experiments were successful with the appearance of cren-specific MDH isozymes in the cytoplasm of med. But in the med, eren, experiments only six out of a total of fifteen were successful transformations. A successful combination of this type only means that the third and fifth cren isozymes disappeared. The appearance of the fourth isozyme band in cren is too variable to be used as a marker. Nothing can be said about the appearance of the med isozymes, for they cannot be distinguished from the corresponding eren isozymes. In the unsuccessful transplants of the med, crene type the pattern was intermediate between cren and med and in some cases the

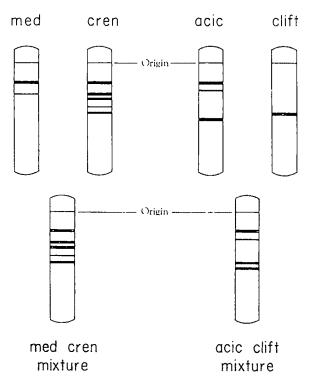
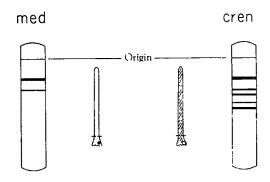


Fig. 1. Isozymes of MDH in Acetabularia and the effect of mixing cytoplasms.



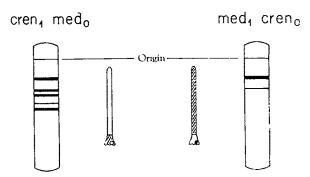
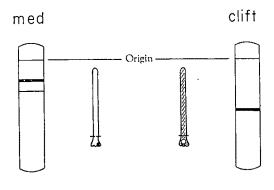


Fig. 2. Transplantation experiments between med and cren; , denotes the presence and denotes the absence of the nucleus of that species.

pattern was more like that of cren than that of med. Intermediate MDH isozyme transformation was accompanied by intermediate morphological changes. Such intermediate morphogenesis of transplants has been described in detail by Werz⁷.

Another series of experiments included combinations of med or cren with acic. All twelve experiments with combinations of the med, acic, type were successful; the second acic-specific MDH isozyme band disappeared with a simultaneous appearance of the faster moving med MDH isozyme (Fig. 1). The typical fast moving acic MDH isozyme band either disappeared or was reduced to a very faint band. In the reverse grafts acic, med, the second med band was considerably diminished but still appeared in most of the grafts, and a faint band corresponding to that of acic was observed after 28 days. In all ten experiments with cren, acic, combinations, the MDH bands specific for crenulata appeared and the acic isozymes disappeared or decreased simultaneously. In the corresponding acic, cren, combinations the isozyme pattern was intermediate between those of acic and cren. The fifth band of cren was considerably diminished with a simultaneous appearance of the fast moving acic MDH isozyme, but the second and the third intense MDH isozyme bands of eren still persisted almost undiminished even after 28 days.

Implantation. To exclude the possibility that the changes of the isozyme patterns after transplanting a heterologous rhizoid are caused by the accompanying cytoplasm, we performed implantation experiments in which an isolated heterologous nucleus was implanted. The eight eren, med, implantations produced the same results as the transplantation experiments and the expected pattern of the eren MDH isozymes was obtained in all the cases. In three out of four med, eren, implantations the eren specific isozymes were considerably diminished and the fourth revealed an MDH isozyme pattern identical to that of med. Results similar to those of corresponding transplantation experiments were also obtained in eight acic, med, and one acic, eren, implantations. The appearance of the fast moving acic MDH isozyme was especially prominent.



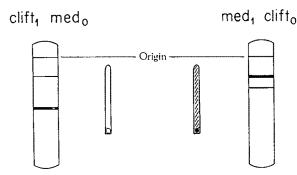


Fig. 3. Implantation experiments between med and clift; , denotes the presence and $_0$ denotes the absence of the nucleus of that species.

P. cliftonii has one very prominent MDH enzyme moving faster than the faster band of med and can easily be distinguished in a mixture of med and clift cytoplasms. Four weeks after nuclear implantations of the clift, med, type, the second med isozyme completely disappeared in five out of ten experiments and the first prominent band was reduced to a very faint band (Fig. 3). The prominent clift band appeared in all cases. The remaining four experiments revealed an intermediate behaviour of different degrees. In the reverse combination of med, clift, the clift band completely disappeared and the two med MDH isozymes appeared in four cases out of eight. In the remaining four cases the MDH pattern was intermediate between med and clift and corresponded quite well with the morphogenetic success of the implant.

Localization of MDH activity. In three species—A. mediterranea, A. crenulata and Acicularia schenckii—MDH activity could be demonstrated in the chloroplasts with a nitro BT stain technique using the same constituents as those for the disc electrophoresis except that the buffer medium was Erdschreiber medium.

An attempt was made to demonstrate MDH activity in isolated cell fractions. In both A. mediterranea and A. crenulata almost all the activity was localized in the particulate fraction sedimenting at about 500g. Quantitative determination of MDH activity using the oxaloacetate-NADH system⁵ showed that in A. mediterranea, A. crenulata and A. schenckii 95 per cent of the activity was in this particulate fraction and only 5 per cent in the supernatant. For further localization, a "cytoplasm fraction" was prepared from A. mediterranea by a method based on that of Thalacker and Behrens*. A crude chloroplast preparation obtained from this "cytoplasm fraction" was subjected to density gradient centrifugation in a carbon tetrachloride-petrol ether system. This method prevents losses of activity by excluding water from the system. Fractionation, after centrifugation, and estimation of chlorophylls and MDH activity revealed that the amounts of chlorophylls and the enzyme activity parallel each other. This provides further evidence that enzyme activity is associated with the chloroplasts (Fig. 4). By subjecting the pooled fractions containing chlorophylls

to disc electrophoresis the two typical med MDH isozymes could be demonstrated. Our results are interesting for several reasons. We have proved at the molecular level that the four species studied differ in their MDH isozyme patterns. The persistence of the MDH isozyme pattern and the increase in MDH activity even 28 days after enucleation again mean either that the isozymes are stable over weeks or, more probably, that synthesis of specific protein takes place in Acetabularia even in the absence of a nucleus¹, in which case the synthesis of all the isozymes of MDH should be under the direction of stable cytoplasmic messenger RNA. This result resembles the observations of Hämmerling¹ on the morphogenesis in Acetabularia in the absence of a nucleus.

The presence of a foreign nucleus in the enucleate cytoplasm results in the appearance of the specific MDH isozymes under the influence of the heterologous nucleus (Figs. 2 and 3) and in the disappearance or decrease of certain previously existing MDH isozymes. It follows that the heterologous nucleus causes the appearance of certain molecular characteristics. Furthermore, our results demonstrate that there is a close relationship between cytoplasmic transformation at the molecular This follows level and morphogenetic transformation. from the fact that in both transplants and implants a full morphogenetic response is accompanied by the complete transformation of the isozyme pattern. An incomplete morphogenetic effect was accompanied by an intermediate isozyme pattern.

It is noteworthy that in the cytoplasm the homologous MDH isozymes disappear under the influence of a heterologous nucleus while the same isozymes not only persist but even increase in amount 28 days after enucleation. This suggests that in the absence of the nucleus the synthesis of the MDH isozymes is directed by the stable messenger RNA in the cytoplasm and that under the influence of a heterologous nucleus these isozymes are eliminated from the cytoplasm by a special regulatory mechanism which is not yet known. It might well be that this type of regulation operates at the translation level. It remains an open question how, if at all, a homologous nucleus influences the isozyme pattern of the MDH in the cytoplasm.

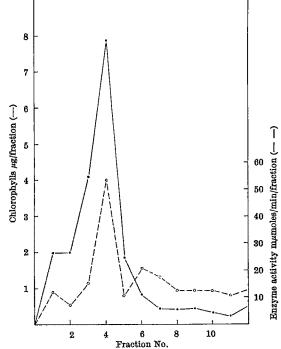


Fig. 4. Density gradient centrifugation in the carbon tetrachloridepetrol ether system of a chloroplast preparation.

The nuclear dependence of the MDH isozymes in Acetabularia raises the question of their subcellular We have shown biochemically and histochemically that these enzymes are associated with the chloroplasts. Because all the isozymes of the different species under investigation are nuclear dependent it is highly probable that there are enzymes in the chloroplasts of Acetabularia which are controlled by the nucleus. The nuclear dependence of a chloroplast enzyme is not surprising if the amount of DNA per chloroplast in Acetabularia is not sufficient to code for all the proteins in these organelles¹⁰. Chloroplast MDH may be synthesized in the cytoplasm and transferred to the chloroplasts, or, alternatively, the messenger RNA directing the synthesis of MDH may be transferred from the cytoplasm into the chloroplasts. Chloroplasts, and probably mitochondria, are not only able to survive and to carry out functions in the absence of the nucleus¹¹ but also multiply¹². means that there is some sort of autonomy. On the other hand, the nucleus is known to direct autonomous functions like RNA synthesis in chloroplasts^{11,13}. The mechanism of nuclear dependency of chloroplast enzymes shown in this article for the MDH might explain the nuclear effect on otherwise autonomous functions.

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Effects of the Food Additive Butylated Hydroxytoluene on Monolayer Cultures of Primate Cells

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Inhibition of monkey kidney cell numbers by the antioxidant food additive, butylated hydroxytoluene, is caused by a rapid depression of cellular metabolism. This effect varies according to sex and may be the result of more rapid metabolism of butylated hydroxytoluene

AUTOXIDATION of unsaturated fatty acids involves the formation of free radicals during the induction period leading to the breakdown of carbon-carbon double bonds and consequent rancidity. Butylated hydroxytoluene (BHT), or 3,5-ditertbutyl-4-hydroxytoluene, acts as an antioxidant by accepting free radicals1. The onset of rancidity in foods containing lipids may therefore be delayed by the addition of BHT. The safety of BHT as a food additive remains suspect. It is not included on the list of permitted antioxidants in many countries2, despite pathological, teratogenic^{3,4} and biochemical tests⁵⁻⁸ which indicate that BHT is a harmless compound in low doses.

Rats are the animals most frequently used for in vivo studies of BHT, and the oral dose required to cause an increase in relative liver weight and in the concentrations of drug metabolizing enzymes and urinary ascorbic acid is about 500 mg/kg body weight. It has, however, long been recognized that experimental animals other than rodents are required for toxicity studies of compounds for human consumption9 because of species differences in the type and rate of metabolism10,11.

In view of the questionable status of BHT as a food antioxidant, the in vivo results, and a possibility of species specific metabolic differences, it was considered important to examine the effects of BHT on primate cells. Primary cultures of monkey kidney (MK) cells were chosen rather than cell lines or strains in order to study cells as closely as possible to the in vivo state.

Initial experiments were designed to find the dose of BHT which stopped cell numbers increasing. When this had been established a more detailed investigation was carried out to study the effects of this dose on primate cell metabolism by measuring the rate of incorporation of radioactive precursors into DNA, RNA and protein.

Solutions of pure grade BHT, the metabolites 3,5ditertbutyl-4-hydroxybenzoic acid (BHT-acid), 3,5-ditertbutyl-4-hydroxybenzyl alcohol (BHT-alcohol) and αtocopherol were prepared fresh for each experiment by dissolving overnight in dimethylsulphoxide (DMSO). All dilutions were made up to contain 0.9 per cent DMSO with 199 tissue culture medium containing 10 per cent calf serum and antibiotics. For the radioactive precursor studies, solutions containing 0.1 µc./ml. of tritiated thymidine-6-T (n) (3HTdR), 0.1 µc./ml. tritiated uridine-5-T (3 H-UR), and 0.05 μ c./ml. D-L-leucine-I- 14 C (14 C-leucine) were made up in phosphate buffered saline (PBS). Eight day old primary monolayer cultures of kidney cells obtained from 2 yr old rhesus monkeys were treated with 0.25 per cent trypsin at 37° C for 5 min and resuspended in growth medium to the order of 5×10^4 - 10^6 cells/ml. Constant stirring maintained a good suspension while aliquots (4 ml.) were removed and seeded into 2 in. diameter plastic Petri dishes. The cells were grown overnight at 37° C in an atmosphere of 5 per cent carbon dioxide in air.

At time zero the supernatant medium was removed, and the monolayers were covered by 4 ml. of growth medium (controls), 4 ml. of growth medium containing 0.9 per cent DMSO (DMSO controls), or 4 ml. of growth medium containing the required dilution of test compound in 0.9 per cent DMSO (test). All media were prewarmed to 37° C, and duplicate cultures were treated for each estimation. To avoid complications caused by individual animal variation, each complete experiment utilized cells from one animal only. After treatment for the required time intervals, the monolayers were labelled for 20 min at 37° C. They were then washed with ice-cold PBS, treated with versene, collected into test tubes and resus-

pended in a known amount of PBS. The cells in each suspension were counted, and 1 ml. was transferred to a small centrifuge tube for extraction. Perchloric acid (0.2 normal) was used to remove nucleotides from the samples, and the acid precipitated proteins and nucleic acids were dehydrated by two washes of ethanol before rendering them soluble in 0.3 ml. of hyamine at 60° C The hyamine was dissolved in 3 ml. of scintillation fluid containing 0.01 per cent POPOP and 0.4 per cent PPO in toluene and was then pipetted into a counting vial. The samples were counted for 10 min in a Packard 'Tri-Carb' scintillation spectrometer. Precursor (0.8 ml.) was added to 3 ml. of Bray's scintillation fluid13 and c.p.m. were measured for each batch of radioactive precursor prepared. Dividing the c.p.m. of 104 cells by the c.p.m. of the precursor used and multiplying by 100 gave a value proportional to the percentage of precursor uptake into 104 cells. Using this value a direct comparison between different experiments was possible.

The MK cell monolayers were treated with 0.034 mmolar, 0.068 mmolar and 0.136 mmolar BHT for 24 h. Each monolayer was detached from the glass with versene and collected in a test tube, resuspended in suitable known amounts of PBS free from calcium and magnesium ions and counted in a haemocytometer. The percentage inhibition of increase in cell numbers was calculated

according to the equation

inhibition was reversible within a further 24 h. Cells treated for 48 h showed a greater inhibition than those treated for 24 h.

Inhibition of cell numbers may result either from cell death or from a sub-lethal depression of cellular metabolism. MK cells were therefore grown on coverslips and treated with 0.136 mmolar BHT for 24 h and stained with May-Grünwald-Giemsa. There was no apparent morphological difference between the BHT-treated and DMSO control cells when examined by light microscopy.

Three separate series containing test, control and DMSO control cell cultures were labelled with ³H-TdR, ³H-UR or (¹⁴C)-leucine after treatment with 0·136 mmolar BHT for 0.5, 3 and 24 h: male MK cells were used. The rate of synthesis of DNA and RNA was also measured in a repeated experiment using female cells after treatment for 0.5, 1, 3, 5 and 7 h.

Fig. 2 shows that the rate of RNA synthesis was most affected by the BHT, male cells showing 50 per cent inhibition and the female 95 per cent inhibition. In the male cells, protein synthesis seemed to be the least inhibited, decreasing by only 15 per cent. In both experiments, depression was rapid, and was maximal within

A foreign compound may be toxic to an organism in its native form, or as a metabolic intermediate before its

increase in cell No. (DMSO control)—increase in cell No. (test) \times 100 (ref. 14) increase in cell No. (DMSO control)

In other experiments, monolayers which had been treated with $0.136\,\mathrm{mmolar}$ BHT for 24 h were washed with growth medium and grown for a further 24 h in fresh growth medium. Simultaneously, other cultures were treated for 48 h. DMSO controls were treated in a similar

The MK cell numbers showed an inhibition proportional to the concentration of BHT used (Fig. 1). This 24 h excretion 15. Because the metabolism of BHT in the MKcell culture system has not yet been proved. It was felt to be important to test two BHT metabolites for inhibitory effects. Each of three groups of MK cell monolayers were treated with either BHT, BHT-acid or BHTalcohol, at a molarity of 0.136 mmolar. After 1, 3 and 5 h the cells were labelled with 3H-UR and the percentage uptake into RNA, compared with DMSO controls, was

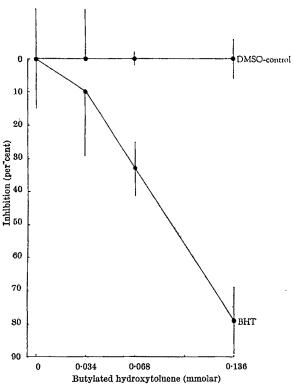


Fig. 1. The percentage inhibition of MK cell numbers after exposure for 24 h to 0.034, 0.068 and 0.138 mmolar BHT (dissolved in 0.9 per cent DMSO in growth medium). Cultures treated with 0.9 per cent DMSO were assumed to show no inhibition. The $95\,$ per cent confidence limits are marked.

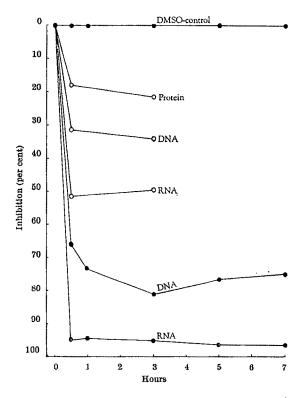


Fig. 2. The percentage inhibition of DNA, RNA and protein synthesis in MK cells treated with 0-136 mmolar BHT. All the values marked were significantly lower than those for the DMSO control. O, Male cells; \bullet , female cells.

calculated. The cells showed little or no significant inhibition of RNA synthesis caused by BHT-acid or BHT-alcohol (Fig. 3).

The possibility that antioxidant activity of BHT induced a metabolic inhibition was tested by comparing the effects of the natural antioxidant α-tocopherol 10,17 and BHT on RNA synthesis. Monolayers of cells were treated with either 0·140 mmolar α-tocopherol or 0·136 mmolar BHT for 0·5, 3 and 24 h, and then labelled with 3H-UR. The results in Fig. 4 show that the α-tocopherol had no significant effect on the synthesis of RNA.

Cells were treated for 1 h with 0·136 mmolar BHT. These cells were then washed with prewarmed growth medium, and grown for a further 23 h in fresh growth medium. At 24 h the same cells were again treated with 0·136 mmolar BHT for a further hour and reversed as before. At each reversal, some treated cultures were left in BHT medium to act as BHT controls. The DMSO control group were treated in a way identical to the BHT group of cultures. Cells were labelled with ³H-UR after 1, 3, 24, 25 and 26 h. The results in Fig. 5 show that a complete and rapid reversal of RNA inhibition occurred after removal of the BHT. In all experiments, the DMSO controls showed only slight, if any, variation from the control results.

In addition to these experiments, a duplicate set of precooled control cells were labelled at $+4^{\circ}$ C for 20 min with 3 H-UR to measure RNA synthesis in cells with a greatly reduced metabolic turnover. These cells showed a 90 per cent inhibition in the synthetic rate when compared with the 37° C controls. A variability in the cellular inhibition caused by 0·136 mmolar BHT was noted in some experiments. This seemed to be correlated with the cell sex; cells from male animals were relatively less affected, and female cells showed a wider range of results. The significant decrease in MK cell numbers treated for 24 h with 0·136 mmolar BHT seems to result from a rapid

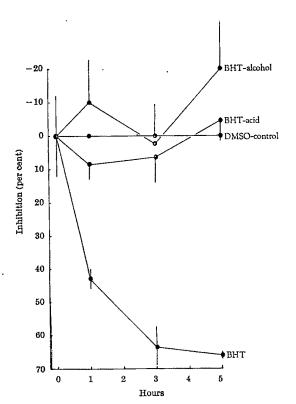


Fig. 3. The percentage inhibition of *H-UR incorporation into MK cell RNA ceused by 0-136 mmolar BHT and the BHT metabolites 3,5-ditertbutyl-4-hydroxybenzoic acid (BHT-acid), and 3,5-diterbutyl-4-hydroxybenzyl alcohol (BHT-alcohol). The 95 per cent confidence limits are given, those of the metabolites and the DMSO control all overlapping.

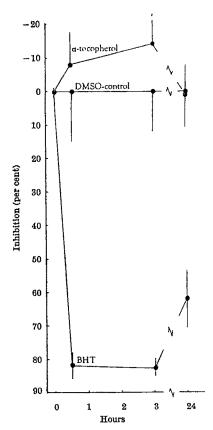


Fig. 4. The percentage inhibition of *H-UR incorporation into MK cell RNA caused by 0-138 mmolar BHT and 0-14 mmolar α -tocopherol. The 95 per cent confidence limits are shown.

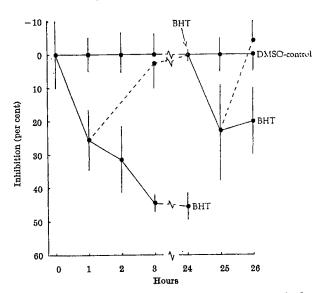


Fig. 5. The reversibility of inhibition of MK cell RNA-synthesis after exposure to 0·136 mmolar BHT for 1 h. The reversed cells were re-treated for 1 h with BHT at 24 h, and again reversed. 95 per cent confidence limits are shown. ————, Reversed cells in growth medium.

depression of DNA, RNA and protein synthesis which would result in a lowered mitotic index¹⁸.

BHT is a highly lipid soluble compound¹⁹, and thus will associate with the lipids of the plasmalemma and the intracellular membrane systems. If this lipid association were to cause configurational changes^{20–22} in biochemically active and/or structural components of the membrane, for example, by altering the membrane water structure²³, then it might interfere with the normal metabolic systems situated partially or wholly in these membranes^{24–26}. Here it is

important to note that two BHT metabolites, 3,5-ditertbutyl-4-hydroxybenzoic acid and 3.5-ditertbutyl-4hydroxybenzyl alcohol, both hydrophobic compounds, did not cause inhibition of RNA synthesis at concentrations of 0.136 mmolar. This difference may be the result of a specific characteristic of the molecule-BHT or, alternatively, of a more rapid excretion of the metabolites.

The capacity of various compounds to prevent oxidation has been measured by the AOM, or "active oxygen method"²⁷. α-Tocopherol has a recorded AOM value of 83, compared with 67 for BHT and 23 for unprotected controls2. In the experiments reported here, however, 0.14 mmolar α-tocopherol does not inhibit RNA synthesis comparable with that produced by 0.136 mmolar BHT, despite its hydrophobic nature and similar antioxidant activity.

The in vivo metabolism of BHT has been studied in rats and rabbits^{6-8,28}. This occurs chiefly in the liver, involving the enterohepatic circulation^{7,29}. The capability of MK cells to metabolize BHT in vitro has not been proved. In both the cell number and labelling experiments, however, the depressions induced by BHT were rapidly reversed by removal of the BHT, and this may be caused by the MK cells converting BHT into non-inhibitory metabolites. A variation in results seemed to be correlated with cell sex; male cells were usually less affected by the BHT than were the female cells. The possibility of BHT being more rapidly metabolized in the male MK cells arises. In vivo, the hydroxylating activity of the liver in untreated rats is greater in males than females³⁰; also Catz and Yaffe³¹ have shown that hexobarbital sleeping times in female rats varied with the oestrous cycle. The concentrations of BHT permitted for use in such

foods as butter, lard and margarine is 160-200 p.p.m., and 1,000 p.p.m. is allowed in essential oils³². In vivo oral doses of 500 mg of BHT/kg body weight cause an increase in the relative liver weight of rats33. In these experiments, 0.136 mmolar BHT, or 30 p.p.m., caused an inhibition of cellular metabolism, leading to a relative fall in cell numbers, in 4×105 cells, weighing approximately 46 mg. Although this in vitro dose is relatively high, it is difficult to make a direct comparison with the in vivo experimental results because of the different effects measured. Also, selective concentration of oral doses of BHT may occur in vivo, such as in the small intestinal

wall during lipid absorption34 and in the liver during metabolism.

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Investigation of Pre-Cambrian Thucholite

by

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Biochemical identification of amino-acids and monosaccharides in carbonaceous materials from the Witwatersrand indicates that there was life in Pre-Cambrian times. Electron micrographs of surface replicas of the surrounding rocks also suggest a probable organic derivation of these carbon concentrations.

THE occurrence of vast amounts of carbonaceous matter or thucholite in the gold-uranium conglomerates of the South African Witwatersrand System has intrigued geologists for a long time, although a systematic mineralogical investigation of these substances was not attempted until 1951 (ref. 1). Earlier this year Hoefs and Schidlowski2 presented isotopic data which seem to support a biological derivation of these carbon concentrations. From the range of the δ^{13} C values (-22 parts per thousand to -33 parts per thousand), it was concluded that photosynthetic processes were operating in those times, bringing about an accumulation of the source material of the polymerized hydrocarbons which now make up the bulk of the carbonaceous matter. Because the sedimentation of the Witwatersrand System was complete about $2\cdot15\times10^9$ yr ago³ it seemed probable that there was a flourishing primitive life in early Pre-Cambrian times. This assumption has recently been substantiated by the discovery of cell-like structures within Witwatersrand rocks⁴ as well as by the occurrence of primitive life forms in strata of a comparable or even older age⁵.⁶. With these preliminary results at hand, the launching of both a biochemical research programme and an electron microscopic investigation seemed to be warranted, the first results of which are communicated in this paper.

Biochemistry of Rock Samples

Amino-acids and carbohydrates were singled out as the primary objects because they are usually synthesized by, and therefore indicative of, life processes. The analytical technique applied for the identification of amino-acids consisted of one or two dimensional descending paper chromatography using a set of different buffered solvents? with a following quantitative colorimetric determination by means of a Beckmann spectral photometer. Carbohydrates were separated by one dimensional descending paper chromatography; the quantitative determination was again colorimetric. A detailed description of the laboratory technique has been given elsewhere.

per cent. Another important group is represented by the sulphur-bearing amino-acids accounting for 20-9 per cent.

In the quartzite rock close to the carbon-rich "B" Reef, cystine and methionine are the most abundant amino-acids, and these sulphur bearing compounds make up nearly 50 per cent of the total amino-acid content of the rock. Tyrosine is another conspicuous constituent of this sample. On the other hand, the quartzite adjacent to the Basal Reef shows a marked preponderance of the oxymonoamino-monocarboxylic acids serine and threonine (31.9 per cent), with tyrosine again occupying the third place in the order of abundance. Altogether, it is obvious that the quartzite samples as well as the carbonaceous matter contain aliphatic amino-acids in excess of aromatic amino-acids; the heterocyclic acids have not been determined in this first set of analyses. Furthermore, it cannot be overlooked that the sulphur-bearing amino-acids are well represented in all samples investigated, accounting sometimes for nearly 50 per cent of the total yield. This seems to emphasize the important role of sulphur in the physiology of primordial life.

In addition to amino-acids, several carbohydrates were recovered from thucholite samples in amounts ranging from 10-26 p.p.m. Seven monosaccharides have been quantitatively determined (Table 2). Among the hexoses, mannose and glucose are the prevalent components, whereas among the pentoses, wood sugar (xylose) plays

Table 1. Amino-acid content of three samples of carbonaceous matter and two samples of wall rock quartzite occurring adjacent to carbon bearing conglomerate horizons or reefs

Sample	Reef	Mining company	δ ¹³ C of carbon in parts per thousand				Aliphatic amino Oxymonoamino- monocarboxylic acids		o-acids Monoamino- dicarboxylic acids		Sulphur bearing amino-acids		Aromatic amino- acids	
			(from ref. 2)	Glycine	Alanine	Valine	Serine	Threonine	Aspartic acid	Glutamic acid	Meth- ionine	Cystine	Tyros- ine	
Thucholite Thucholite Thucholite Quartzite Quartzite	"B"-Reef Basal Reef Basal Reef "B"-Reef Basal Reef	Loraine Virginia Virginia Loraine President	-27·1 -22·4 -22·9	6·6 3·5 6·6 5·7 7·4	9·7 5·8 7·3 6·9 4·0	8·8 3·8 5·7 5·0 3·8	$\frac{-}{13.7}$ $\frac{13.7}{20.8}$	11·6 12·0 17·5 7·9 11·1	13·8 9·9 8·0 8·8 9·5	5·2 3·7 8·9 5·8 8·2	2·0 21·4 10·3 16·7 13·5	19·3 14·6 10·6 29·6 7·5	28-0 25-2 11-3 13-5 14-2	100-0 99-9 99-9 99-9 100-0

The figures represent the percentage (dry weight) of the single compounds within the sample.

Table 1 shows a compilation of amino-acids isolated from three thucholite and two quartzite samples, the latter having been taken from the immediate wall rock portions of thucholite-bearing conglomerates. The applied subdivision has been adapted from Meister. The total content of amino-acids within the carbonaceous matter ranges from a minimum of 2 p.p.m.-20 p.p.m.; the respective values of the quartzites are considerably lower (0.8-3 p.p.m.). Whereas the lowest values lie in the range of a possible contamination, the upper ones should be high enough to exclude such a possibility. An evaluation of the percentage distribution of the individual aminoacids shows that in the first thucholite sample, tyrosine, an aromatic amino-acid, is the most abundant component, accounting for nearly one-quarter of the total amino-acid content. Cystine, a sulphur bearing aminoacid, takes the second place and is followed by aspartic acid. Among the single groups of amino-acids, the monoamino-monocarboxylic acids make up about 25 per cent of the bulk content, while the sulphur-bearing amino-acids are next with 21.3 per cent. The second thucholite sample also shows a predominance of tyrosine (25.2 per cent) among the single compounds, whereas the most important group is represented by the sulphur bearing amino-acids (methionine and cystine) which together amount to 36 per cent. Within the third thucholite sample, threonine takes the lead with 17.5 per cent, other important components being serine (13.7 per cent) and tyrosine (11.3 per cent). This sample is, accordingly, characterized by a preponderance of the oxymonoamino-monocarboxylic acids, serine and threonine, together constituting 31.2

Table 2. CARBOHYDRATES FOUND IN CARBONACEOUS MATTER

	Hexoses						
Galactose	Glucose	Mannose	Arabinose	Xylose	Ribose	Rhamnose	
13.47	19.12	20.60	11.83	14.06	8.59	12-29	99-96

Values are an average for four samples. The figures give the percentages (dry weight) with which the individual compounds are represented in the total yield. The samples are derived from the "B" and Basal Reefs (Loraine Gold Mines, President Brand, Western Holdings).

the most important part. The hexoses constitute the principal group, being about 53 per cent; the pentoses are probably more liable to decomposition and therefore slightly under-represented in the fossil record.

Significance of Results

Biochemical investigations thus strongly support concepts advocating a biogenic derivation of the carbon assemblages which had already been advanced earlier from geological considerations1, putative micropalaeontological evidence4 and from carbon isotope data2. In our opinion the presence in Witwatersrand rocks of aminoacids and monosaccharides can only be explained if life processes operated in those times. Although several workers have pointed out that some amino-acids may be produced abiotically 10, this possibility can be precluded in this case. The early period in the history of the Earth which was possibly characterized by an abiotic production of amino-acids must have been terminated before 3.1×10^{9} yr ago, for organized organic relics of this age have been recently reported from other South African rocks. Moreover, an abiotic synthesis of monosaccharides under primi-

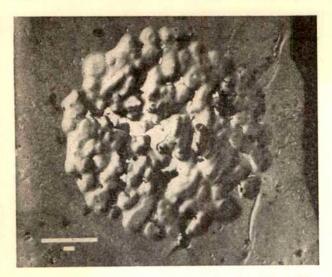


Fig. 1. Electron micrograph of a surface replica taken from a Witwatersrand quartzite neighbouring the Basal Reef. The globose aggregate depicted resembles a cell colony. The scale represents 5μ (long line) and 1μ (short line), respectively.

tive earth conditions appears extremely unlikely. A large scale contamination of the Witwatersrand rocks with these organic chemicals during their post-depositional geological history is also very unlikely, for the compounds are not only restricted to the carbon assemblages (the parent hydrocarbons of which took part in a limited migration, although their source material was certainly indigenous to the sequence), but are also locked up in ordinary quartzite.

Survival of Organic Compounds for a Very Long Time

We have reason to assume that the amino-acids and carbohydrates described originated in their present milieu, and so they must have been preserved-in spite of their intrinsic instability—for more than 2.15 × 10° yr. Although both amino-acids and carbohydrates are well represented in the fossil record down to the Cambrian8,11,12, their occurrence in early Pre-Cambrian rocks must necessarily raise suspicion, even if these rocks have had only a mild thermal history. This suspicion is in particular nourished by the fact that among the amino-acids described are compounds like serine, threonine and tyrosine which have been regarded as relatively unstable by Abelson¹¹. The presence of amino-acids, however, has been reported from other Pre-Cambrian sediments which are only slightly younger than the Witwatersrand System13; furthermore, the putatively unstable compounds serine and threonine have been isolated from rocks at least 1.95 × 109 yr old14. Experimental work on the thermal stability of aminoacids carried out recently has shown that this stability depends largely on environmental factors. For example, in a fine mixture with montmorillonite, amino-acids like cystine, glutamic acid, methionine and leucine may sustain temperatures between 300° and 400° C for about 170 h, although the fusing points of the pure substances (which coincide with the temperatures of disintegration except in the case of glutamic acid) lie at 258°, 225°, 283° and 294° C, respectively15. One should therefore hesitate to extrapolate from the behaviour of the pure substances in vitro on their behaviour in the sediment where the material occurs in a finely dispersed form allowing interactions with other components which might possibly result in a protective effect. It is outside the scope of this preliminary communication to deal at length with all aspects of this problem; in the present stage of the investigation we prefer to present the mere facts, even if the latter are—at least at the moment—difficult to reconcile with certain theoretical considerations. As carbonaceous material from the Witwatersrand is not difficult to

obtain, the reliability of our results can be easily checked by other workers.

Electron Microscope Techniques

In view of the startling results of the biochemical investigations, a systematic search for structurally preserved remnants of early life in the surrounding rocks seemed very promising. Ordinary microscopy had yielded only scanty hints at probable life forms and the application of the electron microscope therefore seemed necessary. This approach was in particular prompted by the fact that electron microscopic techniques have just recently proved to be of outstanding value for palaeontological work on Pre-Cambrian micro-organisms 6,16.

In our attempt to examine the surfaces of quartzite samples from the Witwatersrand System, we followed standard procedures. A relatively even fracture plane of the rock was covered with either a 'Formvar' or 'Technovite' film which was stripped off after polymerization and then shadowed with platinum and replicated with carbon in a vacuum evaporator. The resulting platinum-carbon film was liberated from the underlying 'Formvar' (or 'Technovite') layer in a chloroform bath; the platinum-carbon replicas were washed in acetone and distilled water and finally mounted on microscope grids.

Figures 1-3 show some electron micrographs of surface replicas of samples of Witwatersrand quartzites taken from near carbon bearing reefs. The objects illustrated suggest a probable organic derivation. This applies in particular to the peculiar aggregate resembling a cell colony (Fig. 1), each of its individual elements displaying a characteristic small central hub which might be explained as the nucleus of a shrunken cell. Other cell-like structures are depicted in Fig. 2. The forms at first seem to resemble elongated bubbles, but indications of a certain regular branching cannot be overlooked. It is conspicuous that they are mostly arranged in one preferred direction which coincides with a crack system within the rock. Sometimes the individuals form chain-like aggregates Whether elements like these are actually of biological origin or rather constitute vacuoles within a quartz grain is very difficult to assess at this early stage of the work; we think it advisable, however, to present these forms here in order to bring them to the attention of, and have them discussed by, other

The electron microscopic as well as the biochemical work is being continued and a further report will be given later. Overall, the preliminary results are encouraging, thus corroborating the idea of a high diversity of primordial



Fig. 2. Peculiar elements resembling open, elongated bubbles. Some of them, however, exhibit a kind of branching, thus raising suspicion of an organic derivation (from Witwatersrand quartzite as in Fig. 1; the line represents 1μ).

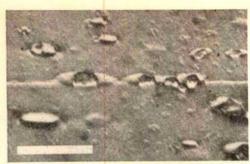


Fig. 3. Chain-like aggregate of the forms depicted in Fig. 2. It is conspicuous that the aggregate is situated on a crack within the rock. Note isolated elements in the surroundings (from Witwatersrand quartzite as in Fig. 1; the line represents 1μ).

life which has been stressed in recent reviews on Pre-

Cambrian palaeontology¹⁷.

We thank Professor F. W. Schlote, Mr R. Dierichs, Mr K. H. Matucha, Mr J. Petermann, Dr J. Pense and

Mr M. Danai for their help. Note added in proof. According to a personal communication by Professor F. M. Swain to one of the authors, amino-acids have meanwhile also been recovered from rocks of the Fig Tree Series (>3×10° yr old) of the Barberton Mountain Land, South Africa; the respective results are not yet published. These findings most decidedly substantiate the data presented in this paper. On the other hand, Kranz¹⁸ has recently advanced the hypothesis that amino-acids may be synthesized abiotically by radioactive irradiation of traces of ammonia and simple alkanes (like methane) which are sometimes present in several minerals and rocks. Even if this concept should prove true, it cannot account for the occurrence of monosaccharides within the carbonaceous material, for the origin of which life processes are a necessary postulate.

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Ice Nucleation and the Substrate-ice Interface

by

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Embryo crystals form by enlargement of one of the normal components of the liquid. This is favoured by reduced molecular motions and a tendency towards more open, hydrogen bonded regions near the interface.

THE heterogeneous nucleation theory, as it is usually expounded, is based on the probability that an embryo crystal forms on the surface of a substrate and grows, by statistical fluctuations, to a critical size beyond which the radius of curvature of some portion of its surface is large enough to favour continued growth to a macroscopic size. In the case of ice nucleation, it is common to visualize that the embryos are attached to the substrate surface where they form a dome of water molecules in which elements of the ice lattice can be identified. It has often been supposed that in order to enhance the probability of embryo formation, a good ice nucleating agent, besides providing a base for embryo formation, should also have certain surface features that match configurations which can be identified in the ice lattice.

The possibility that the water adsorbed by montmorillonite and other clay minerals exists in a more or less rigid, hydrogen-bonded, icelike structure has been suggested by Hendricks and Jefferson1 and Macey2. Soon after this possibility was pointed out, investigations to determine the relative water nucleating efficiency of these and similar substrates were made3,4. Although it was shown that the clay minerals are common nucleators, it became clear that they are not as effective as one might expect if they are coated with a layer or patches of adsorbed water in an icelike form. For this and other reasons, it is now generally accepted that good nucleators need not actually constitute a template for embryo growth, but it is still believed that embryo nuclei form by direct attachment to the substrate. This need not be so. There is reason to believe that with some, and perhaps many, substrates, embryo nuclei form and induce nucleation at a location some distance removed from the substrate surface. Direct contacts with the surface, if they exist at all, are probably never more than tenuous and transitory.

Unfrozen Water in Frozen Mixtures

The existence of substantial quantities of unfrozen water in frozen, clay mineral-water mixtures was first indicated by dilatometric and calorimetric measurements^{5,6}. The results are not entirely conclusive, however, for they may be interpreted in two ways. They are consistent on the one hand with a rigid surface layer, tightly constrained by adsorption forces and cannot be melted or, on the other hand, with a liquid-like layer which because of the presence of exchangeable cations and solutes expelled by growing ice crystals, cannot be frozen. Tabor believed that the unfrozen water forms a mobile surface layer separating mineral surfaces from ice through which water transport to growing ice lenses can occur, and he used this concept successfully to explain the phenomenon of frost heaving. As will shortly become evident, this is the correct view. The amount of unfrozen water present in a frozen sample is usually high for materials which have high specific surface areas and low for those that have low specific surface areas, other factors being equal. According to my own unpublished results, although the unfrozen water content changes with solute content in what seems to be a systematic way, it is practically independent of the ice content. The most important factor, however, is temperature; this is well illustrated in Fig. 1 (ref. 8).

The microscopic mobility of the unfrozen water has been established beyond doubt. Hoekstra⁸ has, for example, recently confirmed earlier reports^{9,10} that the conductance of frozen clay mineral-water mixtures is much higher than that of ice at the same temperature and has demonstrated that the data are best explained by postulating continuous pathways in the frozen specimen through which exchangeable cations and other charge carriers can move. Because the exchangeable cations are constrained by electrical forces to regions close to the mineral surfaces and because solutes expelled from growing ice crystals are also expected eventually to concentrate near these interfaces, it is safe to conclude that at least some of the pathways for electrical conduction are to be found in the unfrozen, interfacial water. Furthermore, nuclear magnetic resonance (NMR) measurements on a variety of minerals at temperatures far below the normal freezing point of water have revealed the very high proton mobility (compared with that in the solid state) of the water in close proximity to the mineral surfaces^{11,12}. The fact that electrical and

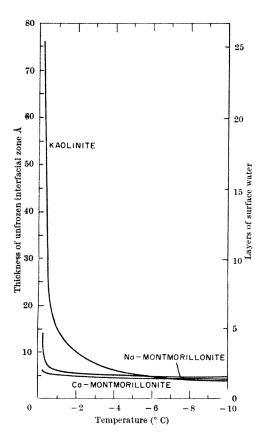


Fig. 1. Relationship between the thickness of the unfrozen interfacial water and the temperature of three representative clay minerals frozen in water.

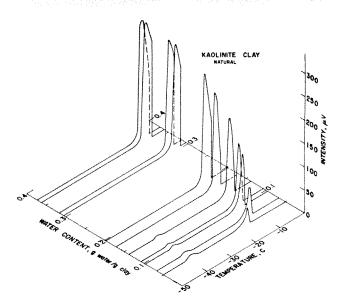


Fig. 2. Graphical summary of results obtained from differential thermal analysis of the kaolinite-water system. Exothermic processes give positive peaks.

thermal osmosis of water is easily accomplished in frozen earth materials demonstrates that the interfacial water molecules possess high mobilities relative to that in ice6,8. The migration of mineral particles suspended in ice, however, offers the most impressive evidence of the fluidity and the continuity of the interfacial water. Corte showed that small mineral particles and glass beads tend to be rejected and pushed ahead of an advancing ice interface when the rate of interface advance is slow and controlled13. Even when the particles become engulfed by ice, subsequent particle migration in response to temperature gradients has been observed and measured14. Migration velocities exceeded 5µ/h at temperatures very near 0° C but amounted to only about $1\mu/\bar{h}$ at temperatures a few tenths of a degree lower. A reason for the decrease can be seen from the data of Fig. 1; near 0° C, decreasing the temperature by a few tenths of a degree markedly reduces the thickness of the unfrozen water layer. migration almost certainly involves the melting of ice in front of a particle and the transfer of water through the unfrozen interfacial zone to the rear where it refreezes. Closer confinement because of a decrease in thickness of the unfrozen interfacial zone brought about by a decrease in temperature must therefore cause a reduction in the particle migration velocities.

Low Temperature Analyses of Clay-Water Systems

The interfacial layer is seen to be many molecular diameters thick at temperatures near 0° C and never seems to be less than one or two molecular diameters thick down to -10° C and lower. Decreasing the temperature probably also causes a decrease in the mobility of the interfacial water. A temperature dependence of this kind is most obvious in the particle migration velocities very near 0° C; it is also obvious in the electrical conductance measurements down to about -20° C. Large decreases in proton motions, however, begin to be evident in the NMR data only at about -80° C. order to locate phase transition temperatures and to define fruitful temperature-pressure fields for more precise calorimetric investigations of interfacial phases, we have made some systematic low temperature differential thermal analyses of representative clay-water systems together with ancillary theoretical studies. Some DTA data bearing on this discussion are summarized in Figs. 2 and 3. The data were obtained as follows. The clay-water contents were fixed at desired values and after a lengthy equilibration period a sample was packed into the DTA cell. The cell and its contents were then cooled at a controlled rate and a continuous recording was made of the cell temperature and temperature differences between the sample and the cell wall. The magnitude of the temperature difference (signal intensity) between the sample and the DTA cell is given in microvolts (positive values correspond to exothermic processes as the temperature was lowered).

The first exotherm quite obviously corresponds to the heat evolved on initial freezing of the sample. The intensity of the exotherm diminished with diminishing water content in accord with expectation. Allowing for a few degrees of undercooling, it can be shown that the lowering of nucleation temperature with diminishing water content (decreasing interfacial water thickness), apparent in Figs. 2 and 3, seems to follow the law of the freezing point lowering. This suggests that embryo formation is dependent on the thermodynamic activity of the interfacial water relative to that of ice, but it can be seen that even at high water contents before nucleation occurred, as was observed earlier. It was necessary to undercool the sample 4° or 5° C.

It can be seen that additional exotherms were detected at lower temperatures. For water contents corresponding roughly to monolayer coverage of the mineral surface and above, these exotherms did not vary markedly with water content and they seem to be distinctive of elay lattice type. The low temperature exotherms were not observed in black runs. Neither were they observed with ice alone or with completely dry clay. It may be concluded therefore that they result from phase changes in the temperature range where evidence at present available suggests that the molecular mobility of the unfrozen, interfacial water becomes very low. It is probable that these exotherms correspond to the development of structural rigidity in the unfrozen, interfacial water. configuration and nature of the structures developed cannot be deduced from the evidence available, but there is sufficient difference evident from Figs. 2 and 3 to sug-

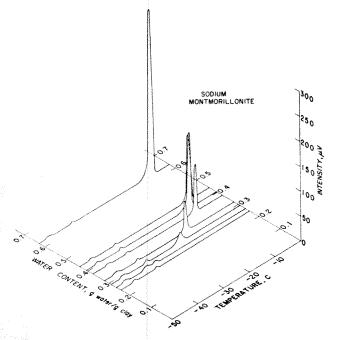


Fig. 3. Graphical summary of results obtained from differential thermal analysis of sodium-montmorillonite system. Exothermic processes give positive peaks. Sodium is present as the only exchangeable cation; no electrolyte present.

Barting a language second by

gest that the structures finally frozen into the interface are different for each substrate. Montmorillonite crystals have both internal and external surfaces exposed to water, whereas kaolinite crystals have only external surfaces. Two low temperature exotherms were observed in mentmorillonite—water systems but only one was observed with kaolinite. One wonders if the exotherm common to both, that at about -39° C, corresponds to the interfacial water on external surfaces. If so, one would then associate the exotherm at about -48° C with structural changes in the interlamellar water of montmorillonite. There is also the possibility, however, that ion dehydration occurs at some point, and that this also contributes to one or both peaks.

In the case of kaolinite, the low temperature exotherm was not observed at water contents exceeding about 0·15 g of H₂O/g of clay. In the apparatus used, the sample size was small (about 5 g) and fixed, so that increasing the water content required a corresponding decrease in the amount of clay present. This in turn resulted in a decrease in quantity of the interfacial water present. At high water contents it is likely that the amount of interfacial water present in the kaolinite-water system became too small to produce a detectable signal. The specific surface area of montmorillonite is about ten times greater than that of kaolinite so that one would only expect the same effect in montmorillonite above about 1·5 g of H₂O/g of clay. This explains why the low temperature exotherms were observed in the montmorillonite-water system at the highest water contents examined.

Nucleating Phenomena

From these results, it seems beyond doubt that in frozen silicate mineral-water systems, the ice crystals are separated from the substrate surface by an unfrozen, fluid-like interfacial zone of water. Considerable fluidity of the interfacial water seems to persist at temperatures at least as low as -10° C; at about -40° C, rigid structures begin to be frozen in. At temperatures near 0° C, the unfrozen interfacial zone is many molecular diameters in thickness and the ice, except possibly for temous and transitory links, cannot be directly bonded to the substrate. In these circumstances, to say that ice nucleation is caused by embryo crystals forming by direct attachment to the substrate and subsequently growing to a size sufficient to nucleate the surrounding water becomes highly questionable.

Many points of similarity in nucleation phenomena induced by organic and inorganic substrates may be pointed out. Space does not permit a full consideration, but one example will suffice to illustrate the point. Evans has reported the results of ice nucleation studies at high pressures involving phloroglucinol dihydrate and some other organic substrates17. He observed that the undercooling required for nucleation was usually about 6° C at atmospheric pressure but became nearly zero at 1,500 bars and higher. This was true of only the first nucleation, however; on melting, if the sample temperature did not exceed +1° C, something less than the original undercooling was required at all pressures. This "memory effect" was attributed to the development of stabilized water structures on the substrate after freezing in which monolayer patches of ice could grow and subsequently serve as nucleation sites. (The possibility of imprinting the ice structure on the substrate itself was considered and rejected for what seem to be good reasons.) Because the temperature at which memory is erased was found to be $0^{\circ} \pm 1^{\circ}$ C regardless of the pressure, Evans argued from the Clausius-Clapeyron equation that the volume change accompanying deactivation of memory in the adsorbed water must be very small and concluded that the only way in which this could be so is for the water structure at the nucleation sites to consist of a single |0001| layer of ice (a second layer would reduce the density of this layer too much) on the substrate surface.

Icelike structures for water absorbed on clay substrates have been proposed on several occasions. It has been shown, for example, that the density of the interfacial water immediately adjacent to montmorillonite surfaces is some 3 per cent less than that of the normal liquid but that it is substantially greater than that of ice18-20. Furthermore, at atmospheric pressure, about 5° C undercooling is frequently necessary to accomplish spontaneous nucleation with silicate substrates, and a memory effect for these substrates similar to that observed by Evans for organic substrates was mentioned recently by Graham et al. 12. Mineral substrates tend to be rejected from growing ice crystals, and frost heaving on a microscopic scale is commonly observed when biological materials are frozen. In all probability, it will soon be demonstrated that, depending on temperature, a liquid-like, interfacial zone of unfrozen water also separates ice from the surface of many, if not all, organic

It may be awkward, but not impossible, to reconcile the idea of embryo formation and growth on the substrate surface with the fact that the ice subsequently formed is, except for possible sporadic, tenuous links, completely separated from the substrate. It seems far more likely that although embryo formation may be induced, and embryos in a sense may be stabilized by the interaction of fluids with substrate surfaces in such a way as to favour further growth, the formation and growth of embryos actually occur at a location several, perhaps many, molecular diameters distant from the substrate. After nucleation and freezing, as the temperature falls the unfrozen interfacial zones must diminish in thickness, and when the temperature is later raised the interfacial zones must be thickened because of the progressive melting back of the ice interfaces, in general accordance with the relationships shown in Fig. 1. The memory effect is explained by the fact that active nuclei may be stabilized by the environment of the interfacial region and even after melting is complete they may remain in certain favourable locations. The size, and hence the potency of these "protected" nuclei is determined by the maximum temperature to which they are exposed.

The concept introduced here is in some respects similar to a theory of heterogeneous nucleation advanced by Dorsey21. He visualized an adsorbed layer of liquid orientated by influences from the substrate and from which small aggregates might be dislodged as a result of molecular collisions or shearing forces. He thought it was likely that some of the aggregates torn loose from the adsorbed layer would be larger than critical size at some temperature below the normal freezing point, and these, possibly by reorientation or further growth, would eventually become viable nuclei. The interface envisaged in his theory is characterized by a relatively strong binding of the absorbant to the substrate which diminishes in intensity in progressively distant adsorbed layers until the state of the bulk liquid prevails. The aggregates are supposed to be dislodged from the outermost layers by molecular collisions or by mechanical shearing. The idea that nuclei formed in this manner could, in the interfacial environment, be stable until heated to the melting point of the solid phase is more or less implicit in Dorsey's theory, and in this respect his theory can be said to accommodate earlier explanations advanced to explain memory effects when a melt is refrozen²².

Formation of Embryos

The theory advanced here requires the stabilizing presence of a substrate but does not require embryo attachment to the substrate, nor does it visualize embryos as being torn out of an extended network of molecules

existing in an ordered array next to the substrate. What is proposed here is that various kinds of ordering are brought about in water by interaction with a substrate and that these may be identified and characterized in localized domains in the interfacial zone. The location and extent of these domains must shift in special ways as the temperature is raised or lowered. Thinking in terms of the "flickering-cluster-mixture-model" of water, at some favourable location in the interfacial zone sufficiently removed from the intense—and perhaps too specificinteractions at the substrate surface, an environment exists in which the lifetimes of icelike flickering clusters are lengthened appreciably. This requires an increase in the coherence and extent of hydrogen bonding of the clusters which in effect creates embryos and facilitates their enlargement by molecular accretion into nuclei. Embryos can thus be said to form by enlargement of one of the normal components of the liquid. The critical aspects of the environment created by the substrate which favour embryo formation no doubt involve reduced molecular motions and a tendency towards more open, hydrogen bonded regions near the interface. Viewed in this way, heterogeneous nucleation is satisfying similarly in concept to homogeneous nucleation. The effect of a substrate is simply to provide a protective environment in which nuclei of critical size may form in the same way but at a temperature substantially higher than that required for embryo growth to similar size in the pure liquid. Once formed, these icelike clusters can easily be incorporated into the ice and on subsequent melting many may remain intact and active at favourable locations in the interfacial zone, provided the temperature is not raised too far above the melting point; hence, subsequent freezings may require little or no undercooling.

This theory of heterogeneous nucleation can account for all the experimental observations so far reported, including those of Evans made with organic substrates. It is also in harmony with the observations that silicate substrates when completely engulfed by ice are nevertheless free of direct attachment to the ice. Although considerably more work may be required to establish its validity, the concept I have advanced is attractive and promises to be useful. One of its most attractive features is its stress of the basic similarity which may exist between the homogeneous and heterogeneous nucleation theory as it applies to water.

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LETTERS TO THE EDITOR

ASTRONOMY

Energy Emission from a Neutron Star

Although there are still many problems concerning the supernovae, there is little doubt that a very dense stellar core has to be left behind after the explosion (at least in some cases). During the contraction of this core, inverse β reactions take place and transform most of the nuclei and electrons into neutrons. If the mass of the neutron star does not exceed a critical value of about one or two solar masses, a stable equilibrium situation can be reached with the gas pressure balancing the gravitational force.

A newly formed neutron star is an excited object. Apart from its thermal content (which will be dissipated very fast because of neutrino processes), there will also be much energy stored in vibrational and rotational form. The problem therefore arises of finding out whether the energy stored in the neutron star plays an important part in connexion with the activity observed in some supernova remnants such as the Crab Nebula.

The vibrations of the neutron star, however, do not last long enough for our purposes. The principal reason for this is that the emission of gravitational waves will damp quadrupole and higher order pulsations in a few seconds (ref. 1 and unpublished work of T. A. Wheeler and A. Zee). Moreover, because the stellar rotation will mix the radial modes of vibrations with the non-radial one, all the vibrations are going to disappear very quickly.

It seems more rewarding therefore to look for some mechanisms by which the neutron star can release either its magnetic or its rotational energy or both. In this communication I would like to outline the principal features of a possible model of this kind.

The existence of very strong magnetic fields in the neutron stars has been suggested as a consequence of the compression of an ordinary stellar field. The underlining assumption here is that the conductivity, σ , of the stellar matter is so high that the decay time for the field exceeds the collapse time. As the collapse time is of the order of seconds, this means

$$\tau_{\rm decay} \sim \frac{4\pi \, \sigma R^2}{c^2} \gg 1$$
(1)

where R is the radius of the neutron star (about 10^6 cm). For any conceivable value of σ this is a very weak requirement. We can therefore expect the field strength to increase as $1/R^2$ during the contraction so that fields as high as $10^{10}-10^{14}$ gauss can be produced².

If we assume that the magnetic field is that of a dipole, the angle between the dipole and the angular momentum is likely to be arbitrary (oblique rotator). Actually, even if the two axes coincided in the pre-supernova star, the mass loss occurring during the explosion is unlikely to be perfectly symmetric, especially in the presence of strong magnetic fields. The mutual inclination of the two axes will then be modified and both the rotation and the magnetic field will tend to flatten the star (but along different directions). The shape of the star is going to be rather complicated and the body will rotate about an axis which is not a principal axis of inertia. This is a non-equilibrium situation and the motion will be such that the total angular momentum is constant while the

instantaneous axis of rotation precesses. Stresses and magneto-hydrodynamic waves are therefore to be expected at the surface of such a star³. This will dissipate energy with the final result of bringing the system into an equilibrium state, that is, the magnetic axis in coincidence with the axis of rotation. Acceleration of particles to relativistic energies is to be expected under these circumstances.

The same picture of an oblique rotator leads also to a different possibility, that is, that the neutron star might directly emit electromagnetic waves of very low frequency (in the kc/s range). This idea has been suggested by Hoyle, Narlikar and Wheeler⁴ as a possible consequence of the vibrations of a magnetic neutron star. Because the rapid damping of the vibrations makes it difficult to retain this suggestion in the original form, I wish to point out that the oblique rotator model also results into an analogous emission of electromagnetic waves.

If d_0 is the projection of the dipolar moment on the plane perpendicular to the axis of rotation and Ω is the angular velocity of the star, there will be a monochromatic emission of electromagnetic waves at the frequency $\omega = \Omega$. The corresponding intensity is given

$$I = \frac{2}{3} \frac{d_{6}^{2} \Omega^{4}}{c^{3}} \tag{2}$$

If we take d_0 to be about $H_0R^3 = 10^{10} \times 10^{18}$ gauss cm² and $\Omega = 10^4$ sec⁻¹, the intensity would be of the order of 2×10^{40} ergs/sec.

We must, however, ask ourselves the question whether this radiation will ever arise. Any variation of the magnetic field can actually be compensated by the electric currents in the surrounding matter. Hoyle, Narlikar and Wheeler⁴ have stated that the strong gravitational field creates a near vacuum immediately outside the star so that in this region no propagation difficulty would arise. This equilibrium picture, however, seems unlikely for a newly formed neutron star, soon after the supernova explosion. It is then necessary to evaluate the maximum gas density which still allows the emission of electromagnetic waves. This is easily done if we note that the maximum current density in the gas is given by $j=n_eec$, where n_e is the electron density. The Maxwell equation

$$\operatorname{curl} \overrightarrow{H} = \frac{4\pi}{c} \overrightarrow{j} \tag{3}$$

gives then the maximum induced field. If we take $\operatorname{curl} \sim 1/r$ (r is a characteristic length of the order of the size of the system) we obtain

$$H_{\max} \sim 4\pi \, n_e \, e \, r \tag{4}$$

Any variation of the magnetic field of the order of the field itself cannot therefore be compensated by the induced currents if

$$n_e < \frac{H}{4\pi e r} \tag{5}$$

Assuming again H to be about 10^{10} gauss and r=R, about 10^6 cm, we must require $n_e<10^{13}$ cm⁻³. This limit is certainly not very stringent and condition (5) is likely to be violated only at the beginning, that is, soon after the birth of the neutron star.

Once the electromagnetic waves are emitted and propagate in the supernova remnant, they will be reflected by the circumstellar gas if the plasma frequency exceeds the radiation frequency. For $\omega=10^4~{\rm sec^{-1}}$ this happens if $n_e>2.5\times10^{-3}~{\rm cm^{-3}}$ which is now a very low figure. The electromagnetic waves would therefore be unable to reach us, but by this means a large amount of energy and momentum could be pumped from the neutron star into the supernova remnant. In particular, the radiation will give an outward momentum to the nebula by being reflected and therefore accelerate its expansion. As a matter of fact, there is observational evidence that the motion of the Crab Nebula has been accelerated after the

original explosion: if we divide the size of the nebula by its present expansion rate, we get too short a lifetime. From a quantitative point of view, no difficulty arises because of the large amount of energy which can be stored in the neutron star under the rotational and magnetic form. As suggested by Hoyle, Narlikar and Wheeler4, generation of high energy electrons can be expected in the region where the electromagnetic waves are reflected and cause a rapid compression of the nebular

It seems therefore that, when the oblique rotator model is realized, it can lead to a release of energy from the neutron star. It is, however, clear that the model is very idealized and requires further investigation. particular, it would be important to evaluate the emission of gravitational waves from the star. This will depend on the mass distribution in the star as influenced by the rotation and magnetic field and will determine the ability of the gravitational waves to carry out rotational energy and angular momentum from the star.

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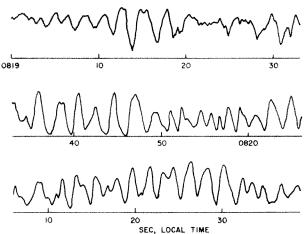
PLANETARY SCIENCE

Ionospheric Irregularities caused by Acoustic Waves

This communication presents some observational evidence for ionospheric irregularities being formed because of the propagation of acoustic waves. The acoustic waves themselves are believed to have been associated with an earthquake experienced at College, Alaska. During the past few years several studies have been devoted to the problem of the generation of acoustic and acoustic-gravity waves by an earthquake. Many of these studies followed in the wake of the Alaskan earthquake of 1964 (refs. 1-4). A number of travelling ionospheric disturbances were observed at different places after the earthquake and these disturbances are believed to be a manifestation of the propagating acoustic-gravity waves^{5,6}. Here we do not intend to explore the problem of the excitation of acoustic waves by an earthquake, and implicitly assume the existence of acoustic waves associated with an earthquake. This assumption seems to be justified in view of the earlier studies and also from our own observations.

The motion of the neutral component in a partially ionized gas affects the ionized components as well. The acoustic waves propagating in the ionosphere may therefore create perturbations in the ionization which can be detected by radio methods. A theory of the formation of ionospheric irregularities by acoustic waves was put forward by Whitehead, in an attempt to explain the origin of sporadic E in the equatorial regions. With certain modification, the theory is also applicable to the auroral EWe present here some results from a partial reflexion experiment which provide observational evidence in support of the theory.

The partial reflexion experiment conducted at College uses a frequency of 3 Me/s and is primarily intended for the study of horizontal motions in the lower ionosphere by the spaced receivers method. During most of the time, echoes from both D and E regions are present. For recording purposes the receivers are gated to accept only one of the echoes, and the outputs from the three receivers are recorded on a multichannel Sanborn recorder. On the morning of June 21, 1967, rapid periodic fading, with periods from less than 0.5 sec to 2 sec, was observed in the wake of an earthquake felt at College. The earthquake occurred in a series of four shocks between 0804 and 0825 A.S.T. The equipment was switched on just after the second shock, which was felt at about 0814 h. The first indication of the disturbance in the reflecting region of the ionosphere was noticed at about 0819 h. Before this the signal was quite steady. The receivers were gated for the E region echo with its peak at 105 km. The signals recorded after the onset of the disturbance are shown in Figs. 1 and 2. The fading patterns in all the receivers are identical. The fading starts with a period of about 1 sec; the period as well as the depth of fading increases with time. After about 15 sec, a more rapid



Fading pattern of the E region echo (105 km) received at College soon after the earthquake on June 21, 1967.

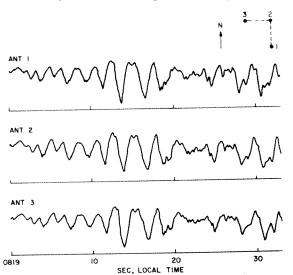


Fig. 2. Simultaneous recording of the signal in three spaced receivers. The north-south and east-west spacing between the antennas is one wavelength,

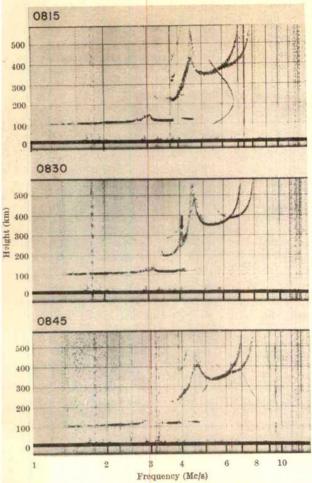


Fig. 3. College ionograms for 0815, 0830 and 0845 A.S.T. on June 21, 1967.

fading with a period of about 0.5 sec appears superimposed on the longer period variations. These short period variations last for 15 sec, after which only the long period fading remains. After another 15 sec, short period variations with a period of 1 sec appear; the period and the amplitude again increase with time. This sequence amplitude again increase with time. appears to be repeated and the signal level gradually Thus the disturbance seems to have arrived at the reflecting region in a series of pulses. The fact that the ionosphere was disturbed by the earthquake is also borne out in the regular ionosonde observations at College. Fig. 3 shows the ionograms taken at 0815, 0830 and 0845 h; the 0830 h ionogram shows an additional cusp at about 300 km which is not noticed in the earlier or later observa-

The rapid periodic fading of the reflected signal is not unusual. The regular sequence of increasing period, however, has not been observed on any other occasion during 1 yr of operation of the equipment. The association of these observations with the earthquake is immediately suggested. The time lag between the second shock and the observed fading is about 5 min. This roughly corresponds to a speed of propagation of about 350 m/sec, which is quite close to the acoustic speed. The 0830 h ionogram also agrees with the assumption that the disturbance propagated from below at the acoustic speed.

In view of the very short periods, the fading cannot be caused by periodic variations in the electron density at the reflecting level, for the relaxation period for the ionosphere at this level is much longer. Because the experiment uses vertical incidence, the fading is attributed to Doppler shift caused by vertical motions in the scat-

tering (or reflecting) region. The vertical motion must be in the range of 25-100 m/sec in order to give the observed Doppler fading. This motion cannot possibly be the physical motion of the medium itself. Vertical motions of the medium may be caused by oscillations of the ionospheric layer. These oscillations can result from acoustic gravity waves. The period of these waves has a lower limit which is of the order of a few minutes, while the periods encountered in the present observations are in seconds.

Because the observations are believed to be associated with the earthquake, it appears more reasonable to look for an explanation in terms of acoustic waves. acoustic waves propagating through the reflecting region will generate electron density irregularities which will be moving with the phase speed of the waves. The irregularities will be more intense for waves propagating at low angles to the horizontal and beyond some critical angle they will not be noticeable. Thus the observed fading can be produced by acoustic waves propagating at angles below the critical angle. The waves propagating with higher angles (within this range of angles) arrive first and cause the shorter period fading; these are followed by the waves propagating at progressively lower angles. This accounts for the gradual increase in the fading period. The increase in the fading amplitude is then accounted for by the fact that the irregularities are more intense for lower angle waves. According to this viewpoint, then, the vertical motion causing the Doppler shift is the vertical component of the wave velocity. A comparison of the fading pattern at the three antennas also gives confirmatory results. The horizontal motion is towards the north with a speed of 300-350 m/sec. Also the horizontal speed is less for the shorter period fadings which imply a larger vertical component. The periodic repetition of this sequence is in all probability caused by the pulsed nature of the acoustic wave excitation.

The role of the ionospheric irregularities in this case is different from their role in VHF scattering. relatively low frequency used here (3 Mc/s), the aspect sensitivity of these irregularities does not play a crucial part. The irregularities are not essential for obtaining the scattered signal; they act only as a perturbation on the existing mechanism of partial reflexion.

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Terrestrial Origin of Aluminium-26 and Oceanic Sedimentation Rate

The recent detection of aluminium-26 in marine sediments by Amin, Kharkar and Lal¹ has posed a question about the origin of this nuclide. Lal and Venkatavaradan2 pointed out that the amount of aluminium-26 in sediments is an order of magnitude higher than expected, as a result of its production by cosmic ray interactions in the terrestrial environment. Thus they concluded that the large amount of aluminium-26 can only be explained by postulating an influx with extraterrestrial cosmic dust which

has been exposed to a significant flux of energetic particles

capable of producing nuclear interactions.

I have recalculated the figures of Lal and Venkatavaradan in the light of new cross-section measurements carried out by Yiou et al. (ref. 3 and private communication), and I have obtained a result which suggests that the large amount of aluminium-26 can be explained by its production through cosmic interactions in the terrestrial atmosphere.

Lal et al. found that the mean ratio of activity of aluminium-26 to beryllium-10 in a South Pacific core (0-110 cm deep) and a North Pacific core (0-120 cm) was 0.12 ± 0.04 , but the mean ratio in the cores 1 m deep is not very significant because the rates of decay of these nuclides are very different (Fig. 1). I have therefore used the measurements of beryllium-10 and aluminium-26 activities in the surface layer of the South Pacific core^{1,2}— 4.7 ± 0.5 and 0.78 ± 0.34 d.p.m./kg of dry sediment, respectively. Thus the ratio is 0.17 ± 0.08 and, after correcting for decay, the ratio of aluminium-26 to beryllium-10 in freshly deposited sediment is 0.2 ± 0.1 .

Lal and Peters⁴, however, estimated the global production rates of beryllium-10 and aluminium-26, by spallation of atmospheric nuclei, to be 4.5×10^{-2} and 1.4×10^{-4} atoms cm⁻² sec⁻¹, respectively, and these correspond to an activity of 7.5×10^{-7} and 7.9×10^{-9} d.p.m. cm⁻² yr⁻¹, respectively. Thus in a freshly deposited sediment, the activity ratio of aluminium-26 to beryllium-10 should be about 0.01. From this expected ratio, Lal et al. concluded that the observed ratio of aluminium-26 to beryllium-10 (about 0.1 or 0.2) is an order of magnitude higher than the expected ratio (about 0.01) and that most of the aluminium-26 may have an extraterrestrial origin.

Lal and Peters estimated rates of production based on the formation cross-section of beryllium determined by Honda and Lal⁵. Recent measurements, however, made by Yiou et al.3 with an elaborated method using a mass-spectrometer, have shown that the formation

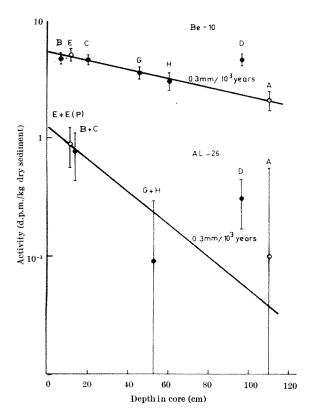


Fig. 1. The activity of beryllium-10 and aluminium-26 as a function of depth in oceanic sediments; ♠, South Pacific core (57° 35′ S., 174° 15′ W., 4,760 m); ○, North Pacific core (8° 20′ N., 145° 24′ W., 5,110 m).

Table 1. FORMATION CROSS-SECTION OF BERYLLIUM-10 Target \mathbf{C} CN₃O Cross-section (mb.) Honda and Lal (Ep., 220 MeV) Yiou et al. (Ep., 155 MeV) $^{1.8}_{0.3}\!\pm\!0.6$ 4 ± 2

0.4 + 0.2* * The formation cross-sections at E_P (proton energy) = 600 MeV and 19 BeV are about 0.6 ± 0.4 mb.

cross-section of beryllium-10 is an order of magnitude lower than the value obtained by Honda and Lal (Table 1).

Although the formation cross-section from nitrogen has not yet been measured, Yiou et al. tell me that it can safely be estimated to be of the same order as the cross-section from oxygen, using a systematic prediction. The low value of the new cross-section is consistent with an empirical rule on the systematics of spallation crosssections of light elements, proposed by Reeves: the cross-sections of the reactions which produce a nuclide having an isobaric spin of 1 (such as beryllium-10) from a nuclide having an isobaric spin of 0 (such as nitrogen-14 and oxygen-16) are, in general, an order of magnitude lower than the cross-sections of the reactions which produce a nuclide having an isobaric spin of 0 or 1/2 (for example, beryllium-7) from the same target.

The global production rate of beryllium-10 by spallation of atmospheric nuclei should therefore be an order of magnitude lower than the value expected by Lal and Peters. Thus, in a freshly deposited sediment, the activity ratio of aluminium-26 to beryllium-10 is expected to be about 0.1. An uncertainty factor of 2 may be involved in this expected ratio, because the formation cross-section of aluminium-26 is not actually measured but evaluated

from the semi-empirical rule of Rudstam.

This expected value agrees with the observed ratio, 0.2 ± 0.1 , within the experimental errors. Thus we can conclude that most of the aluminium-26 activity, found in marine sediments, can be explained by its formation from the spallation reactions in the terrestrial atmosphere, and we need no influx with cosmic dust.

Because of a slight excess of the observed ratio over the expected ratio, some cosmic dust contribution to the aluminium-26 cannot be excluded, but the uncertainty factor does not justify it. It would therefore be desirable to determine the formation cross-sections (particularly of aluminium-26) and the activity in sediments with more precision in future experiments.

Recent experiments of Nilsson⁷ suggest that previous estimates of the accretion rate of cosmic dust on the Earth may be exaggerated, for he has found that the counting rates of microphonic dust counters probably resulted from noise, provoked by temperature gradients rather than from the impact of micrometeoroids. influx rate of aluminium-26 with cosmic dust may therefore be considerably lower than the value estimated by Lal.

Apart from the origin of aluminium-26, and because of its revised cross-section, I have re-evaluated the rate of sedimentation by the beryllium-10 method. The aluminium-26 method was also used. This evaluation was carried out in two ways. (a) The production of beryllium-10 or aluminium-26 (in d.p.m./cm² yr⁻¹) was divided by the volume concentration of beryllium-10 or aluminium-26 (in d.p.m./cm3) in the freshly deposited sediments to obtain the sedimentation rate (cm/yr). The density of the core material, defined as the ratio of the dry weight of the core to the volume occupied by it in situ at the ocean floor, is about 0.5. This value was used to convert weight concentrations (d.p.m./kg) to volume concentrations (d.p.m./l.). Thus, from the data of beryllium-10, we found a value of about 0.3 mm/1,000 yr both in the South Pacific core and in the North Pacific core. The previous value by Lal was about 4 mm/1,000 yr.

From the aluminium-26 data, the sedimentation rate was found to be about 0.2 mm/1,000 yr. This rate agrees with the beryllium-10 rate, for it is within the experimental errors. These rates should be considered as the sedimentation rate of the surface layer.

(b) In the second evaluation of the rate of sedimentation the decay of beryllium-10 (aluminium-26) was plotted as a function of the core depths (Fig. 1). A regular decrease in the activities with the depth was found except in the fraction D (83-110 cm depth) of the South Pacific core. In fraction D, both the beryllium-10 content and the aluminium-26 content were exceedingly high. Although the reason for this irregularity of the fraction D is not known, we have eliminated temporarily the data of the fraction D.

The rate of sedimentation is obtained from the gradient of the decay curves. We found a rate of about 0.3 mm/ 1,000 yr from the decay of beryllium-10, which should be considered to be the mean rate of sedimentation between cores 0 and 1 m deep. Good agreement was found between this rate and the sedimentation rates of the surface layer determined by method (a). This means that there has been a constant sedimentation rate in these cores, and a constant cosmic ray intensity during the last 3×10^8 yr, with the reservation of fraction D.

The activity of aluminium-26 in each fraction was recalculated from the data of Amin et al.1. Because of the large statistical errors involved in measuring the rate of counting, it was not possible to determine the gradient of the decay curve, but a sedimentation rate of 0.3 mm/ 1,000 yr seems to be consistent with the decay curve. The decrease of the aluminium-26 to beryllium-10 ratio with depth was also consistent with this rate of sedimentation. All values from the beryllium-10 and aluminium-26 methods therefore indicate a sedimentation rate of about 0.3 mm/1,000 yr for these two cores. This rate may have an uncertainty factor of 2, because of the experimental errors in measurements of the cross-sections and the activities.

These figures obtained from the beryllium-10 and aluminium-26 methods agree well with the sedimentation rates, determined by Goldberg and Koides with the ionium to thorium ratio method, of 0.3 to 0.6 mm/1,000 yr in South Pacific cores.

Although discrepancy has sometimes been reported between the results by the ionium to thorium ratio method and those obtained by the protoactinium and carbon-14 methods, recent studies, have indicated the ionium to thorium ratio method or the ionium method with the protoactinium method are consistent. On the other hand, the carbon-14 method is not adequate to determine the very low sedimentation rate, because of its short halflife. We can therefore consider the low (about 0.4 mm/ 1,000 yr) sedimentation rate of the South Pacific Ocean as a well established fact.

The agreement of the sedimentation rates, measured by the beryllium-10 method and the aluminium-26 method, with the rates by the ionium to thorium ratio method indicates the usefulness of the former two methods in the measurements of the sedimentation rates and also the time variation of cosmic rays over the past few million years.

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Emission of BO₂ from Injection of Triethylborane into the **Upper Atmosphere**

Hoffman, Palmer and Smith¹ recently presented a low resolution emission spectrum obtained from the sunlit release of triethylborane at a height of between 90 km and 178 km above the Earth. They assigned the observed bands to an $A^2\Pi^{-2}\Pi$ transition of boron oxide, even though they noted the absence of a band expected at 6025 Å on the basis of this assignment. Their communication does not make clear which oxide of boron is

assigned as responsible for the emission.

I believe the observed emission spectrum is that of the boric oxide fluctuation bands—a spectral system arising from a transition of the BO₂ radicals. A comparison is given in Table 1 between the wavelengths of maximum emission observed in ref. 1 and those reported in absorption for flames containing boron3. The correspondence between these data is as good as can be expected when a complicated spectrum of many lines is observed at low resolution. The 6025 Å band shown in ref. 4 and mentioned by Hoffman et al. is not a part of the BO₂ A² II-X2∏ system; therefore its absence in the upper atmosphere emission supports this assignment rather than presenting a difficulty.

> Table 1 Emission maxima from upper atmosphere release 1 Absorption maxima for 1 BO $_{2}$ in flames 8 6,4206,200 5,795 5,465

The boric oxide fluctuation bands were erroneously assigned to B₂O₃ for many years^{5,6}. The correct assignment of the spectrum to the BO2 radical was made independently in Russia⁷ and in the United States⁸ on the basis of spectral and chemical kinetic studies. This assignment was confirmed when this electronic spectrum was resolved, analysed and the molecular constants of BO2 determined. The key to success in this work lay in production of the emitter with a low rotational temperature by flash photolysis of boron trichloride-oxygen mixtures at low pressures. Those conditions are strikingly similar to those which obtained in the upper atmosphere release of Hoffman et al. Both experiments probably involve non-thermal excitation processes which obviate the use of the relative band intensities for thermometry.

In spite of its recent discovery, the properties of the BO_2 molecule are now well known. This is partly a result of the fact that its electronic spectrum is one of the best examples of the Renner effect. The thermodynamic properties of BO₂ have been computed¹⁹ and its heat of formation determined11. It may therefore usefully serve as a chemical probe for species with which it reacts in the upper atmosphere.

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Hydrothermal Melting Curves in Silicate-Water Systems at Pressures greater than 10 Kilobars

Fusion curves, or melting intervals, for many elements, minerals and rocks have now been measured experi-

mentally to high pressures, but most determinations of melting relationships in the presence of water vapour have been limited to pressures below 10 kbar. It is well known that the solubility of water vapour under pressure in silicate liquids produces a marked depression of melting temperatures, as illustrated by the negative slopes, dP/dT, of the univariant curves for the reactions: albite+vapour= liquid (Fig. 1); albite + nepheline + vapour = liquid (Fig. 2). Barth1, Smith2 and Kadik and Khitarov³ have predicted that, because of the progressive change in the relative properties of water in vapour and liquid phases with increasing pressure, the slopes, dP/dT, of hydrothermal melting curves in silicate-water systems (solid-liquid-vapour) should change from negative to positive at moderate pressures, probably within the interval 3 kbar-10 kbar, with the curves passing through a temperature minimum where dT/dP = 0. We have recently extended water saturated univariant melting curves in the system $NaAlSiO_4$ - SiO_2 - H_2O from 10 to 35 kbar, using piston-cylinder apparatus, and none of the curves exhibited a temperature minimum where dT/dP = 0. Figs. 1 and 2 show some of our results. These are not complete phase diagrams; they show only the reactions en-

countered by two composition joins through the ternary system. Further reactions required by the existence of invariant points, I, and a singular point, S_5 , will be

illustrated elsewhere.

The reactions for mixtures on the composition join

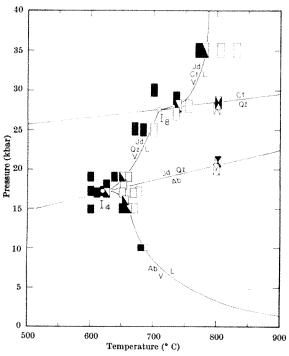


Fig. 1. Univariant reactions in the system NaAlSiO₄-SiO₂-H₂O encountered by mixtures on the composition join NaAlSi₂O₃-H₂O. The inflexion on the curve for Ab+V=L where this approaches the invariant point I_4 involves a change to the reaction Ab+V=Jd+L, which is not labelled. Abbreviations: Ab, albite; Jd, jadeite; Qz, quartz; Ct, coesite; L, liquid; V, vapour (aqueous fluid phase).

NaAlSi₃O₈-H₂O are shown in Fig. 1. This join is effectively binary up to 17 kbar, where the curve for the hydrothermal melting of albite, with negative gradient, terminates at the invariant point I_4 as albite breaks down to jadeite and quartz. At pressures above I_4 , the composition join is ternary and mixtures are involved in the hydrothermal melting reaction jadeite + quartz + vapour = liquid, which

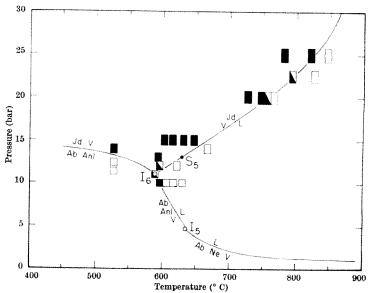


Fig. 2. Univariant reactions in the system NaAlSiO₄-SiO₆-H₂O encountered by mixtures on the composition join NaAlSi₂O₄-H₂O. I_5 and I_6 are invariant points, and S_5 is a singular point. Abbreviations as in Fig. 1, with Ne, nepheline, Anl, analcite.

has a positive slope. The melting curve crosses the quartz-coesite transition at invariant point $I_{\mathfrak{s}}$ with another marked change in slope.

The reactions for mixtures on the composition join $\operatorname{NaAlSi}_2\operatorname{O}_6-\operatorname{H}_2\operatorname{O}$ are shown in Fig. 2. The phase relationships are ternary up to the invariant point I_6 at 11 kbar, and effectively binary above it. The hydrothermal melting curve for jadeite, jadeite+vapour=liquid, extending above the invariant point I_6 , has a positive slope (dP/dT), with dT/dP about 17 degrees C/kbar , which is similar to the gradient of fusion curves for many dry silicate minerals, but quite different from any previously put lished hydrothermal melting relationships. The familar pattern is illustrated by the curves below I_6 , where jadeite is unstable. Mixtures with compositions on the join $\operatorname{NaAlSi}_2\operatorname{O}_6-\operatorname{H}_2\operatorname{O}$ are involved at low pressures in the reaction albite+nepheline+vapour=liquid, which terminates at the invariant point I_6 where analcite becomes stable⁴ Between I_4 and I_6 is the curve for the reaction albite+analcite+vapour=liquid.

The results shown in Figs. 1 and 2 establish that the slopes of hydrothermal melting curves for silicates can change from negative to positive at high pressures, but the change is imposed, in this system, by phase transitions involving a large volume decrease, $-\Delta V$: albite = jadeite + quartz (Fig. 1); albite + nepheline = jadeite, which passes near I_6 in Fig. 2. The change is not produced by a progressive change in the properties of water in liquid and vapour phases. This does not preclude the possibility that a hydrothermal melting curve could pass smoothly through a temperature minimum where dT/dP = 0, in a system where the minerals do not undergo phase transitions until much higher pressures. At pressures above 15 kbar, we have found that the water content of the silicate liquids is of the order of 30 per cent by weight, and the aqueous vapour phase contains a high, but undetermined, proportion of dissolved solids; thus the physical properties of liquid and vapour phases become similar with increasing pressure.

These results indicate that temperatures at which silicate rocks in the presence of water begin to melt continue to decrease with increasing pressure through the thickness of the Earth's crust (equivalent to about 10 kbar pressure on average). Predictions that temperature minima occur on melting curves in this pressure range are not substantiated by experimental studies.

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PHYSICS

Relationships between the Masses of Subatomic Particles*

Two recent publications^{1,2} have listed the most reliable experimental figures for the rest masses of the known subatomic particles, and it would appear that the changes in the individual masses of these particles can be explained very simply by postulating the ubiquitous existence of a small particle of mass equivalent to approximately 26 MeV. In this communication, I shall call this minute particle a "tamaid" (Welsh, tam-ed: "a small piece"). The neutral tamaid is given the symbol t^0 and by analogy with baryons, mesons, and so on, the possibility of the existence of t^\pm tamaids cannot be discounted.

One singular advantage in dealing generically with uncharged particles is that complicating factors which result from electromagnetic mass effects are probably either eliminated or considerably decreased^{3,4}. I shall show that the masses of the known, neutral subatomic particles can be calculated with considerable exactitude if it is assumed that each particle consists of a π^0 meson (134.97 MeV) associated with an integral number, n, of neutral tamaid particles of mass, $t^0 = 25.95$ MeV, that is

 $M = \text{mass of a subatomic particle} = 25.95 \ n + 134.97$ (1

The magic number n has typical quantum characteristics and adopts values $n = 0, 1, 2, 3, 4 \dots$ and so on.

Table I shows the numerical application of this simple equation and shows that the calculated values for the individual rest masses, expressed in MeV, agree quite well with the experimental figures.

Table 1 UNCHARGED PARTICLES Mass M (MeV) (experimental) Mass M (MeV) (calculated) Difference (MeV) Integer, Symbol Type (experimental)
134-97 ± 0-015
497-82 ± 0-25
548-6 ± 0-4
756-4 ± 3-2
783-4 ± 0-9
939-55 ± 0-005
£,115-63 ± 0-05
1,192-2 ± 0-2
1,254 ± 12 134.97 Meson Meson Meson Meson 498·4 550·3 757·9 783·8 939·6 1,017·4 14 16 24 25 +0.58+ 0.58 + 1.7 + 1.5 + 0.4 + 0.05 - 1.2 η e° ω° N φ Δ f° E° Y* Neutron Meson Baryon 31 34 38 1,121·3 1,199 1,251 41 43 45 48 49 60 69 85 ±,254 ± 12 £,314·7 ± 1·0 £,385 £,405 £,688 £,990 Baryon Meson $\begin{array}{r}
-3.0 \\
-11.7 \\
-4.0 \\
+2.0 \\
+4.0 \\
+6.0 \\
+1.0
\end{array}$ Meson Baryon Baryon Baryon Baryon Baryon Baryon 1,303 1,303 1,381 1,407 1,692 1,926 2,341 $\frac{1,920}{2.340}$ ± 20

There is some experimental evidence for the existence of a \times meson of rest mass equivalent to (731 ± 2) MeV (ref. 5). A value of n=23 gives a calculated mass of $\times = 731.05$ MeV.

It could therefore be argued that the neutron, N, consists of a π^0 meson, associated with thirty-one tamaids

of type t^o , either as a static conglomerate or having the minor masses rotating in individual orbits of quantum number n=0, 1, 2, 3 and so on, the outward centrifugal forces, $t^o V^2/R$, being balanced by equivalent inward forces produced by mass interactions. Gravitational forces are usually considered negligible in nuclear calculations, but they could become enormous if the electromagnetic shape of the atomic particles were such that they permitted the close approach of the centres of mass of the particles concerned. Even for charged particles, the distance between the centres of mass need not necessarily be associated with the distance between the centres of electrostatic charge.

When applying the tamaid theory to the structure of the positively or negatively charged subatomic particles, one cannot ignore the possibility of the existence of tamaids of t^{\pm} type which would probably have masses of the order of $t^0=25.95$ MeV. The existence of quantum numbers for hypercharge, isotopic spin, angular momentum and parity would also have to be considered. By analogy with equation (1), π^{\pm} mesons (139-577 MeV) could be assumed to form the nuclei of charged subatomic particles, the n factors remaining the same as those taken for the uncharged sister particles, that is

mass of charged subatomic particle= M=25.76 n+139.577

If M for the positive subatomic particles is plotted against n the slope of the line is slightly less as shown in equation (2). Table 2 shows the application of this equation to present experimental data.

	Table	2. POSITIV	TELY CH.	ARGED PARTICLES	
Symbol	n	M (obse	rved)	M (calculated)	Difference
π^+	0	139-58	±0-014	139.6	+0.02
K+	1.4	493.78	±0-17	500-3	十多古是
e+	24	758-3	± 2·8	757-9	0-4
K	29	891.7	± 0.7	886.7	-5.0
P	31	938-256	± 0.005	938-28	+0.02
Σ^{+}	41	1,189.53	±0.08	1,195-6	+61
Σ_{γ}	59	1,660		1,659-6	(1+4
$\Sigma_{\mathcal{B}}'$	63	1,767	± 4	1,762-6	-44

The calculated value for the Σ^+ baryon is slightly high but is near to the experimental value for $\Sigma^-=1,197\cdot3$ MeV. The chief percentage divergence occurs with the K⁺ meson which has a calculated mass of 500·2 MeV. It is interesting that on the basis of the much more complex quark theory, Zeldovitch and Sakhurov² have also recently calculated a value of M=500 for this particle. The quark has not yet been discovered, but it is believed to have a mass equivalent to about one-third a nuclear mass, that is, about 330 MeV (ref. 3). The data of Tables 1 and 2 appear to confirm that changes in the masses of subatomic particles occur in such a way that the different

tial $\frac{\mathrm{d}M}{\mathrm{d}n}$ is of the fairly constant order of 26 MeV where n is a member of an integral series. It is remarkable that no subatomic particles are known which fall into the range n=1 to n=13, but there may be some basic physical reason for this peculiarity. For a tamaid of rest mass equal to 25.95 MeV, the de Broglie wavelength is of the order of $\lambda=4.78\times10^{-12}$ cm, which may be large for existence within a subatomic particle. If the tamaids agglomerate within a subatomic particle. If the tamaids agglomerate and if one considers the wavelength associated with the upper (n=13) tamaid agglomerate, then λ falls to 3.68×10^{-13} cm, a more reasonable value.

It could be argued that nuclear structures n=1 to n=13 are "forbidden" for these relativistic reasons, but once n=13 has been passed, the tamaid agglomerates can link up with the π meson in the range n=14, 15, 16, etc., to give the known mesons and baryons. It may be purely coincidental that the tamaid agglomerate at the end of the forbidden range would have a mass of (13×25.95) MeV = 337.3 MeV, which is almost exactly the hypothetical mass of the quark. According to the present theory, this mass, in association with a tamaid, or other masses, could pass

^{*} A further contribution will appear in a forthcoming issue of Nature.

the "forbidden" barrier and exist in the nuclei of sub-

Note added in proof. The value for the tamaid mass developed in this paper refers to the bound state, there being insufficient data to assess the binding energy. Should this particle ever be detected experimentally, serious consideration would have to be given to its possible occurrence in the core structure of the muons (4 tamaids) and the π mesons (5 tamaids).

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Growth of Single Crystals of Silver lodide in Silica Gel

The great solubility of silver iodide in concentrated potassium iodide solution and the rapid decrease of this solubility on dilution with water suggested that single crystals of silver iodide could be grown by a gel method1 similar to that used for growing crystals of cuprous chloride. Indeed, small silver iodide single crystals have been grown from solution by a dilution method. They appear as hexagonal prisms up to 20 mm in length and 2 mm in diameter or hexagonal pyramids up to 3 mm long and 1.5 mm across the base and take about 6-8 weeks to reach this size3. It seemed plausible that a combination of the two methods would yield larger and better crystals.

Gels were prepared from 7 ml. sodium silicate solution (244 g Na₂SiO₃·9H₂O+500 ml. water), 8 ml. 2 M potassium iodide and 15 ml. 2 M acetic acid. The gelling mixture was allowed to set in a 25 x 200 mm test tube at 45° C, and a solution made from 57 g silver iodide, 260 g potassium iodide and 250 ml. water was added carefully when the gel had set. This solution was allowed to diffuse into the gel for about a week, poured off and replaced by water. The temperature was kept at 45° C throughout the experiment. Hexagonal plates appeared within a few hours and grew to about 5 mm diameter. One side of these crystals was smooth, the other showed ridges along the diagonals between corners of the hexagon and along lines parallel to the sides of the hexagon, suggesting that growth occurs along only one direction of the c-axis.

Electron microprobe analysis confirmed the crystals to be silver iodide. No contaminating silicon from the gel was found (to an accuracy of a few p.p.m.), but potassium was found in quantities up to 6 per cent in certain regions of the crystals, chiefly along the valleys between the ridges.

Smaller hexagonal plates (about 3 mm) were obtained by a slightly different and shorter procedure. Gels were prepared as described, except that potassium iodide solution weaker than 2 M, or water, was used in making the gelling mixture. When the gel had set, addition of the silver iodide-potassium iodide solution resulted in the growth of these smaller crystals in the gel within a week.

A similar set of experiments at room temperature (about 23° C) gave different results. The gels were again made with water instead of potassium iodide solution. Crystals grew in the aqueous layer above the gel as well as in the gel. The crystals in the gel appeared as completely clear small pyramids and prisms (about 0.05 mm). The crystals in the aqueous layer were hexagonal pyramids,

5 mm in diameter and 5 mm high. They had good surfaces, but were translucent rather than transparent.

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THE SOLID STATE

Growth of Sapphire Filaments from the Melt

WE wish to report the growth of continuous sapphire (a-Al2O3) filaments from the melt with diameters in the range 0.05-0.50 mm, at rates up to 150 mm/min. The growth process almost certainly takes place (at the tip of the advancing filament) below, rather than above, the melt surface. There is an obvious similarity between the process described here and the dendritic growth of germanium and silicon previously described by various authors1.

Crystal growth experiments were conducted in an apparatus similar to a Czochralski crystal puller, using RF coupled to a carbon susceptor to heat the melt in a molybdenum crucible. It has been reported2 that sapphire crystals grown from molybdenum crucibles have a slight grey cast but do not contain significant amounts of molybdenum. Our experience is somewhat similar, though the grown crystals show no visible departure from transparency.

Fig. 1 shows an almost classical dendrite produced by allowing a melt of aluminium oxide to solidify onto a mass of solid aluminium oxide immersed in it. Dendrites were observed to propagate rapidly along the surface of the melt when the RF power was switched off. Careful X-ray examination of dendrites, such as the one shown,

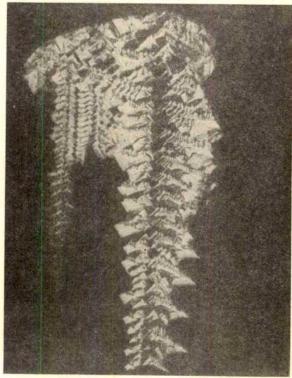


Fig. 1. Sapphire dendrite which grew in the c-direction (\times 40).

clearly demonstrates that most of the dendrites grow in the c-direction < 0001>. Other dendrites were found growing perpendicular to the very high index planes. Elongated dendrites growing straight downward were observed by rapidly pulling a mass of aluminium oxide from a rapidly cooling melt. These, too, were found to

be single crystals oriented in the c-direction.

Continuous c-direction growth of filaments has been achieved by properly controlling the thermal gradients in the melt. When the filaments are accurately oriented in the c-direction, the external surface is smooth, and they exhibit excellent mechanical properties. When the orientation departs slightly from the c-axis, the external surface of the filament reflects this in its morphology. This is demonstrated in Fig. 2, which shows four filaments (0.20-0.50 mm in diameter). Two are accurately aligned in the c-direction and two depart from it by small amounts, 9° and 11°. When the orientation departs even further from the c-direction, relatively gross jogging of the crystal occurs. The straight portions of the filament are inclined at an angle to the axis of growth but tend to grow in the c-direction. From the evidence presented, we conclude that the growth process is probably dendritic; and, because it does not require a particular twin geometry for its propagation, as is the case in germanium and silicon, the process may be described as auto-dendritic in the c-direction. Further demonstration of the dendritic nature of the process comes from an examination of filaments pulled rapidly from the melt. The tip morphology, under these circumstances, is strongly reminiscent of that reported for germanium dendrites1.

Mechanical measurement of the filaments at room temperature yielded a value for the average tensile strength of 300,000 lb./in.2, with high values of about 500,000 lb./ in.2 in filaments having diameters in the range 0·1-0·13 mm, as shown in Fig. 3. The modulus of elasticity was determined by a number of methods (including tension, bending, and ultrasonic propagation), yielding values ranging from 40-68 × 10⁶ lb./in.2. Because the filaments are single crystals, however, we believe that the modulus will approach the value characteristic of alpha alumina namely 67.5 × 106 lb./in.2, in the c-direction3. For comparison, similar strength measurements on centreless ground, flame polished, Verneuil grown single crystals have yielded tensile strengths in the range 72-102,000 1b./in.2, depending on orientation4. Sapphire whiskers, on the other hand, have exhibited tensile strengths in the range 1-2 million lb./in.2, but only where the diameter is very small⁵. For vapour-grown whiskers of diameters similar to those of our melt-grown filaments, values of 100,000 lb./in.² have been reported³. In brief, therefore,

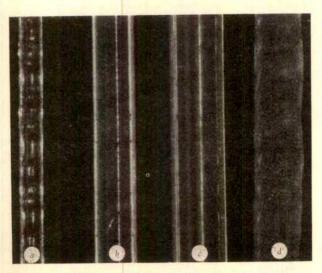


Fig. 2. Sapphire filaments which grew (a) 9° , (b) 0° , (c) 0° , (d) 11° to the c-axis (\times 30).

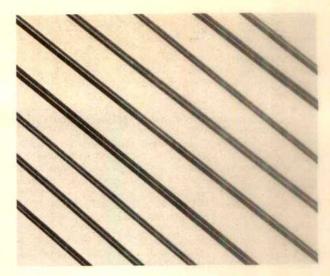


Fig. 3. Sapphire filaments 0·10-0·13 mm in diameter (×15).

the relatively massive filaments grown by our technique exhibit properties much closer to those of relatively perfect, very small whiskers than to those of larger diameter crystals obtained by another method.

In a more detailed account to be published later, the kinetics of the growth process are discussed and it is shown that very fast rates of growth may be possible, especially if forced convection is used to augment heat loss from the growing filaments. Because of the many potential technological uses for sapphire, it is interesting to note that we have already demonstrated multiple filament growth and the growth of continuous lengths of arbitrarily chosen cross sections. It is also clear that the process need not be limited to alumina but should be applicable to the growth of other materials which meet the basic crystallographic criteria for auto-dendritic growth.

We thank Dr Gunther A. Wolff, who has rendered invaluable assistance in X-ray crystallographic analysis, Dr George Hurley, who has provided much of the mechanical data, and Mr John Bailey, who has ably assisted in experimental phases of the programme.

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Voids in Irradiated Stainless Steel

During development work on fuel elements for fast reactor applications, electron microscope examination by the thin foil technique has been carried out on samples of stainless steel irradiated in the Dounreay Fast Reactor, either in the form of cladding on experimental fuel elements or as specimens intended for mechanical property tests. The steel had a composition falling within the American Iron and Steel Institute type 316 specification as shown by the analysis given in Table 1.

Tabl 1. ANALYSIS OF STEEL IN WEIGHT PER CENT

A common feature of all specimens examined after irradiation to total neutron doses greater than 1022 n.cm⁻² in the temperature range 400°-610° C was the presence of spherical or polyhedral cavities ranging in size from the smallest observable to 1500 Å. Fig. 1 is an electron micrograph of one such specimen irradiated at 510° C to a total neutron dose of 4.7×10^{22} n.cm⁻².

Bubbles have been seen before in irradiated stainless steel1,2 and their origin has been ascribed to the segregation of helium generated by (n, α) reactions, involving boron-10 if the flux is chiefly thermal, but embracing all the components of the alloy when the incident neutrons have energies greater than about 3 MeV (ref. 3). In the present case, however, the void population is too great to derive solely from helium.

It has been demonstrated that the size of helium bubbles formed in metals in conditions of vacancy mobility is determined by equilibrium between surface tension and gas pressure. We can therefore estimate the apparent helium content of a specimen from bubble sizes and numbers, assuming that the gas pressure P in the bubble is $2 \gamma/r$, where γ is the surface energy and r is the bubble radius. The number of moles of gas, n, in a bubble is given by the Van der Waals formulae

$$P(V-nb) = nRT$$

where V is the bubble volume, b the Van der Waals constant, and T the temperature.

Application of this analysis to the present observations using a value for γ of 5,500 erg cm⁻² (ref. 5) gives a much greater quantity of helium than is known to be present, both from theoretical considerations³ and from gas analysis, the discrepancy in some cases being a factor of several hundred. For example, in the case of a typical fuel pin at the point where void volume was at its maximum, atomic fraction of the helium calculated from neutron flux and nuclear cross section data was 4.7×10^{-6} atoms per atom, while the fraction of helium deduced from the void population was 3.1×10^{-3} atoms per atom. The gas pressure within the voids must therefore be much less than that expected from surface tension considera-

The voids are thought to develop through the condensation of vacancies, which are generated prolifically during irradiation in a fast neutron flux, on the helium nuclei produced by (n, α) reactions.

This conclusion is supported by the behaviour of the voids on annealing. Specimens containing voids of average diameter 210 Å and occupying 1.5 per cent of the total volume were annealed for 1 h at 700° C and 900° C.

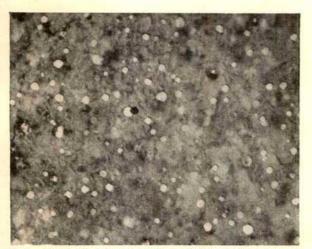


Fig. 1. Sample of fuel element cladding irradiated at 510° C to a neutron dose of 4.7×10^{22} ncm⁻² (\times 60,000).

	Table 2		
Total neutron dose $(n \times \text{cm}^{-2})$	Estimated mid wall can temp. (°C)	Average void diameter (Å)	Total void volume (per cent)
4·0×10 ¹²	270	_	
4.9 × 10 ¹¹	310	-	_
$5-3 \times 10^{22}$	380	_	-
5·2×10**	450	135	0.8
4·5×10 ²²	510	252	1.5
3·2×10**	560	258	0.2

While the 700° C treatment left the voids relatively unchanged, they were almost completely removed by the 900° C treatment, leaving a residue of porosity confined mostly to the grain boundaries and which could reasonably be equated with the helium content.

In typical irradiation experiments an axial temperature gradient is maintained in fuel pins which are exposed in flowing sodium, and one such pin was used to investigate the effect of irradiation temperature on void population. Specimens were taken at intervals of 3 in. along a 22 in. fuel element, the clad of which was originally in the solution treated condition, irradiated in the temperature range 270°-560° C. Average void diameters and estimated total void volumes are given in Table 2 together with irradiation temperatures and total neutron dose data.

Voids were seen only in the three hottest specimens, average void size increasing with irradiation temperature. The total void volume was peaked at 510° C.

The maximum void volume observed in any specimen so far examined was 7 per cent, this figure being confirmed by density measurement. The average void diameter in this sample was 1100 Å. This remarkable example of clad swelling occurred in a fuel pin at a point where the irradiation temperature was 500° C and the total neutron dose was 7.8×10^{22} n.cm⁻².

The cladding of this type of fuel element is subjected to considerable stress and strain during irradiation and there can be no doubt that these will influence void growth. Voids, however, have also been seen in unstressed material after irradiation, although the void fraction was smaller than in the samples from fuel pins. Thus it can be concluded that although stress may influence the subsequent enlargement of voids, it is not an essential prerequisite for void formation.

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CHEMISTRY

Use of Ethylene as a Gas Phase Dosimeter for Accelerator Radiation

The radiolysis of ethylene has recently been extensively studied1-6 and the production of hydrogen has been increasingly used as a gas phase dosimeter, principally for irradiation with electrons and with gamma radiation. We have extended the previous dosimetry studies by measuring the yields of hydrogen from ethylene irradiated with about 1.5 MeV protons from a Van de Graaff accelerator and with 4.3 and 6.8 MeV helium ions from the Harwell Variable Energy Cyclotron, over a range of dose rates from about 6×10^{18} to 6×10^{20} eV g⁻¹ sec⁻¹.

Our need for a reliable gas phase dosimeter arose from the continuing use of all-silica irradiation vessels? in which the thin silica windows (2-3 mg/cm²) are distorted during fusion to their mountings. The most convenient method

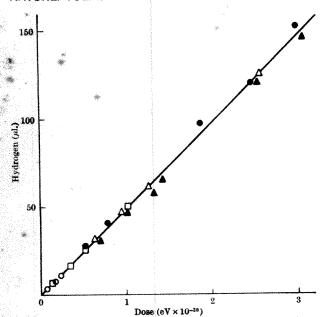


Fig. 1. Production of hydrogen from the radiolysis of ethylene. 1-5 MeV H⁺: \bigcirc , 5-75×10¹⁸ eV g⁻¹ sec⁻¹; \bigcirc , 5-75×10¹⁹ eV g⁻¹ sec⁻¹. 4-3 MeV He⁺: \bigcirc , 6-5×10¹⁹ eV g⁻¹ sec⁻¹. 6-8 MeV He⁺: \bigcirc , 4-1×10¹⁹ eV g⁻¹ sec⁻¹.

to estimate the loss of energy in the resulting non-uniform window is to measure the product yield from a gaseous dosimeter under the identical conditions used for subsequent irradiations of other gases. In order to establish reliable yields over the range of anticipated conditions, irradiations were carried out in a glass cell with a mica window of precisely known thickness cemented into place with 'Araldite' adhesive. The incident energies of the ion beams are known precisely and the energy losses in the mica window are calculated from range-energy datas and from the measured thickness of the window (2.69 mg/cm²). The geometry of the irradiation vessel is such that the ion beam is completely absorbed by the irradiated gas in a volume which is significantly less than the total volume Thus the insulated irradiated vessel corresponds to a Faraday cage and beam current measurements are made with a d.c. current integrator by inserting a platinum or tungsten electrode outside the irradiated volume in the vessel. The He+ ions lose an electron to form He²⁺ ions on passage through the mica window, but as the irradiation vessel is electrically earthed through the collector electrode the measured current corresponds to particles with only one positive charge.

Preliminary measurements with nitrous oxide over a range of dose rates were unsatisfactory principally because of the need to restrict doses to low values (<1020 eV/g) where the true initial yields can be measured. This requirement limited the irradiation times to a few seconds at the higher dose rates used, and while initial yields could be measured more accurately in a fast flow gas system this technique removes the elements of acceptable convenience and simplicity for a secondary dosimeter.

Measurements of the production of hydrogen from ethylene have, however, proved to be eminently satisfactory over a wide range of dose rates and of total dose. Ethylene from a cylinder (B.O.C.) was simply purified by two vacuum distillations at -78° C, collecting only the middle fraction (about 40 per cent) each time, and used without further treatment. The irradiations were carried out at ambient temperature and the radiolytic gases analysed by gas chromatography after passage through a liquid nitrogen trap; residual polymer on the walls of the cells had no effect on the yields, provided that the vessel was not opened to air between irradiations. The polymer could be removed eventually by treatment with chromic acid, or in the case of all-silica vessels by heating to 750° C with oxygen in situ. Irradiations were usually

carried out at a gas pressure of about 40 cm of mercury, but in one series of experiments with protons at a dose rate of about 2×10^{20} eV g⁻¹ sec⁻¹ the yield of hydrogen was unaffected by variations in gas pressure over the range 15-45 cm of mercury.

The experimental data are summarized in Fig. 1 for the two different ions (H+ and He+) from the two accelerators; only about 50 per cent of the data are included for clarity of presentation. It is clear that the production of hydrogen is a linear function of dose over the range of dose rates from about 6×10^{18} to 6×10^{20} eV/g and over the range of linear energy transfer for our experimental conditions—that is, from about $1.1 \times 10^{-3} \text{ eV}/\text{Å}$ (1.5 MeV H⁺) to about $7 \cdot 1 \times 10^{-3}$ eV/Å (4·3 MeV He³⁺) for ethylene at about 20° C and a pressure of about 40 cm of mercury. The slope of the line in Fig. 1 corresponds to an average yield of $G(H_2) = 1.31 \pm 0.02$

Perhaps the most striking feature illustrated by Fig. 1 is the linearity of the hydrogen yield up to high doset corresponding to ≤ 5 per cent decomposition. This results is probably unique to the irradiation conditions used because the polymer which is formed in high yields (G>11) settles onto the walls of the irradiation vessel and thus its concentration in the irradiated gas volume is always low and largely independent of dose. Even at 5 per cent decomposition the amount of radiation energy absorbed by the ethylene is only decreased by about I per cent as result of the accumulation of hydrogen and other gaseous products, under conditions where the ion beam still completely absorbed by the irradiated gas.

The measured yield agrees with values for other types of radiation at markedly different dose rates and values of linear energy transfer, namely

 $G(\mathbf{H}_2)$ Radiation 1·14 ± 0·28 (at 75 mm) 1·28 ± 0·19 (at 150 mm) (ref. 1) 1·2 (refs. 3 and 5) 1·2 (refs. 3 and 5) 2 MeV electrons 1 MeV electrons Gamma-radiation 1.2 ± 0.1 (ref. 6) 1.2 ± 0.1 (ref. 6)

Moreover, the independence of the yield with dose rate is in accord with previous evidence that the hydrogen is formed principally by a molecular detachment pro-These data demonstrate the usefulness of ethylene as a simple gas-phase dosimeter for work with accelerator beams and extend its increasing range of usefulness for dosimetry purposes in radiation chemistry.

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Formation of Solid Free Radicals by Mechanical Action

THERE is a marked reduction of the molecular weight of nitrocellulose on pulping, for the length of fibres is considerably reduced in the process1.2. Staudinger et al.3 have found a similar effect when polystyrene, cellulose and nitrocellulose are ground in a colloid ball-mill, and have also found that nitrocellulose is partially denitrated when grinding is prolonged. These observations indicate that purely

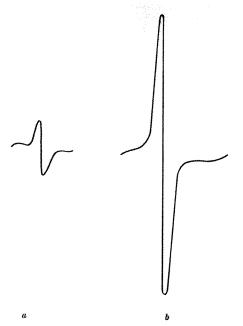


Fig. 1. a, Amber in grains 2–3 mm, amplitude 36 mm. b, Amber ground to $10-20\mu$, amplitude 170 mm.

mechanical cutting or breaking action can cleave covalent bonds.

I have now been able to show that free radicals are also formed by grinding solids. Materials, such as anthracene, acridine, cellulose and polyvinyl chloride powder, were ground in an agate mortar and free radicals were detected by electron spin resonance. An experiment with Baltic amber was particularly striking, for, after grinding a 0.0576 g sample of 2-3 mm diameter grains to $20-30\mu$, the intensity of the signal increased by 6.3 (Fig. 1a and b). The spectrum in Fig. 1a clearly indicates the existence of free radicals in the unground sample, confirming previous findings4. The intensity of the peak in Fig. 1a was calculated by taking Mn²⁺ in zinc sulphide (10¹⁵) as standard. The number of spins was 10¹³.

Although the intensity of the signal increased with grinding, it decreased with time and after a week was only 2.7 times its former, unground value. On further grinding the signal increased. Grinding once a week for three weeks to give a final diameter of 1-5µ gave about 10¹⁴ spins, ten times that of the original unground sample. Assuming that amber is a polyabietic acid, this led us to the figure 0.87×10^{-4} mol. per cent of abietic acid units in a free radical form.

Lagercrantz and Yhland⁴ have described how melted and solidified samples of calophony did not show the presence of free radicals, but grinding, however, gave an ESR signal, a finding with which our experiments agree. Powdered charcoal, $50-200\mu$ diameter grains, gave a strong signal-in agreement with previous findings with various types of coal⁵. Grinding the sample to 30-100u diameter grains gave in our experiments about an 18 per cent increase in the signal. The ESR measurements were carried out on an X-band apparatus having double modulation at 50 and 100 c/s.

I believe that the formation of free radicals by mechanical breaking and cutting, or scratching and polishing, could throw some light on phenomena, such as the charging of solids by friction6, and triboluminescence, which have not been fully explained. They both seem to result chiefly through a homolytic breaking of covalent bonds and formation of ions or radical-ions from free radicals. The charging of gases and liquids might also be explained on the basis of bond scission, the formation of free radicals and eventually ions or radical-ions, as a

result of thermal effects and a considerable increase of the temperature from the mechanical action.

The free radicals, caused by mechanical action, might lead to a reverse phenomenon by forming covalent bonds. This might explain the adhesion of polished surfaces in close contact; the "caking" of finely powdered substances (this would include powder metallurgy); the chemissorption of gases or liquids by solid adsorbants; and various phenomena associated with the beating of cellulose fibres^{7,8} such as an increase in the hygroscopic properties of cellulose pulp and an increase in strength of sheets made of cellulose.

Attention should also be given to the properties of charcoal as an ingredient in pyrotechnic compositions, such as gunpowder. Charcoal helps these mixtures to burn, and it is known that free radicals are essential intermediates in combustion and other chain reactions. The explosive nature of dusts, particularly coal dust, in air might be explained in terms of free radical reactions.

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PHYSIOLOGY

Psychopharmacological Profile of Molindone

RECENT clinical reports1-3 have indicated that the combination of a neuroleptic and an antidepressant affords an added measure of therapeutic effectiveness in the treatment of certain mental disorders. In the course of our investigations on new psychoactive agents, we have found a compound (molindone hydrochloride, EN-1733A) with a profile in animals which predominantly resembles that of the neuroleptics and yet which contains some of the elements characteristic of antidepressants. eally, molindone (3 - ethyl - 6,7 - dihydro - 2 - methyl - 5morpholino - methylindol - 4 - (5H) - one) is unlike any of the psychotherapeutic drugs at present available.

When administered orally to rodents, dogs and monkeys, molindone induces phenothiazine-like behavioural changes. In mice (CF1 males) and rats (CFE females), molindone suppresses spontaneous locomotion, blocks a conditioned avoidance response and antagonizes stereotyped behaviour induced by amphetamine. Although less potent than chlorpromazine in mice, molindone is approximately two to three times more potent in rats (Table 1).

Table 1. RELATIVE POTENCIES OF MOLINDONE AND CHLORPROMAZINE IN RODENT NEUROLEPTIC TESTS

Test	Species	Relative potency (95 per cent confidence limits) molindone/chlorpromazine
Locomotor suppression*	Mouse Rat	$0.23 (0.13-0.46) \\ 2.97 (2.19-3.89)$
Avoidance blockade†	Mouse Rat	$0.17 \ (0.12-0.24) \ 1.78 \ (0.92-3.58)$
Amphetamine antagonism ‡	Mouse Rat	$0.31 \ (0.23-0.44)$ $2.38 \ (1.90-2.98)$

Determined during a 30 min period using four mice or one rat/photocell

Animals trained to avoid a shock by jumping to a ledge at the sound of a

buzzer.

† Intensity of sniffing, licking and biting estimated 1 h after subcutaneous injections into mice and rats of 12 and 6 mg/kg, respectively, of d-amphetamine sulphate.

Molindone was administered orally 1 h and chlorpromazine 2 h before each determination.

determination.

In mongrel dogs or beagles, 1-2 mg/kg of molindone causes a behavioural depression for about 3 h, characterized by decreased locomotion, miosis and reduced arousability. Higher doses intensify and prolong these effects and simultaneously induce such others as catalepsy, palpebral closure and, in some dogs, tremors or motor restlessness. In cats, the oral administration of 5-10 mg/kg is necessary to suppress motor activity and arousability and to cause miosis. Palpebral closure was rarely observed in cats, even at 20 mg/kg, but staring, vocalization and emesis occurred in more than half the animals tested. In rhesus monkeys, 0.5-1 mg/kg of molindone curtails aggressiveness for 2-3 h. With increasing dosage, the degree and duration of docility are correspondingly increased but more gradually than after similar increments in the dosage of chlorpromazine.

Molindone also affects other responses frequently used to identify neuroleptics4. For example, the emesis induced in dogs by intravenous injections of 0.1 mg/kg of apomorphine hydrochloride is antagonized by molindone, orally administered at 0.5-1 mg/kg. Catalepsy and hypothermia can be demonstrated in rats and mice at oral doses of about 5-15 mg/kg. Finally, molindone inhibits the pressor responses of epinephrine and 5-hydroxytryptamine in anaesthetized dogs, but at intravenous doses which are about ten times higher than equiactive doses of chlor-

promazine.

Table 2. EFFECTS OF MOLINDONE IN RODENT ANTIDEPRESSANT TESTS

Test	Species	ED ₅₀ , mg/kg (95 per cen confidence limits)*
Tetrabenazine antagonism	Mouse Rat	2·5 (1·5-4·1) 2·6 (1·4-4·9)
5-HTP potentiation	Mouse Rat	10.0 (7.1-14.0) 20.0 (14.1-28.2)
l-DOPA potentiation	Mouse Rat	12·0 (8·6-16·8) 8·8 (4·6-16·7)

* Estimated by the method of Litchfield and Wilcoxon⁵.

Molindone was administered, orally, 1 h before intraperitoneal injections of 5-HTP and t-DOPA and 5 h before tetrabenazine.

In addition to these characteristic neuroleptic actions, molindone modifies certain responses in rodents which are similarly affected by antidepressant agents (Table 2). The ptosis induced by intraperitoneal injections of 40 mg/kg of tetrabenazine methanesulphonate in mice or 15 mg/kg in rats is antagonized by oral pretreatment with molindone. Using 50 per cent lid closure as the criterion for ptosis, peak activity was observed at 3-5 h in mice and 24 h in rats. Molindone also produces tremors when administered up to 6 h before subthreshold intraperitoneal injections of 50 mg/kg (mice) and 25 mg/kg (rats) of 5-hydroxytryptophan (5-HTP), peak effects occurring after 1-3 h pretreatment. Finally, molindone potentiates certain effects induced by intraperitoneal injections of 100 and 25 mg/kg of 3,4-dihydroxyphenylalanine (DOPA) into mice and rats, respectively. The criterion for DOPA potentiation in mice was the presence of two or more of the following symptoms: pupillary dilatation, exophthalmos, Straub tail, salivation and aggressiveness. In rats, pupillary dilatation alone was used as the criterion for potentiation because most of the symptoms mentioned occur sporadically after independent administration of either molindone or DOPA.

The effects of molindone summarized in Table 2 are similar to those induced by antidepressants of the dibenzoazepine class (for example, desmethylimipramine⁶) as well as by inhibitors of monoamine oxidase (MAO)7. Molindone scarcely affects rat brain MAO activity in vitro, however (50 per cent inhibition at 9.8×10^{-3} molar), using kynuramine as a substrate in the assay system of Krajls; nor does this agent substantially inhibit kynuramine oxidation by brain MAO after oral doses of 20 mg/kg in rats (maximal inhibition of 30 per cent over a period of 24 h). The possibility that molindone is converted into an active metabolite, in vivo, which then competitively reduces enzyme activity cannot be discounted. Yet when molindone is administered orally to rats 30 min after intraperitoneal injections of 50 mg/kg of SKF 525A, the usual potentiation of 5-HTP and DOPA is unaffected while inhibition of a conditioned avoidance response is enhanced and prolonged.

Work is now in progress to measure concentrations of amines in rat brain after treatment with molindone and to attempt to clarify the apparent paradox concerning tetrabenazine antagonism and 5-HTP and DOPA potentiation, on one hand, and antagonism to the effects of amphetamine, on the other. Although the ultimate clinical disposition of this unusual psychopharmacological agent has not been determined, a recent preliminary investigation has demonstrated its antipsychotic activity.

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Reversal of Reserpine-induced Hypothermia by Pharmacological Agents other than Antidepressants

It is known that antidepressants reverse or antagonize many of the effects of reserpine. A widely used test for the detection and evaluation of antidepressants is based on their calorigenic action in reserpinized mice1. In this test all types of clinically useful antidepressants are active but the test is not specific for this class of compound. Potent pharmacological agents such as aspirin, chlorpromazine and morphine, none of which is a clinically useful antidepressant, are accepted as positive by the test. Jori, Paglialunga and Garattini2 have shown that the calorigenic action of desipramine in rats was antagonized by α and β-sympathetic receptor blocking agents. Interest in the mechanism involved, and the possibility of making the test more specific, prompted an investigation of the action of α and β -blocking drugs on the calorigenie action of non-antidepressant compounds in mice.

Male mice (Alderley Park, No. 1, specific pathogen free) weighing 19-21 g were treated with reserpine (2 mg/ base/kg, subcutaneously) and 17 h later were dosed with saline (controls) or a subcutaneous injection of an a or β-blocking drug. Fifteen minutes later, the drug or saline was given orally. Gastric temperatures were

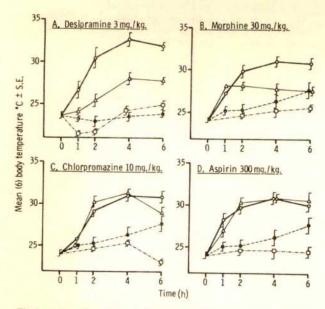


Fig. 1. Antagonism of the calorigenic action of compounds given orally. $A, \bigcirc -\bigcirc$, Desipramine hydrochloride, 3 mg base/kg; $B, \bigcirc -\bigcirc$, morphine hydrochloride, 30 mg base/kg; $C, \bigcirc -\bigcirc$, Chlorpromazine hydrochloride, 10 mg base/kg; and $D, \bigcirc -\bigcirc$, aspirin, 300 mg/kg. Groups of six mice on each treatment received reserpine phosphate, 2 mg base/kg subcutaneously 17 h before 0 h. Phenoxybenzamine, 30 mg base/kg, $\triangle -\triangle$, and propranolol hydrochloride ('Inderal'), 30 mg base/kg, $\square --\square$ were given subcutaneously 15 min before animals treated with A, B, C or D at 0 h. Controls, $\blacksquare ---\blacksquare$, received reserpine 17 h before, and saline at 0 h.

measured using an oral probe and electrical thermometer as described previously³. Temperatures were taken immediately before dosing with the calorigenic agent and at intervals of 1, 2, 4 and 6 h thereafter.

Fig. 1A shows the calorigenic effect of desipramine and Figs. 1B, C and D that of three compounds which, although they are potent pharmacological agents, are not clinically useful antidepressants. The α -receptor blocking agent phenoxybenzamine given at a dose of 30 mg/kg only partially blocks the calorigenic action of desipramine and morphine. The calorigenic effect of chlorpromazine was not significantly affected by phenoxybenzamine at the dose used (30 mg/kg).

The β -blocking drug, propranolol, at a dose of 30 mg/kg completely antagonized the calorigenic action of desipramine and the other compounds. The temperature of control mice rose significantly during the course of the experiment (Figs. 1B, C and D), but the mean temperature of the propranolol-treated mice was lower than that of controls and was not significantly different from the temperature at 0 h.

Selective blockade of either α or β -sympathetic receptors does not help in differentiating between the action of antidepressants and non-specific compounds in the reserpine hypothermia test. The results are compatible with the hypothesis that antidepressants and non-antidepressant compounds such as aspirin, chlorpromazine and morphine are, like antidepressants, producing their calorigenic action in reserpinized mice through an adrenergic link. The sympathetic receptors involved in the mediation of this effect appear to be more sensitive to β -blocking drugs than to α -blocking agents.

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Opening and Closing of Eyes and Signs of Psychomotor Excitation resulting from Stimulation of the Putamen in Cats

EVIDENCE from electrophysiological and behavioural experiments and from pathological conditions in man suggests that the functions of the putamen and caudate nucleus are inhibitory in nature. Electrical stimulation of the caudate, however, also gives rise both to electrophysiological excitation¹⁻³ and, from the point of view of behaviour, to facilitatory effects involving contraversive turning of the whole body⁴⁻⁶. We have shown that the inhibitory phenomena previously associated with the putamen⁷ are similarly concerned with excitatory effects.

The putamen was stimulated electrically in conscious unrestrained cats by means of implanted electrodes in which the tips (1 mm uninsulated) were located 2 mm apart one above the other. Square wave pulses lasting 3 msec were applied at frequencies varying from 2-30 c/s, the parameters being monitored by an oscilloscope. The effects of stimulating at 180 different foci, verified by subsequent histological examination, were examined by analysis of filmed records of behaviour taken at the time.

In the resting cat stimulation of certain foci, detailed later, gave rise to excitatory behavioural effects: the ears were flattened and retracted, the palpebral fissures (but not the pupils) were dilated (Fig. 1f and g) and sometimes piloerection occurred. When stimulation was continued

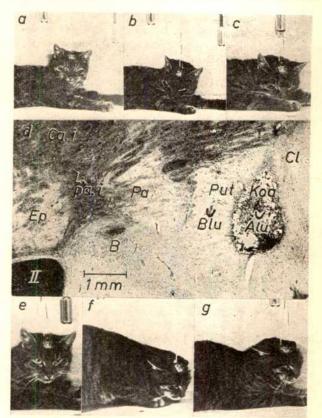


Fig. 1. Stimulation of the points Alu and Blu (see d) at 30 c/s causes an arousal effect with eye opening (a). During stimulation at 8 c/s at the same points the eyes are half closed (b), whereas stimulation at 4 c/s causes a closing of the eyes and a sleep like attitude (c). (d) Sagittal section through the brain of a cat showing some of the stimulation points which produced these effects. The track of the stimulating electrode and point Blu are located in the middle of the putamen. The stimulation point Alu lies in the rostral putamen within the coagulation focus produced by electrocoagulation at the end of the experiment. B, Basal nucleus; Ca. i, internal capsule; Cl, claustrum; Bp, entopeduncular nucleus; L. pa. i, lamella pallidi interna; Pa, pallidum; Put, putamen. Stimulation of the point Blu also elicited a backward dropping of the ears (f) as well as a moving backward of the cat (g) as a sign of excitation.

Compare the attitude before the stimulation (e).

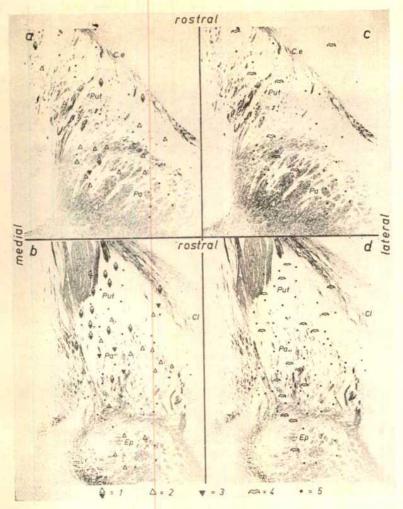


Fig. 2. Horizontal sections at two different levels of the basal ganglia (1.5 mm apart) showing the sites of the stimulating points producing contrary eye effects (opening and closing of the eyes) and/or of psychomotor excitation. The contrary effects (a and b, 1) are mostly elicited in the putamen and opening of the eyes alone (2) chiefly in the pallidum and entopeduncular nucleus. The points producing psychomotor excitation (4) are chiefly found in the putamen (c and d). In the pallidum only the medial region contains similar points. These, however, could be a result of the structure of the efferent pathway of the caudate (c) and putamen (d). (3) Closing of the eyes; (5) negative effects; C.e, external capsule; Cl, claustrum; Ep, entopeduncular nucleus; Pa, pallidum; Put, putamen.

or repeated, the animal also raised its body from the ground. These movements differ from those of the ears and head which are elicited by stimulating the basal nucleus8 or midbrain9 and from those which follow excitation in the region of the posterior commissure10 in so far as the palpebral fissures are then narrowed rather than dilated as in this experiment.

The foci from which such signs of a psychomotor excitation could be obtained were distributed unsystematically throughout the putamen. Seven of the eleven foci (Fig. 2c and d) giving rise to this effect were located within the basal putamen and, as can be seen in Fig. 2d, were particularly concentrated in the marginal regions of the putamen. Outside this area foci from which this effect could be elicited included the region occupied by the basal pallidum, the ansa lenticularis and the entopeduncular nucleus, which corresponds anatomically to that traversed by efferent fibres from the putamen. Several points responding to stimulation were also found in the dorsal margin of the pallidum and the entopeduncular nucleus; they correspond to the position of efferent fibres from the caudate nucleus.

Stimulation of other points within the putamen at a frequency of 30 c/s was, in contrast, followed by opening of the eyes and dilation of the pupils. This was sometimes

accompanied by a behavioural arousal which was demonstrated by the posture of the head. Stimulation of the same points with low frequencies (2-6/sec), however, caused a narrowing both of the palpebral fissures and of the pupils until finally the eyes were closed, although in these conditions the head did not always assume the posture characteristic of sleep (Fig. 1c). The effect of stimulation at 8 c/s on the palpebral fissure depended on the degree to which the eyes were open (wide or half) at the moment the stimulation commenced. Thus an incipient dilation of the palpebral fissure might be converted into a narrowing of the fissure and vice versa

We found no foci in the putamen from which stimulation alone at any frequency would cause the eyelids to close; such foci, however, were found near the putamen. During medium frequency stimulation opening of the eyes without the closing effect was seen six times following stimulation of the putamen, eighteen times from the pallidum and four times from the ansa lenticularis. Such points of stimulation were also found basal and rostral to the putamen.

The finding that both opening and closing of the eyes could be obtained from the same focus depending on the frequencies of stimulation supports the idea that the neuronal elements of the putamen mediate not only an inhibition of gazing movements but also a control mechanism for the reception of visual stimuli. It is surprising that there seems to be no description of this particular phenomenon in the literature dealing with stimulation of the caudate, but similar contrary effects involving sleep and alertness can be elicited from the hypnogenic zone if a supraliminal stimulus is applied11,12 centrum medianum, part of the hypnogenic zone, is known to project to the putamen and caudate nucleus13,14 so that the effects from the putamen described here could belong to the neuronal apparatus representing the hypnogenic system. The relatively frequent arousal and opening of the eyes induced by stimulating the pallidum and the ansa-lenticularis (without eye closing in the

case of low frequency stimulation) cor-respond to the activating influence of the pallidum and its efferent fibres15.

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Respiration and Clicking in Quail Embryos

In chicken embryos it is known that lung ventilation begins when, or just before, the embryo pierces the membrane dividing it from the air space, and is well established before hatching. Breathing is intermittent and irregular at first, becoming more regular and more rapid as hatching approaches^{1,2}. It has also long been known that respiration in chicken embryos may be accompanied by a loud regular clicking^{3,4}. Apart from the connexion with respiration, it is not known how these clicks are produced, although in duck embryos observed through holes made in the shell clicks are reported to coincide with movements of inspiration or expiration or both⁴.

In bobwhite and Japanese quail, we have been able to observe that lung ventilation begins about 2.5 and 1.5 days, respectively, before hatching, and it has been shown that the embryos produce many sounds, although usually only of moderate intensity, until about 10-15h before hatching when regular loud clicking begins. Usually these loud regular clicks end before the chick emerges. Recently. we have made simultaneous recordings of clicking and breathing which have thrown more light on their relationship and perhaps also on the development of lung ventilation in avian embryos. Methods of recording will be described elsewhere. Briefly, for sound the egg rests on three mutually perpendicular piezoelectric ceramic ele-The system gives a calibrated flat frequency response over the audio range. A second instrument measures the pressure changes in the air space occurring with breathing (and, of course, also heart and limb) movements. This system can measure to about 0.01 mm of water differential and can be used at the same time as the sound recording. Apart from a small hole in the shell over the air space, which is sealed by the pressure transducer, there is no interference with the embryo.

Records show intermittent and irregular patterns of early respiratory movements (Fig. 1) and the type of simple pattern which occurs as breathing becomes more regular (Fig. 2). An increase in pressure is taken to indicate inspiration. In all illustrations, the top line gives time in seconds and the second shows pressure changes (a rise in pressure is indicated by a deflexion away from the time marks). Of the pair of lines at the bottom, the top line indicates a push and the lower one a pull of the embryo against the transducer. These two lines together form one of the three sound channels (sound envelope only). Figs. 1 and 2 show that this early breathing, which may last for up to about 2 days, occurs without any regular clicking.

Clicks first occur in short bursts and may become loud and regular quite suddenly. We have evidence that the

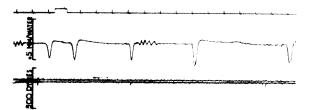


Fig. 1. Bobwhite quail, 49.5 h before hatching.

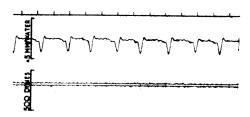


Fig. 2. Bobwhite quail, 17.5 h before hatching.

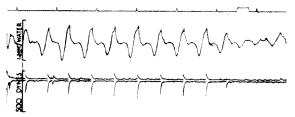


Fig. 3. Bobwhite quail, 5 h before hatching. Compare amplitude of silent (last 3) breaths with that of those accompanied by a click.



Fig. 4. Japanese quail, 4.75 h before hatching.

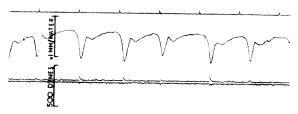


Fig. 5. Bobwhite quail, 29-25 h before hatching.

beginning of loud clicking accompanies or follows a rise in the respiration rate. Moreover, recordings show an increase in amplitude of pressure change (Fig. 3), as well as an alteration in the respiratory pattern, when breathing is accompanied by a click (Figs. 3 and 4). Here the pattern is more complex than that in Figs. 1 and 2, which indicate breathing of the in-out-pause type, although the in-out phase is still apparent. The pattern of pressure change varies somewhat from one egg to another, as the embryo's position within the egg is asymmetrical, and the position of the hole for the pressure transducer will also vary. What is invariable in these records is the timing of the click signals on the sound and pressure records; the click just precedes a sudden rapid change in pressure. This is almost always a change to a rapid rise in pressure (presumed to be a change to breathing in) and occurs between successive in-out movements. during or after the period of regular loud clicking, similar sounds of lower intensity may occur during the in-out phase of breathing (Fig. 5), but in the quail these are less frequent than the regular loud clicking, chiefly considered The sounds associated with this second type of click also show forces of polarity different from the loud regular clicks.

More detailed records can be obtained by recording from only one side of the air space—that is, from the side where the beak protrudes through the torn membrane, or from the opposite side, excluding the beak and below the membrane. For this purpose the shell is removed over that part of the air space to be excluded, and a thin strip of rubber stretched across the gap and glued to the shell. When the edges are sealed with 'Vaseline', it is possible to record from the remaining part of the air space. Records excluding the beak are of the form shown in Fig. 6 (fast recording) where the click coincides with a very rapid lowering of pressure preceding the rapid rise. This suggests that the click accompanies, or could be produced by, an extra, sharp exhalation and inhalation between successive in—out phases of breathing. Recordings from the side of the air space including the beak (Fig. 7) provide a mirror image of this pattern. This mirror image

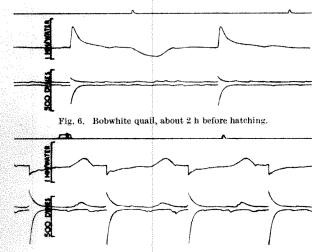


Fig. 7. Bobwhite quail, 15 h before hatching.

effect is to be expected if the greater part of the pressure change recorded now comes from below the membrane. These very fast recordings show best the actual relationship between the sound and pressure signals. Calibration tests show that any delay in the pressure as compared with the sound channel is less than 0.25 msec. sudden lowering of pressure seems to be smoothed over when recordings are taken from the whole air space as in Figs. 3 and 4.

This coincidence of clicking with a change in the rate, amplitude and pattern of breathing suggests that the clicks are produced by a specialized form of breathing in the embryo. This type of breathing begins many hours after lung ventilation has begun and has been stabilized, and usually ends before hatching

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Visual Feedback in Hand Tremor

In the experimental situation with which we are concerned here a subject holds a short, stiff, vertical joystick with the finger tips of his right hand and attempts to press to the left with a constant force of, say, 1 kg. His forearm is supported in a horizontal trough. The force exerted is measured by a strain gauge and is made to deflect the spot of a cathode ray tube watched by the subject. The subject's task is to keep the spot at a mark on the tube face corresponding to the required force. He does not succeed, the spot dancing unceasingly around the target position. These errors we refer to as tremor.

Sutton¹ recorded the tremor with this experimental arrangement and performed a frequency analysis. He found (as have other workers in other tasks) that some subjects have a pronounced peak at about 9 c/s in their tremor spectrum. Sutton and Sykes² subsequently made the surprising discovery that if such a subject closed his eyes, or if (without any other alteration in the conditions) the cathode ray tube spot was extinguished, the 9 c/s peak disappeared.

Attempts were made^{2,3} to reconcile this finding with the current view that the 9 c/s peak represents an oscillatory tendency in the stretch reflex loop at spinal level; but experiments, which we need not go into now, led us to doubt this interpretation and to test the alternative hypothesis that the 9 c/s peak involves activity in the larger, visual, feedback loop joystick-display tube-eyebrain-hand-joystick. The test we applied was to introduce time delays into the loop between the joystick and the display tube and to show that this shifted the frequency of the 9 c/s peak. Time delays were simulated by a Padé network giving a phase shift nearly proportional to frequency over the range 1 to 15 c/s. (The principal results have been confirmed in later experiments using genuine time delays from a magnetic tape device.)

The results of a typical experiment are shown in Fig. 1. The spectrum for the first control run (at the top) has a peak at 8.8 c/s. Introducing a delay of 25 msee shifts this to 8.0 c/s. Larger delays caused larger shifts, roughly in proportion. A quite unexpected feature was the emergence of a second peak at a higher frequency. Occasionally with the longest (100 msec) delays even a third peak appeared, at about 13 c/s. (A questionable example of such a peak is seen in Fig. 1.)

Such experiments show without much doubt that under these conditions the frequency of the 9 c/s peak is determined by visual feedback, but the nature of the potential instability in the visual loop thus revealed is uncertain. Consider a negative feedback system with a time lag in it oscillating in the simplest manner at the frequency at which the time lag introduces π radians (180°) phase

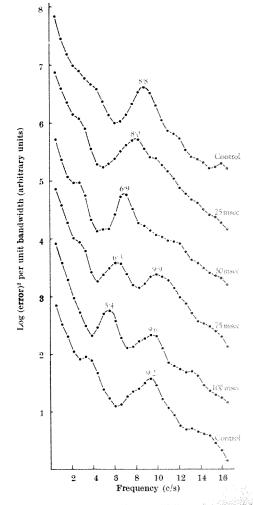


Fig. 1. Power spectra of hand tremor with the various time delays shown inserted between the joystick and the display cathode ray tube. Each recording run lasted 30 sec. The series began and ended with a control run and the runs were made in the order shown, starting at the top. To avoid overlap consecutive spectra are displaced one log unit downwards. The frequencies of the main peaks are given above them in cycles per second. The data were digitized, punched on to paper tape and the power spectra computed on Edsae II in Cambridge.

shift. A frequency of 9 c/s corresponds to 55 msec lag. Adding an additional lag of 100 msec would reduce the frequency of oscillation by a factor of nearly three; but the shift in our experiments was always much less than this. Hence the 9 c/s peak is not of this nature. Suppose instead that the 9 c/s peak is a harmonic of some fundamental mode of oscillation at a lower frequency. turns out that if it were the third harmonic, the fundamental being at about 3 c/s (corresponding to an inherent lag of 167 msec), the expected shift in the 9 c/s peak with added delay would be reconciled with the experimental results, and, furthermore, the two higher frequency peaks, when they occur, would fall into place as the fifth and seventh harmonics. This identification has therefore much to recommend it, but, from other considerations, we prefer a different, but closely related, working hypothesis in which the fundamental (if present) corresponds to an oscillatory tendency with π radians of phase shift round the loop, but the higher peaks are not harmonics but separate modes of oscillation with 3π , 5π and 7π radians phase shift. We prefer this hypothesis because it can account (a) for the apparent lack (in most experiments) of a peak at 3 c/s (any mode may be absent if the gain at that frequency is low), (b) for the absence of peaks corresponding to the even harmonics (instability only results when the phase shift is $\pi + 2n\pi$ radians) and (c) for the occurrence of multiple peaks not in any simple harmonic relation when frequency dependent lags are introduced. These last observations will be described in the full account of this work now in preparation.

It should be mentioned that not all 9 c/s peaks are visually determined. By slightly altering the mechanical conditions of the task Dymott and Merton⁴ have produced good 9 c/s peaks in runs with the eyes shut.

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Dissociation of the Pressor and **Antidiuretic Activities of Synthetic** Arginine Vasopressin by Heating at pH 10

Zaoral, Kolc and Šorm¹ showed that the analogue of arginine vasopressin in which the arginine is in the D rather than the L-configuration, [8-D-arginine]-vasopressin, has ten times as much antidiuretic as vasopressor activity. This finding allows an explanation for the early report by Heller² that a similar dissociation of activities was obtained when 'Pitressin' (Parke, Davis and Co.) was heated at alkaline pH. One of the effects of such treatment on polypeptides is racemization of their constituent amino-acids, and, if one can generalize from the behaviour of adrenocorticotrophin (ACTH), one of the amino-acids most likely to racemize is arginine3.

Fraser⁴ confirmed Heller's² results when he assayed antidiuretic activity by subcutaneous injection of the hormone into rats or dogs. When, however, he administered the hormone intravenously, to dogs he found that the antidiuretic potency of the treated hormone, so determined, was the same as its vasopressor potency. He attributed the discrepancy between the antidiuretic potencies, determined by the subcutaneous and intraven-

Table 1. Effect of heating synthetic arginine vasopressin at ph 10 in a boiling water bath on the ratio of its antidiuretic and vasopressor activities

Time of		Potency	Potency (v/ml.)*			
heating (min)		Antidiuretic	Vasopressor	Vasopressor		
Unheated	(a)	2.147 (1.863-2.438)	1.944 (1.572-2.393)	1.06		
	(b)	2.510 (2.241-2.846)	2.172 (2.041-2.314)	1.15		
15		0.468 (0.410-0.526)	0.371 (0.330-0.422)	1.26		
30	(a)	0.117 (0.092-0.156)	0.038 (0.034-0.045)	3.08		
	(b)	0.079 (0.065-0.094)	0.048 (0.040-0.059)	1.65		
50		0.073 (0.057-0.098)	0.041 (0.035-0.049)	1.78		
60		0.061 (0.046-0.075)	0.041 (0.028-0.060)	1.49		

* Each result is from a 2+2 assay design with at least three comparisons; values in parentheses are the 95 per cent fiducial limits of the mean potencies.

ous routes, to the presence of amino-acids derived from hydrolysis of the hormonal peptide, because Noble, Rinderknecht and Williams⁵ had shown that amino-acids enhance the antidiuretic effects of subcutaneously injected vasopressin. Noble et al.5, however, found that an aminoacid concentration of about 1 per cent was necessary to demonstrate this effect and this would represent a concentration of the vasopressin used by Fraser ('Postlobin-V') of $2{,}000$ U/ml., whereas Heller's incubation mixture contained only 0.5 U/ml.

In view of the recent findings of Zaoral et al. we decided to reinvestigate the action of mild alkaline treatment on synthetic arginine vasopressin (provided by Dr D. R. Chambers of Hoechst, Ltd.). The peptide (1.15 mg) was dissolved in 5 ml. of 0.05 normal acetic acid and this served as the stock solution. For each experiment, 1 ml. stock solution was diluted to 10 ml. with a carbonate-bicarbonate buffer of pH 10 (made by diluting 10 ml. of molar sodium carbonate and 20 ml. of molar sodium bicarbonate to 500 ml.) and a sample was immediately placed in the cold until assayed. The remainder of the solution was distributed in 1 ml. portions among ampoules which were then sealed and heated in a boiling-water bath for the required time. At the end of the heating period the ampoules were cooled and stored in the refrigerator until assayed-which in all cases was on the same day. Antidiuretic activity was determined by intravenous injection into rats according to a modification of the method of Jeffers, Livezey and Austin⁷. Vasopressor activity was also determined in rats by Dekanski's method. commercial preparation of natural arginine vasopressin, 'Tonephin' (Hoechst AG, Frankfurt), was used as standard in all assays.

Table 1 shows that heating at pH 10 does cause a dissociation of the antidiuretic and pressor potencies of arginine vasopressin even when the former is measured by an intravenous method. Heating the hormone for periods of 30 min or longer increased the ratio of antidiuretic to vasopressor activity from approximately 1:1 to just under 2:1, although the absolute amount of each activity was considerably reduced. This result is compatible with the formation of some [8-D-arginine]vasopressin as one of the reaction products. The ten-fold difference between antidiuretic and vasopressor potencies of heated hormone found by subcutaneous antidiuretic assay^{2,4} may be due in part to a delayed absorption caused by the amino-acids derived from hydrolysis of the hormone⁴, but perhaps more likely to the slower absorption of a modified hormone.

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Anti-inflammatory Activity of d- α -Tocopherol (Vitamin E) and Linoleic Acid

CELLULAR membranes, especially those of lysosomes, become unstable in a variety of conditions—reviewed by Thomas—for example, the Arthus and Schwartzman reactions; exposure to streptolysin, ultraviolet light and carbon tetrachloride; and the muscular dystrophy of tocopherol deficiency1. Cortisone is inhibitory in most of these conditions, and has been shown to have antioxidant properties2, so we thought that other antioxidants might be effective anti-inflammatory agents. Lysosomal membranes, as well as other membranes, are composed largely of lipids and we also thought that their lysis could result from damage to such structural lipids. This idea is supported by work of Austin and Brocklehurst3, showing that the release of histamine and slow reacting substances (SRS-A) from sensitized guinea-pig lung is inhibited by long chain fatty acids. Accordingly d-a-tocopherol (vitamin E) and linoleic acid were tested as anti-inflammatory agents and found to be effective.

Male Holzman rats weighing 120-160 g at the beginning of the experiments were given adjuvant consisting of 3 mg of heat killed Mycobacterium butyricum (Difco) in 0.1 ml. of olive oil injected into the subcutaneous tissues of the tail (day 1). The rats were then divided into test groups. The lesions were graded from 0 to 4 on the following basis: 0, no apparent lesions; 1, up to four lesions up to 3 mm in diameter; 2, more numerous or larger lesions than in grade 1, up to a maximum of 1 cm in diameter; 3, lesions larger than 1 cm often becoming confluent but not yet involving an entire extremity; 4, confluent involvement of one or more extremities with swelling of the entire extremity of two or more times the normal diameter. The scores of rats in the different groups on days 12, 13, 14, 15, 17 and 19 were treated statistically to provide the mean and standard deviation for each group. The treated groups were compared with the controls by the t test.

This adjuvant treatment produced lesions between the eighth and twelfth days and they rapidly grew worse, reaching a maximum usually before the nineteenth day. Most animals in the control groups developed diffuse swelling of the extremities which was so severe that the limbs were three to four times the normal diameter within 5-6 days of the onset of swelling. These rats had severely impaired mobility. Only one of the twenty-one rats in the control group developed no gross lesions, while 85.7 per cent developed grade 4 lesions.

Linoleic acid and d- α -tocopherol were given intraperitoneally in attempts to inhibit the development of lesions. Twenty-four rats were each given 0.6 ml. of tocopherol/kg of body weight once a week, beginning on the day of administration of adjuvant. Of these (Table 1), 29.2 per cent developed no detectable lesions, 54.2 per cent developed grade 1 to 2 lesions and 16.7 per cent developed grade 3 and 4 lesions. The differences between controls and the tocopherol-treated groups on days 12, 13, 14, 15, 17 and 19 were well below the 0.001 level of

significance.

Twenty-four rats were each given linoleic acid, 1.33 ml./kg of body weight on the day when adjuvant was given (day 1) and 0.5 ml./kg of body weight daily thereafter. Previous testing indicated that linoleic acid induced local bleeding and haematoma formation, and that this dosage approached the limits of tolerance for the duration of the experiments. The inhibition produced (Table 1) was similar in degree to that produced by tocopherol and the difference from the controls on each of the respective days was significant below the 0.001 level.

The effect of delaying the initiation of treatment to the fifth or ninth day after adjuvant administration was investigated. Delaying the first injection of linoleic acid to the fifth day produced results similar to those after giving the first injection on day 1. Delaying the first administration of tocopherol until the fifth day, or of linoleic acid to the ninth day, however, resulted in the development of lesions with an average severity intermediate between the controls and the other treatment groups. Delaying the first tocopherol treatment until the ninth day resulted in no significant difference from the controls, and continued treatment produced no gross evidence of improvement. The rats, however, seemed to suffer less discomfort and immobility, and to be less irritable.

One possible explanation for the decreased influence of the linoleic acid and tocopherol when treatment started late is that these substances must be incorporated into cellular structures to be of maximum benefit. If this explanation proves to be correct, the time required for incorporation of tocopherol would be longer than for linoleic acid.

It is possible that the phagocytic or intracellular handling of the adjuvant, or the immunological response to the constituents of the adjuvant, is altered by the treatments outlined, and tests are in progress to determine whether this is so. On the other hand, tocopherol is well known as a biological antioxidant and free radical seavenger*. Linoleic acid is an effective inhibitor of damage by Xirradiation⁵, which is mediated by free radicals. facts together with the anti-inflammatory activity that we are reporting here suggest that inflammation of this type involves lipid peroxidation and its associated production of free radicals. As mentioned here, cortisone is a stabilizer of membranes against a variety of agents, and it is also effective in inhibiting adjuvant-induced arthritis. Weissmann and Thomas have reported that cortisone also possesses antioxidant properties2.

Linoleic acid may also act either as a competitive substrate for whatever processes attack cellular lipids, or as a ready replacement for damaged cellular lipids.

Table 1. DEVELOPMENT OF LESIONS IN TREATED RATS

Group	Total No. in group	No. o	f rats in e	each grou espective 2		1 to 4	Mean	Signt- flexace level
1	21	1 (4·8%)	-	(4·8%)	1 (4·8%)	18 (85·7%)	3-7	Special
2	24	7 (29·2%)	6 (25·0%)	7 (29·2%)	(4·2%)	(12·5%)	1.6	Less than 0-001
3	24	11 (45·8%)	3 (12·5%)	(4.2%)	4 (16•7%)	(20·8%)	1*5	Less than 0.001
4	6		1 (16·7%)	2 (33·3%)	(33·3%)	(16.7%)	2.5	0.01
5	6				4 (66•7%)	(33·3%)	3*3	Greater than 0.5
6	6		(16·7%)	4 (66·7%)	(16•7%)		2-0	Less than 0 001
7	6	1 (16·7%)		1 (16·7%)	(33·3 %)	(33·3%)	2-7	Greater than 0-05

Groups 2-7 received adjuvant plus the treatment described here. The score for each group was compiled from the highest individual scores within the group. The significance level is that for day 19, comparing the respective groups with the control group (1). (1) Adjuvant only; (2) tecopherol, 0.6 ml./kg body weight/week, starting on the day of adjuvant administration (day 1); (3) linoleic acid, 1-33 ml./kg body weight on day 5; (5) as group 4, but starting on day 9; (6) linoleic acid, 1-33 ml./kg of body weight on day 5 followed by 0-5 ml./kg body weight daily thereafter; (7) as for group 6, but starting on day 9;

Lipids are important both in the structure and function of mitochondria, and mitochondrial oxidative phosphorylation declines with peroxidation. Tocopherol also may function in conjunction with sulphydryl sites associated with respiratory enzymes. Lysosomal instability and enzyme loss may also result from lipid peroxidation, and lysosomal enzymes may attack mitochondrial components, so that the conditions are available for a vicious cycle in which impairment of cellular metabolism and attack on structural elements may each cause further damage to the other. Such a cycle could be inhibited or broken by antioxidants.

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Types of Secretory Neurones in the Pre-optic Nucleus of the European Eel, Anguilla anguilla L.

Knowles and Vollrath¹, working on the ultrastructure of the neurohypophysis of the eel, Anguilla anguilla, have shown that it contains three distinct types of neurosecretory axons separable by the size and appearance of their elementary granules, and termed types A1, A2 and Brespectively. A type fibres contain elementary granules of diameter greater than 1000 Å, whereas in the B type fibres they are less than 1000 Å. There is some evidence that only the A type fibres are stained by so-called neurosecretory dyes. For this reason and because the neurones of the pre-optic nucleus are the only ones in the hypothalamus of fishes that stain with these dyes, it seems likely that the A type fibres originate from these neurones. The detailed morphology of the entire hypothalamohypophysial complex of the eel has been described2 and a good deal is known of the histology of the pre-optic nucleus from optical microscopy (see ref. 2 for references) although these studies have so far failed to identify two cell types that might give rise to the two A type fibres of the neurohypophysis.

We have now examined the pre-optic neurones of the eel by electron microscopy and by optical microscopy of

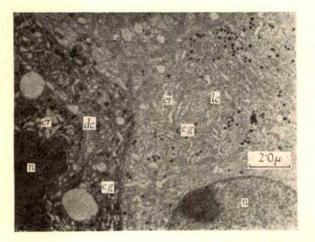


Fig. 1. "Dark" cell and "light" cell adjoining. Note differences in electron density of both cytoplasm and nucleus. dc. Dark cell; eq, elementary granules; er, endoplasmic reticulum; lc, light cell; n, nucleus. Stained with uranyl acetate.

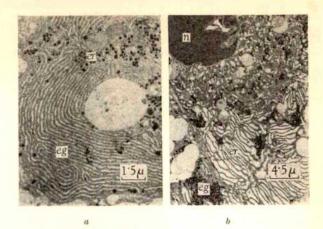


Fig. 2. a, Peripheral region of "light" cell showing endoplasmic reticulum, elementary granules and other inclusions. Stained with uranyl acetate, b, Peripheral and perinuclear regions of "dark" cell showing endoplasmic reticulum, and high electron density of cytoplasm and nucleus. Stained with uranyl acetate. eq. Elementary granules; er, endoplasmic reticulum; n, nucleus.

sections cut about 1 µ thick from material fixed and embedded for electron microscopy and stained with Azur II. These studies have revealed two easily separable types of neurones. At the level of ultrastructure the perikarya of both are divisible into two distinct regions, a zone in contact with the nucleus and continuous with the axoplasm and a polar cap of endoplasmic reticulum containing some large electron lucent vesicles. In the perinuclear zone, mitochondria, Golgi bodies, inclusions of differing sizes and electron densities and the so-called elementary granules are present. The most immediately obvious difference between the two types of cell is that one is appreciably more electron dense than the other, especially after staining with uranyl acetate, this difference extending to both cytoplasm and nucleus (Fig. 1). The functional significance of the two cell types is unknown, and so they are named "dark" and "light" cells, respectively. It may be noted that the dark cells also stain more deeply with Azur II and that both cell types are present in both regions of the pre-optic nucleus.

A further difference between the two cell types concerns the appearance of the endoplasmic reticulum. In the dark cells the cisternae are swollen and irregular whereas in the light cells they are narrow and regular, the lamellae being arranged in parallel concentric rows in "fingerprint' fashion (Fig. 2). The difference between the sizes of the elementary granules is, however, the most important distinguishing feature. By measuring the largest elementary granules in a number of each of the cell types a mean diameter of 2150 ± 30 Å was obtained for the dark cells and of 1627 ± 31 Å for the light cells. These sizes are identical to those obtained for the elementary granules in the A1 and A2 type fibres respectively in the neurohypophysis and the difference between them is highly significant (P < 0.001). Although the sizes recorded here are slightly larger than those given by Knowles and Vollrath¹, the difference between the diameters of the two kinds of granules is closely similar and it seems possible that the discrepancy can be accounted for by differences in fixation techniques.

Our first interpretation of the differences between the two cell types was that they represented different levels of activity, but both cell types appear highly active and there are no intermediate stages. Furthermore, the difference in size between the elementary granules of the two types seems to confirm that they are distinct and it can be supposed that each gives rise to one of the two A type fibres described by Knowles and Vollrath¹ in the neurohypophysis of the eel.

The physiological significance of the two neuronal types has not been determined although, because two octapeptide hormones-arginine vasotocin and ichthyotocin-have been identified in the neurohypophysis of fish³, and Lederis⁴ has shown that at least the former resides in elementary granules, one of the neurone types may be vasotocinergic and the other ichthyotocinergic. Such a possibility can be tested only by isolation of the two types of neurones and identification of the hormones they produce.

We thank Dr Gunther Sterba for allowing us to report that in recent work on the pre-optic nucleus of the carp, Cyprinus carpio, he has identified dark and light cells

very similar to those described above.

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Enzyme Induction in Man caused by Smoking

HIGHER animals may change in their response to drugs because of a decrease in the sensitivity of drug receptor sites in the organism, or because of accelerated drug metabolism. The latter has been explained on the basis of drug induced stimulation of liver microsomal enzymes, for example, many compounds have been listed which induce microsomal oxidizing enzymes in rats, and phenobarbital has been shown to stimulate the metabolism of the anti-coagulant coumarin in man2. We now report the induced enzyme metabolism of nicotine in man by tobacco smoking.

The recovery of nicotine from urine after administration by (a) intravenous injection; (b) inhalation of nicotine vapour; and (c) smoking to male subjects (age 21-40 yr, not taking other drugs) whose urine was maintained acidic was determined by gas-liquid chromatography3. Maintenance of an acid urine minimizes intra- and intersubject variations in excretion of bases4-6, and in these conditions the excretion of unchanged nicotine is virtually complete in 8 h. (Smoking (c) was measured from the amount of nicotine in the main stream smoke7.)

The results of the nicotine experiments are shown in Fig. 1; variation between subjects was small. Whatever the route of administration, the percentage recovery of nicotine in the urine remained constant within ±8 per cent for each subject, and was greater for non-smokers (55-70 per cent) than for smokers (25-50 per cent) (Fig. 1). Non-smokers were not given nicotine more than once every 3 weeks during the trials; nicotine recoveries were the same throughout the trial period.

The increased metabolism of nicotine by smokers cannot be attributed to the increase in the nicotine dose resulting from inhalation, because the recovery of nicotine excreted unchanged in the urine was the same for subjects after intravenous injection and smoking, and was also the same when subjects inhaled or deliberately non-inhaled while smoking, that is, recoveries of nicotine in the ranges of doses used were not affected by the dose of nicotine absorbed.

Subjects were classified as non-smokers if they did not smoke more than thirty eigarettes or three eigars a year. One current non-smoker (G. O. J., Fig. 1) had been a heavy smoker some years before the trials; a lower recovery of nicotine was observed in this subject than in other non-smokers. It is possible that the tolerance to

nicotine lasts for at least 2 months, for one smoker (J. F. T., Fig. 1) abstained from normal eigarette smoking during a 2 month period of the trial; the nicotine recovery remained as expected for a smoker.

Thus the clear difference in nicotine recovery in smokers and non-smokers indicates that habitual smoking induces enzyme metabolism of nicotine. Preliminary results indicate that it is not by an increased metabolism to cotinine because recoveries of cotinine from the urine of smokers and non-smokers were comparable.

Others have demonstrated for the dog and rabbit, and for the rate, that chronic exposure to nicotine leads to a decreased percentage excreted unchanged in the urine. Werle and Uschold injected rats with nicotine daily for 10 days and from their results concluded that progressively

less was excreted in the urine.

Indirect evidence of an acquired metabolic tolerance to nicotine in man by tobacco smoking has been presented by Rottenstein and co-workers10; intravenous injection to smokers did not cause nausea but in non-smokers the same dose produced nausea and vomiting.

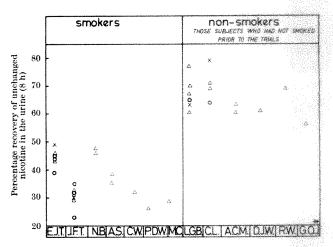


Fig. 1. The percentage recovery of unchanged nicotine in the urine (8.b) of human subjects with various routes of drug administration; urine maintained acidic. \bigcirc , Intravenous administration (1-0-20 mg); \times , inhalation of nicotine (0-1-0-5 mg); \triangle , smoking (0-05-28 mg); * This subject had been a heavy smoker, but had stopped smoking some years before the trials.

The rate of development of the acquired tolerance to nicotine and the possibility that nicotine or other constituents of tobacco smoke stimulate the metabolism of other compounds is being investigated.

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Voltage Fluctuations of Neural Membrane

THE resting membrane potential of the frog Ranvier node shows fluctuations for which, between 1 and 10,000 rad/sec, the power/cycle of bandwidth is inversely proportional to frequency (l/f noise), and for frequencies of less than about 1 rad/sec there may be an increase in the negative slope of the plot of log noise power against log frequency^{1,2}. This communication reports experiments on the amplitude distributions of the membrane noise voltage.

The arrangement was similar to that described before¹: a three node preparation and a three terminal version of the electronic feedback isolation arrangement according to Frankenhaeuser² were used. The membrane potential of the middle node, lying in a pool containing Ringer or a testing fluid, was measured through one of the adjacent nodes, bathed in isotonic potassium chloride, by means of KCl-agar bridges to silver-silver chloride electrodes connected to a low noise, chopper stabilized electrometer amplifier. The membrane potential could be varied by means of d.c. injection through a 1,000 megohm series resistor through the other adjacent node. After filtering through a high pass filter (time constant 2 sec) to eliminate the d.c. component of the membrane potential and after further amplification the amplitudes of the noise voltage were sampled with 1.5 µsec pulses at a rate of 1,000/sec. The amplitude of each sample was stored in the memory of a 128-channel scaler-analyser, with a channel width of 100 µV of the input. From the resulting amplitude distribution the standard deviation, indicative of noise intensity, and the third moment $(\Sigma x^3/N)$, where x is the noise amplitude and N the total number of samples). indicative of degree of skew, were calculated.

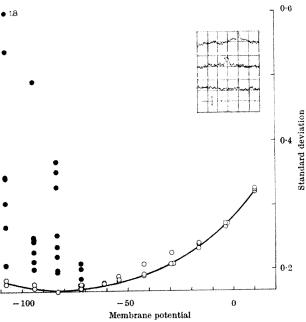


Fig. 1. Standard deviation of amplitude distributions of membrane noise voltage plotted against membrane potential.
O. Gaussian distributions;
o. positively skewed distributions. Length of noise tracks analysed at each point was 12·5 sec. Inset is membrane noise (bandwidth 0·5-3.000 c/s) at a membrane potential of -90 mV. Units: 1 mV and 10 msec. A single miniature depolarizing potential is visible in the upper track, and a group of two in the second, while none are present in the third track.

The relationship between noise intensity and membrane potential is shown in Fig. 1. Overall noise intensity is lowest for membrane potentials between -60 and -70 mV for this particular node. For smaller membrane potentials the spread of the intensities increases. This is because of a pronounced positive skew in the amplitude distributions. The intensity of the Gaussian noise component is minimal

at the (supposed) level of the potassium equilibrium potential, which suggests that there is a relationship between Gaussian noise and passive potassium ion flux through the membrane.

Interfering with active ion transport (by the addition of 2,4-dinitrophenol to the Ringer in the bath together with an atmosphere of pure nitrogen) did not influence the Gaussian noise, nor did replacement of sodium chloride by saccharose. Replacement of sodium chloride by potassium chloride, however, shifted the Gaussian noise minimum towards zero membrane potential, the minimum either coinciding with the small remaining resting potential or lying between this potential and zero. Likewise it was found that 5 per cent tetra-ethylammonium Ringer, which is known to reduce the potassium conductance of frog node^{5,6}, reduces the intensity of the Gaussian noise component.

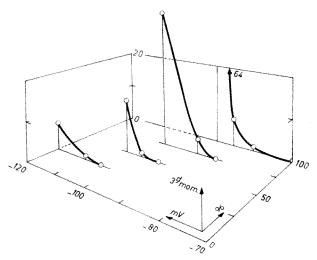


Fig. 2. Three dimensional representation of the relation between skewness (third moment), sodium chloride concentration in the bathing fluid (percentage of that in Ringer) and membrane potential. Length of noise tracks analysed at each point was 100 sec.

We conclude that the Gaussian noise component is related to the passive flux of potassium ions and that its minimum indicates the potassium equilibrium potential. The position of the range of transition from Gaussian into skewed noise is independent of the actual resting membrane potential recorded in the absence of d.c. injection. The tracks of skewed noise (Fig. 1, inset) show that skewness is caused by the irregular occurrence of miniature depolarizing potentials, spiked and with amplitudes of the order of 1 mV and lasting about 1 msec. These potentials occur both singly and in clusters, which vary from groups of a few additive miniature depolarizing potentials to high-amplitude, long-duration depolarizing bursts of noise (described by del Castillo and Katz⁴) in which the unit potentials cannot be distinguished. These phenomena suggest that passive membrane sodium mechanisms are involved.

Interfering with active ion transport did not influence skewed noise, which excludes active transport and neither did replacement of chlorine ions by sulphate ions nor the addition of 5 per cent tetra-ethylammonium to the bathing Ringer solution, which excludes the involvement of chlorine and of potassium ions. Partial replacement of sodium chloride by saccharose resulted in a reversible shift of the transitional range towards more hyperpolarized levels, the shift increasing with the amount of sodium replaced (Fig. 2).

We conclude that skewed noise is related to spontaneous batchwise fluxes of sodium ions through passive transport sites within the membrane.

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BIOLOGY

DDT Residues in Pacific Sea Birds: a Persistent Insecticide in Marine Food Chains

THE accumulation and biological concentration of DDT and its metabolites* within food chains of terrestrial1, freshwater² and estuarine³ ecosystems have been well documented. Significant residue concentrations have occasionally been reported in marine organisms, but these have usually been attributed to local sources of contamination4. The present study, which is based on analyses of collections of birds, fish and invertebrates from Pacific Ocean localities, indicates that DDT is also a component of marine ecosystems and that pelagic species may accumulate high concentrations of DDT residues. results of the work with fish will be published elsewhere.

Specimens collected for analysis were frozen and stored at -15° C. Some conversion of p,p'-DDT to p,p'-DDD may occur in these conditions, and so the reported values of these two compounds may be somewhat lower and higher, respectively, than those occurring in nature. Tissues, eggs and whole birds were digested with a mixture of acetic and perchloric acids, and the lipid fraction containing the pesticide was extracted from the diluted digestion mixture with petroleum ether or N-hexane7. Recovery of DDT from fortified tissue samples averaged 96 per cent. Lipids were removed by passage of the extract through a modified Davidow column. Because aldrin, dieldrin and similar compounds are destroyed by this treatment, some extracts were prepared by the method of Bligh and Dyers, and were cleaned on 'Florisil' columnss. Analyses of the purified extracts were performed on a Microtek 220 gas chromatograph, equipped with an allglass system and an electron capture detector. chromatograms were performed isothermally (190°C) on 3 per cent QF-1 or 5 per cent SE-30 on 'Chromosorb W', 80-100 mesh treated with hexamethyldisilazane or on 10 per cent DC-200 on 'Gas-Chrom Q' (ref. 5). The carrier gas was nitrogen. Identification of the chlorinated hydrocarbons recorded in the western gull and brown pelican was confirmed by thin-layer chromatography10.

Residue levels in the resident California species (Table 1) were considerably higher than those in the northern migrants (Table 2) which spend the winter months off the California coast. It is not possible to estimate how much of the residue carried by the northern birds originated in California waters. The shearwaters (Table 2), which are

* DDT residues include the two isomers of DDT, p, p'-DDT and o, p'-DDT and the metabolic derivatives of p, p'-DDT: p, p'-DDE, p, p'-DDD, and p, p'-DDMU: p, p'-DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; o, p'-DDT, 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)-hane, p, p'-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene, p, p'-DDD, also known as TDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene; p, p'-DDMU, 1-chloro-2,2-bis(p-chlorophenyl)ethylene.

strictly pelagic migrants from the southern hemisphere. contained as much as, or more pesticide than, the local birds. The sooty shearwater and the slender-billed shearwater are known as the mutton birds of New Zealand and of Bass Strait, Australia, respectively, and are a traditional source of food for the local human populations. After the nesting season both species migrate northwards across the Equator. During the summer months many thousands of sooty shearwaters are present in Monterey Bay. DDT has been extensively used for mosquito control in many countries and may therefore be relatively more prevalent in some areas of tropical seas.

A significant fraction of the pesticides present in breeding females may be passed on to the eggs. Three female Cassin's auklets, which lay a single egg each season, were collected with their eggs from burrows on the Farallon Islands. The total concentrations of residue in the eggs ranged from 9.4 per cent to 32 per cent of the total present in the adult female.

A mean value of 3·1 μg of dieldrin was found in three eggs of the western gull, but dieldrin was not present in the breast muscle of the brown pelican, or in fat samples of the Cassin's auklet. DDMU was detected in almost all avian tissues. It is part of the pathway by which p,p'-DDT is degraded to the water-soluble compounds

Table 1. DDT RESIDUES (p,p'-DDT,o,p'-DDT,p,p'-DDE,p,p'-DDD,p,p'-DDME) IN SEA BIRDS RESIDENT IN CALIFORNIA

Total

Sample	Total DDT residues (p.p.m.)	Percenta $p,p' ext{-DDT}$		sidue as DDD
Ptychoramphus aleuticus (Cassin's auklet), nine adults*	5.1	0.4	95	1.1
Ptychoramphus aleuticus, one adult found dead*	15.4	0-1	96	1.0
Ptychoramphus aleuticus, two adults † Ptychoramphus aleuticus, three adults*	1.0	3.8	87	4-0
Breast muscle Brain Liver	2·0 0·7 1·0	11 32 9·7	81 54 79	1.0 5.9
Subcutaneous fat Ptychoramphus aleuticus, fourteen	56 10·8	1·5 0·3	92 96	1.5 1.0
eggs* Larus occidentalis (Western gull), two adults*				
Breast muscle Brain Subcutaneous fat	9·2 1·8 211	0·1 2·2 0·4	89 83 94	6-9 4-4
Larus occidentalis, nine eggs, one-egg clutches*	6.5	$2.\overline{9}$	82	7.3
Phalacrocorax pelagicus (pelagic cormorant), one adult ‡ Breast muscle	0-8	1.3	83	8 % . Ch
Liver Phalacrocorax penicillatus (Brandt's	0.7	0.0	84	10-9
cormorant), five adults§ Breast muscle Liver	4·4 3·3	0.0	91 85	6-2 0-2
Brain (three birds) Pelecanus occidentalis (brown pelican)†	1-2	0-0	92	6.2
Breast muscle Uria aalge (common murre)†	$84.4 \\ 7.3$	1·4 0·3	91 93	4·8 4·0

* Farallon Islands, April and May 1966; † Monterey Bay, November and December 1966. Although these species breed in California, the individual birds may have come from elsewhere; † Tomales Bay, Marin Co., March & 1966; § Tomales Bay, December 31, 1965.

Concentrations are expressed in parts per million, wet weight. Unless otherwise indicated the whole bird was analysed. Concentrations in eggs are based on the entire contents of the egg. The proportions of DDT (p, p'-DDT), DDE (p, p'-DDE) and DDD (p, p'-DDD) are expressed as a percentage of the total. o, p'-DDT and p, p'-DDMU constitute the balance.

Table 2. DDT RESIDUES IN NON-RESIDENT SEA BIRDS

Sample	DDT residues (p.p.m.)	Percen p,p'-DD	tage of r T DDE	esidue as DDD
Synthliboramphus antiquus (ancient murrelet)*	0.75	0-2	90	4:5
Phalaropus fulicarius (red phalarope), two birds*	1.0	7.2	82	0.7
Cerorhinea monocerata (rhinoceros auklet), two birds*	2.7	0.0	92	0.2
Fulmarus glacialis (fulmar), three birds*	1.9	7.5	85	5-1
Rissa tridactyla (kittiwake)†	1.3	3.7	76	13-5
Puffinus griseus (sooty shearwater), three birds*	8-4	5-6	88	
Puffinus tenuirostris (slender-billed shearwater) †	32	4.4	92	2.7

* Monterey Bay, November 1, 1966; † Monterey Bay, December, 18, 1966. Notation as in Table 1.

DDA and dichlorobenzophenone in the chick embryo11, and so we assume that its presence indicates some amount of DDT metabolism.

We have reported the pesticide content and the individual variation of pesticide content and concentration in collections of eight species of Pacific marine fish⁵. Total levels of DDT residue in tissues of the blue fin tuna (Thunnus thynnus) and the yellowfin tuna (Thunnus albacares) from Baja California, Central America, and the Galapagos Islands ranged from 0·1 to 0·6 p.p.m. Skipjack tuna (Euthynnus pelamis) from waters off Hawaii, the Galapagos Islands, and mainland Ecuador contained somewhat lower concentrations of DDT residue, which ranged from 10 to 100 parts per billion. Fish from California coastal waters contained more residue, but in general total concentrations were 10-20 per cent of those in the Collections of the northern anchovy (Engraulis mordax), English sole (Parophrys vetulus), Pacific jack mackerel (Trachurus symmetricus) and of hake (Merluccius productus) from offshore waters between San Francisco and the Channel Islands north of Los Angeles averaged between 0.2 and 2.8 p.p.m. of total residue: 12.7 p.p.m. were present, however, in anchovies taken off Terminal Island, Los Angeles. Significantly lower concentrations of residue were found in anchovies and English sole from San Francisco Bay. San Francisco Bay receives drainage water from the Sacramento and San Joaquin Valleys, and so it seems unlikely that agricultural run-off can account for the observed distribution of DDT residues in the seas.

Residue levels in several marine invertebrates from coastal localities between Monterey and Point Arena were ten to fifty times lower than those in the fish analysed (Table 3). Most values ranged between 20 and 100 parts per billion. The snail (Thais emarginata) and the common starfish (Pisaster ochraceus) both feed on the mussel Mytilus californianus. No biological accumulation could be demonstrated in the starfish, but the snail contained two to five times the concentrations of residue present in its principal food source collected at the same localities. All the DDT recorded in the purple urchins was present in the gonads at a concentration of 5 parts per billion. No residue, or less than the detectable limit of 1 part per billion, was present in the other tissues of the urchins.

Table 3. DDT RESIDUES IN MARINE INVERTEBRATES

Samples		Percenta p,p'-DDT		sidue as DDD
Mytilus californianus (common mussel)* Thais emarginata (short-spired purple snail)†	19 94	26 33	26 29	26 26
Pisaster ochraceus (common starfish), eight animals ‡	20	15	24	32
Mitella polymerus (Pacific goose barnacle), forty animals ‡	27	7	56	22
Crassostrea gigas (giant Pacific oyster), five animals?	29	42	24	17
Strongylocentrotus purpuratus (purple urchin)!	5	100	0	0
Patiria miniata (sea bat starfish), ten animals¶	78	19	55	14
Loligo opalescens (squid), thirteen animals**	28	36	32	14
Slichopus californicus (sea cucumber), three animals † †	93	43	25	25
Pugettia producta (kelp crab), six animals ‡ ‡	42	16	62	10
Thais emarginata (short-spired purple snail), Monterey&	163	28	45	15
Mytilus californianus (common mussel), Monterevill	84	32	38	18
Mytilus californianus Ensenada, Baja	34	21	53	9
California ¶¶ Mytilus californianus, Farallon Islands***	34	0	84	0

Mytilus californianus, Farallon Islands*** 34 0 8 4 0

* Point San Pedro, San Mateo Co., May 26, 1966, pooled sample of twenty animals, shells removed before analysis; † Point San Pedro, San Mateo Co., May 26, 1966, pooled sample of eighty animals, shells removed before analysis; † Point San Pedro, May 26, 1966; \$ Tomales Bay, Marin Co., September 15, 1965; || Point Arena, Mendocino Co., September 16, 1966, opads of eight animals; ¶ Monterey, March 6, 1966; ** Monterey, June 16, 1965; †† Monterey, March 12, 1966; †† Monterey, March 12, 1966; †† Monterey, March 12, 1966; †† Monterey, March 13, 1966, pooled sample of thirty-six animals, shells removed; ||| Monterey, April 11, 1966, pooled sample of to 108 animals; ¶ July 17, 1965, pooled sample of fifteen animals; *** April 6, 1966, pooled sample of eleven animals.

Notation as in Table 1.

Unfortunately, our present knowledge permits only an imperfect estimate of the present and future effects of DDT accumulation in any species. Within the past few years the numbers of the peregrine falcon and the bald eagle have declined and both species have essentially disappeared as breeding birds on the Channel Islands of California (personal observation and records of Mr W. G. Abbott). There is no evidence that the fanatical efforts of egg collectors in the early years of the century or recent pressure from hunters and falconers have been a significant factor. The comparatively high amounts of DDT and its metabolites in fish from the vicinity of the Channel Islands suggest that food chain concentration would result in high levels of residue in fish-eating birds or in species such as the peregrine falcon which feed on other birds. Whether the high pesticide content of the single Cassin's auklet found dead on the Farallon Islands contributed to its death is a matter for speculation.

Reports on levels of residue in tropical areas where DDT is used for mosquito control are as yet lacking. The total DDT residues in the eggs of British sea birds12 and in the tissues of birds from the coastal regions of Holland¹⁸ suggest that DDT contamination is less in European seas, although more dieldrin and endrin are present. The recently published values of DDT concentrations in organisms of a Long Island, New York, estuary³ suggest that contamination in the offshore waters of New England may be as high as in California.

Although chlorinated hydrocarbons in solution or adsorbed to particulate matter are brought by rivers to the sea14, transport by water circulation can scarcely account for the observed distribution of DDT in the sea and its occurrence in very remote areas¹⁵. Chlorinated hydrocarbons readily evaporate with water from the surfaces of marshes and soil16, are found associated with particulate matter in the air17, and are present in the atmosphere as a result of air-spraying operations. Wind transport and subsequent fallout in rain18 might therefore account for the apparent universal occurrence of DDT and its metabolites in the ocean.

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Initiation of Courtship by Hypothalamic Implants of Testosterone Propionate in Castrated Doves (Streptopelia risoria)

DAVIDSON¹ in 1966 and Lisk² in 1967 suggested that a system sensitive to androgen, situated in the preoptic and anterior hypothalamic regions, is associated with mechanisms underlying copulatory behaviour in the male rat. This system would seem to be the counterpart of the oestrogen-sensitive systems known to be associated with patterns of female sexual receptivity in the rat3, the rabbit4 and the cat⁵. The problem arises as to whether the male courtship patterns which often precede copulation are similarly linked to discrete androgen-sensitive regions of the central nervous system (CNS). This seems doubtful in male mammals, for mechanisms underlying such patterns are thought to be functionally independent of gonadal hormones. There is preliminary evidence, however, that in the male fowl simultaneous androgenic activation of the preoptic region and an area extending from the palaeostriatum to the lateral diencephalon is required for courtship display?.

My unpublished observations of the decline of courtship after castration in male Barbary doves have shown that chasing, bowing and nest soliciting (equivalent to the patterns termed "driving", "bowing" and "nest demonstration" in the pigeon by Fabricius and Jannsen⁸). displayed by a sexually active male to a female, declined and disappeared within 10 to 20 days of the castration operation, and that such a castrated male became unresponsive to females. The display of these courtship patterns could be re-established by means of daily intramuscular injections of testosterone propionate, which implies that the systems underlying courtship behaviour in this species are androgen-dependent. In order to examine further the possibility that androgens influence discrete regions of the CNS to promote male courtship in birds, the effects of intracerebral implants of testosterone propionate in castrated Barbary dove males have been assessed.

The experimental males were maintained in visual isolation in separate cages. Light was supplied for 13 h/ day and the temperature maintained at 18°-23° C. Each male was tested for 3 min with one of four test females on 4 consecutive days: the females, known to be sexually active, were alternated so that each male interacted with each female. The duration of each male courtship pattern was recorded on a pen-recorder (Cambridge Instruments) from a hide. Males which failed to show chasing, bowing and nest soliciting at each test were not used. Each male was castrated and given four successive daily tests for courtship beginning 25 days after the castration operation. The majority (97 per cent) were unresponsive to the female: the remainder, which showed short durations of chasing, were discarded. At the time of castration, the light was reduced to 8.5 h/day to discourage regeneration of the testes, and maintained at this level for the rest of the experimental period. On the thirtieth day after

castration, each male was implanted stereotaxically with crystalline testosterone propionate fused to the end of stainless steel tubing (33 gauge), one implant being positioned unilaterally in each male and left in place until the end of the experiment. Implanted males were tested on the day after the operation and on 14 successive days after that; on the sixteenth day, experimental males Transverse sections (25μ) were prepared were killed. from the brain of each male and stained for histological examination to localize the position of the implant. The nomenclature used to describe the position of implants in the brain is after Huber and Crosby. No evidence of regeneration of the testes was found from an autopsy carried out on each male; the vasa deferentia of each male were atrophic.

A group of castrated males (N=5) was implanted with testosterone propionate in the region of the nucleus preopticus medialis (PM), and a further group (N=0) in the region of the nucleus hypothalamicus anterior medialis (HAM). All these males showed a response to the hormone: most displayed chasing and nest soliciting, whereas markedly fewer males displayed bowing (Table 1). courtship patterns displayed by implanted males were similar to their normally occurring counterparts, and the sequence of response of males of both groups followed the same trend of development and decline (Fig. 1): thus few males showed a response on the first or second days after implantation, but the majority had displayed chasing and nest soliciting by the fourth and fifth days after implantation. In both groups, the response declined and disappeared within 8-12 days of implantation. There were no significant differences between the peak durations, the response period or the response latency of any of the courtship patterns displayed by the two groups and for further comparisons (Table 2) the results obtained from the two groups have been combined (PM+HAM). These males displayed significantly shorter peak durations of chasing (Wilcoxon matched pairs test, two-tailed, P < 0.05) and bowing (P < 0.01) after implantation than prior to castration; there was no significant difference between peak durations of nest soliciting displayed before castration and after implantation.

Of males (N=8) implanted with slightly larger implants (Table 1) of testosterone propionate 2.5 mm dorsal to the anterior hypothalamus in a region of the neostriatum intermediale (NI) adjacent to the hippocampus and forebrain ventricle, four males gave no response, and the remainder displayed no bowing and only short durations of chasing and nest soliciting. The peak durations and the response periods (Table 2) were significantly shorter

Table 1. IMPLANT WEIGHTS AND PERCENTAGE RESPONSE OF IMPLANTED CASTRATES

Group:	PM	Testost HAM	erone pro HPM	pionate NI	PAL.P	Choles- terol HAM
Implant weight* (µg) Chasing (per cent) Bowing (per cent)	100 40	$39 \pm 4.5 \\ 100 \\ 33$	36 ± 4·3 0 0	52 ± 2·5 50 0	40 ± 3·7 0 0	59±7-4 0 0
Nest soliciting (per cent)	100	83	40	25	0	0

Table 2. COMPARISON BETWEEN THE COURTSHIP RESPONSES OF MI AND PM + HAM MALES

Expressed as mean ± standard error of the mean.

	Peak duration* (sec)		Response† period (days)		Response ; latency (days)	
	Chasing	Nest solicit.	Chasing	Nest solicit.	Chasing	Nest solicit.
PM + HAM median	37	64	4	2	2	8.5
P§	< 0.002	< 0.002	< 0.02	< 0.02	ns	13.8
NI median		0	1.5	0	1.5	1-6

Longest total period of display of a pattern by an individual male within

縧

Total number of days before the first appearance of a pattern.

§ Mann-Whitney U test, two-tailed; ns, not significant.

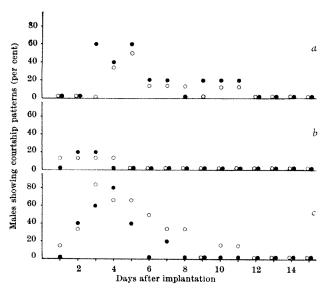


Fig. 1. The development and decline of response to hypothalamic implants of testosterone propionate. The percentage of males that showed courtship patterns has been calculated for each post-operative day. a, Nest soliciting; b, bowing; c, chasing. •, PM males; O, HAM males.

than those displayed by males implanted in the anterior hypothalamus and preoptic regions (PM+HAM).

Males (N=5) implanted in the region of the nucleus hypothalamicus posterior medialis (HPM) displayed significantly shorter durations of nest soliciting (Mann-Whitney U test, two-tailed, P < 0.05) than males implanted in the anterior hypothalamus and preoptic regions (PM+HAM). No other courtship behaviour was recorded from HPM males. A group of males (N=4) implanted in the palaeostriatum primitivum (PAL.P) with testosterone propionate showed no response. A further group (N=5) implanted with cholesterol in the region of the nucleus hypothalamicus anterior medialis also gave no response.

These results indicate that an area incorporating the anterior hypothalamus and preoptic regions of castrated male doves is directly sensitive to testosterone and that this sensitive area is closely associated with systems controlling courtship. At present, the low levels of courtship obtained from males implanted in the neostriatum intermediale and in the region of the nucleus hypothalamicus posterior medialis would seem to be the result of diffusion of hormone to the sensitive area. The androgensensitive area described here corresponds well with the neural area from which Åkerman¹⁶ obtained the most intense responses of bowing and nest demonstration by means of electrical stimulation from the pigeon. The neuro-anatomical representations of the androgen-sensitive systems associated with courtship in the male dove and fowl7, however, clearly differ.

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Continuous Recording of Bird Nesting Visits using Radioactive **Tagging**

In view of the successful application of radioactive tagging in entomology in Central Africa, I have investigated the potentiality of the method as a tagging technique in ornithology. Because the detection range of small amounts of radioactive material is only of the order of feet, the method requires the close proximity of the labelled bird and the radiation detector. It was decided to use the method to obtain data on the bird's nesting visits, and the tests not only demonstrated the potentiality of the method but also provided useful data on behaviour. search of the literature revealed that Barbour¹ made similar studies on the prairie vole (Microtus ochrogaster). Barbour used cobalt-60 as the radioactive tagging material, and the signal from the radiation detector was recorded on a tape recorder. Griffin² has given a thorough discussion on the maximum permissible dose rate to be used for tagging animals under natural conditions, and mentions, without going into detail, an experiment on the semipalmated plover Charadrius semipalmatus.

In this work, I used short lengths of small diameter wire, consisting of tantalum-182 sheathed in platinum. Tantalum-182 has a half-life of 115 days. The lengths are fixed to the usual aluminium bird rings with a suitable adhesive. By using rings with two strengths of radioactive label-one is usually twice the length of the otherit is possible to distinguish between the two parent birds. The radioactive strength of the label is governed by the maximum distance at which it is required to detect the bird compatible with minimization of the health hazard to the bird. In the tests described here, pieces of wire 1 mm in length (100 μ c.) were used for the "strong" rings and 0.5 mm in length (50 μc.) for the weak rings. Measurement even of the weak source was possible at 6 ft. with a scintillation counter, so that the detector could be kept well away from the nest. Although the radiation hazard to the operator is negligible, the bird itself receives a considerable dose of radiation. For example, using a 100 µc. tag of tantalum-182 on Merops nubicoides, the gonads would initially be in a radiation field of about 100 mr./h and at the lenses of the eyes the field would be about 10 mr./h. By arranging the detector to be only 2 ft. from the nest, it would be possible to reduce the size of the radioactive label by a factor of ten.

Two facts about the use of radioactive labels of this size are very encouraging. Both species of birds used for the tests remained healthy for the period of observation and raised healthy broods. Recently, 12 months after the tests, both sexes of one species were seen to be alive and behaving normally. A careful check using tagging with radioactive labels of this size in entomology showed no significant decrease in longevity in caged tsetse

The radiation detector was linked to a portable ratemeter and recorder by 50 m of cable. Use of a 12 V lead acid accumulator allowed the ratemeter to operate continuously for 10 days without recharge. A pen and ink recorder with a chart speed of 6 in./h was found to provide adequate time resolution to measure the frequency and duration of visits. Using the standard chart length-100 ft.—it was possible to obtain a continuous record of the presence or absence of either bird over a period of

The first experiment with this technique was with the white-flanked flycatcher (Batis molitor). The nest was located 5 ft. above the ground and it was possible to leave the radiation detector on the ground directly below the nest. Both parents were caught and ringed a few days before the eggs hatched, so that data were obtained on both brooding of eggs and feeding of young. Fig. 1A shows a typical recorder trace for Batis molitor with

characteristic frequent visits to the nest. In order to obtain an exact departure time, the two young were ringed with radioactive tags of low strength shortly

before their expected departure.

The second experiment was with a tunnel nesting species, the carmine bee-eater (Merops nubicoides). Observations of nesting activity by visual methods are complicated because: (a) visits to the nest must be inferred from entries to and exits from the tunnel; (b) the species nests colonially and confusion frequently results during active periods at the colony; and (c) the sexes are alike. During a week of intensive study of the nesting behaviour of Merops nubicoides, a method was developed for catching birds from a particular nesting burrow. The parents were labelled with radioactive rings and, as before, one was tagged with a ring twice the strength of the other. The burrow selected for the test was 2 ft. below the top of the vertical river bank in which the colony was situated. The recording apparatus was in a hide on top of the bank and was linked by cable to the detector situated on the ground exactly over the nest chamber. The gamma radiation from 50 µc. of tantalum-182 was sufficiently energetic to reach the detector through 2 ft. of soil with adequate signal strength. A marked improvement was achieved, however, by making a short auger hole, large enough to pass the scintillation probe, the bottom of which was 1 ft. from the nest chamber. Data were obtained for a week of continuous recording and a typical part of the chart is shown in Fig. 1. The rather infrequent feeding visits may have been because there was only one chick in the nest. At the end of the week, the parents and young were collected to establish which sex had the strong label and which the weak one.

Information on both the duration and frequency of visits is available from the charts and an investigation of attentiveness at the nest has been made and will be published elsewhere. The analysis gives percentage incubating and brooding times and information on the role of the parents of each species in feeding and brooding during the nesting period. During the nesting period visual observations for periods of several hours were undertaken and these provide a valuable key to assist in interpreting the trace. Typical behaviour patternsfor example, feeding of the female by the male in Botis molitor-are confirmed by the recorded data. Roosting behaviour in the case of Merops nubicoides is easily investigated to establish whether only one parent stays in the nest at night.

Other possible applications of radioactive tagging in ornithology include the investigation of roosting

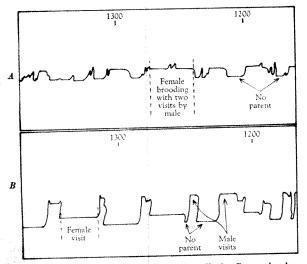


Fig. 1. A, White-flanked flycatcher (Batis molitor); B, carmine bee-eater (Merops nubicoides).

times and behaviour at communal roosts and heronries, and of the nesting behaviour of nocturnal species.

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Fourth Haemoglobin Type in Sheep

Three haemoglobin phenotypes accounted for by the existence of two alleles have been demonstrated in various breeds of sheep1-4. Animals studied have included those homozygous for haemoglobin A (Hb-A) or haemoglobin B (Hb-B) while others were heterozygous for these two haemoglobins. In 1963, a new haemoglobin type was reported in sheep carrying the Hb-A gene after they had been made anaemic by severe bleeding. A new type of haemoglobin (designated Hb-C) was later reported in sheep with experimental anaemias. Haemoglobin C completely replaced Hb-A in the severely anaemic animals. No such variant was detectable in sheep homozygous for Hb-B after severe loss of blood. This Hb-C is probably the same as Hb-N described by Brænd et al.7 in a highly anaemic lamb, and occurring rather commonly in Norwegian breeds of sheep8,8. Studies of the chemical structure of the previously mentioned ovine haemoglobins have shown that they differ in the non-alpha chains (Hb-A = $\alpha_2^A \beta_2^A$, Hb-B = $\alpha_2^A \beta_2^B$, Hb-C = $\alpha_2^A \beta_2^C$) (refs. 6, 10–12).

This communication describes the discovery of a new sheep haemoglobin type, designated haemoglobin D, which was found in three sheep during a survey of a native flock of approximately 500 adults. Fig. 1 shows a starch gel electrophoretic separation using tris-EDTA-boric acid buffer, pH 9·0; the haemoglobin bands were stained with benzidine. The samples for this electrophoresis were selected to demonstrate the relative mobilities of the various sheep haemoglobins (A, B, C, D, F). Haemoglobin D has the fastest electrophoretic mobility at pH 9·0, followed in decreasing mobility by Hb-A, Hb-F, Hb-B and Hb-C.

Table 1. HARMATOLOGICAL DATA OF SHEEP WITH Hb-D

Sheep	Erythrocytes (10°/mml.°)	Hb (g/100)	PCV (%)	Retic.	Haemog A	dobin (p B	ercent) D
G-157 G-261 G-301	7·62 8·91 10·67	$8.4 \\ 8.7 \\ 11.3$	29 33 39	2 1	50 50 50	30 40 36	20 10 14

Haemoglobin D was found only in those animals which seemed to carry the haemoglobin AB genes. The Hb-D band was 10-20 per cent of the total haemoglobin while that of the Hb-B band was between 30 per cent and 40 per cent. No changes were detectable in the quantity of the Hb-A band which constituted the remaining 50 per cent (Table 1). The results of the haematological studies of the sheep with Hb-D are given in Table 1. The data show that two animals (G-157 and G-261) were moderately anaemic, while the third (G-301) was haematologically The red blood cells of the three sheep were separated by centrifugation into populations of different mean ages by the method of Borun et al.13. There was no significant difference in the percentage of Hb-B and Hb-D in the young and old red blood cells. aforementioned sheep gave birth; one lamb was found to have Hb-B and the other Hb-AB. No Hb-D was detected in either lamb.

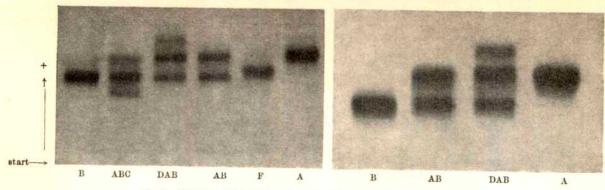


Fig. 1. Relative position of sheep haemoglobins; tris-EDTA-boric acid buffer, pH 9-0.

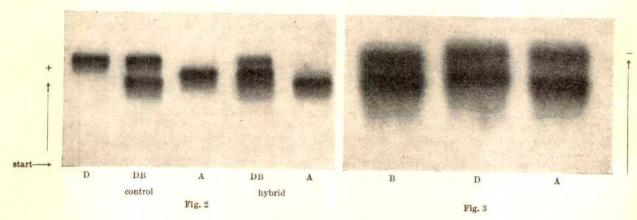


Fig. 2. Results of hybridization experiments of Hb-D with Hb-B.

Fig. 3. Electrophoretic separation of the peptide chains of Hb-D, Hb-A and Hb-B in acetate-phosphate-urea-hydrochloric acid buffer, pH 1-8.

In vitro hybridization experiments14,15 with haemoglobins A, B and D isolated by starch block electrophoresis indicated that Hb-D is an alpha chain variant (α⁰₂). Fig. 2 shows the results of such an experiment with Hb-B and Hb-D. A component with the relative mobility of Hb-A (α,β, is produced, indicating the association of the normal alpha chains of Hb-B $(\alpha_2^A \beta_2^B)$ with the normal beta chains of Hb-D $(\alpha_2^D \beta_2^A)$. The second hybrid haemoglobin (α β β) probably has the same net charge and therefore the same electrophoretic mobility as Hb-A. No hybrid haemoglobins were detectable in similar hybridization experiments with haemoglobins A and D in our experimental conditions.

Starch gel electrophoresis of isolated Hb-D in acetate, phosphate, urea and hydrochloric acid buffer¹⁶ (pH 1·8) revealed the presence of one sub-unit with a slightly faster mobility than the alpha-A chains, and another sub-unit with a mobility indistinguishable from that of the corresponding sub-unit of Hb-A (Fig. 3). From these studies Hb-D seems to be composed of altered alpha chains $(\alpha_2^{\rm b})$, and similar beta chains as in Hb-A (Hb-D = α^D₂β^A₂). Sheep with the three haemoglobins A, B and D would be double heterozygotes for the alpha (α_2^A, α_2^D) and This would yield the following beta (β_2, β_2^8) chains. haemoglobins: Hb-A $(\alpha_2^A \beta_2^A)$; Hb-B $(\alpha_2^A \beta_2^B)$; Hb-D $(\alpha_2^D\beta_2^A)$; Hb-B/D $(\alpha_2^D\beta_2^B)$. This suggests that sheep heterozygous for the alpha chain variant, and homozygous for the beta-B chain variant, are erroneously identified as AB sheep because Hb-A and Hb-B/D probably have the same electrophoretic mobility. In sheep heterozygous for the alpha chain variant and homozygous for the beta-A chain, only Hb-A and Hb-D would be present. The results of this study, although somewhat

limited in the number of animals studied, indicate the existence of a fourth haemoglobin allele, HbD, in sheep.

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Shell Movements of a Wood **Boring Bivalve**

Some bivalves of the super-family Adesmacea are known to penetrate wood by means of mechanical action of the shell valves. The process has been described by various authors, the most comprehensive account being that of Miller¹ for Teredo. Although rocking movements of the shell valves about a dorso-ventral axis are known to be important in penetration of this substratum2, there seem to be no records for any wood boring bivalve of the type and frequency of the shell movements involved.

We have recorded and filmed the shell movements of Xylophaga dorsalis Turton. Adult specimens of this bivalve were obtained from laminated timber test panels exposed at Keppel Pier, Millport, on August 9, 1966, and recovered in August 1967. Xylophaga was found readily boring through such laminated panels, unlike certain species of Teredo whose burrows do not extend through the laminations. By careful separation of the individual laminae specimens of Xylophaga were exposed without any damage and with minimum interference to the animal in the burrow. Specimens removed from the burrows and replaced in smooth cylindrical holes drilled in pieces of cork soaked in sea water were also found to resume boring activity. Records of movements of the shell valves were obtained on a pen recorder (E. and M. Instrument Co. Inc., 'Physiograph'), using an isometric myograph, attached by fine thread to the posterior wing of one valve of the shell.

During several days, when the animals were kept and observed in a continuous flow of sea water, the shell movements chiefly consisted of irregularly spaced periods of vigorous activity (Fig. 1) interspersed with periods of relative inactivity, during which only slight or infrequent larger movements were made. Stoppage of water flow resulted in a slowing down or even cessation of activity

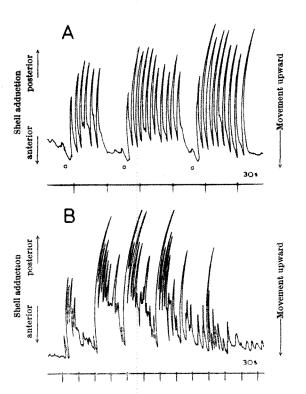


Fig. 1. Two extracts from a recording of the activity of the wood boring bivalve Xylophaga dorsalis. A. Three cycles of boring activity comprising six, eleven and nine rocking movements of the shell valves respectively, caused by successive contraction of the posterior and anterior adductor muscles. Before each group of rocking movements the foot is first relaxed, causing upward movement of the shell in the burrow (a) and the shell is then immediately drawn downwards to the base of the burrow. B. One complete group of boring cycles showing the variation in number and strength of the rocking movements involved.

which was, however, rapidly resumed when the water flew was restarted. During the periods of activity characteristic shell movements were recorded which differed from any hitherto recorded from bivalves, and which are clearly associated with enlargement of the burrow. The shell is drawn towards the blind end of the burrow by the contraction of the pedal retractor muscles, the sole of the foot being attached to the walls of the burrow by mucus secretion. The shell valves then rock about a dorso-ventral axis by the alternate contraction of, first, the posterior and then the anterior adductor muscles (Fig. 1B), the umbones and ventral articular knobs forming a fulcrum for this During the contraction of the posterior movement. adductor muscles the denticulate ridges sculpturing the anterior outer faces of the shell valves are drawn across the walls of the burrow with an effective abrasive action. Considerable variation was recorded in the number of alternate contractions of the adductor muscles comprising each boring cycle as well as in the strength of adduction of the shell valves. Small rotatory movements of the animal were also evident (not shown in the recordings) occurring between the series of rocking movements.

These recordings illustrate the specialization of the movements involved in boring into wood in the Adesmacea. Each series of rocking movements is apparently homologous to the boring cycle of the less specialized Pholadidae, which in turn has characteristics in common with the digging cycle of those species which burrow into soft substratas. The movements recorded therefore fully support the view that rock boring in this group has evolved as a specialization of a deep-burrowing habit4.

The mechanisms of boring in Xylophaga and the rela-

tionships of these to burrowing and boring in other bivalves will be reported in greater detail elsewhere. This work was carried out during the tenure of a Royal Society and Nuffield Foundation Commonwealth Bursary held by one of us (N. B. N.).

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Virus-like Particles in a Weed Mould growing on Mushroom Trays

During investigations of virus diseases of the cultivated mushroom, Agaricus bisporus (Lange) Sing., samples were collected of a species of Plicaria which is probably similar to Plicaria fulva described by Schneider¹ [synonym Peziza atrovinosa Cooke and Gerard]. This fungus is considered to be the perfect stage of Botrytis crystallina (Bon.) Sacc.², the brown mould. Apothecia of P. fulva, collected from various mushroom farms, were tested for the presence of virus particles to investigate the possible role of this weed mould in the spread of viruses of cultivated muchrooms.

Purification was carried out by a method found satisfactory for mushroom viruses3: about 10 g of apothecia was homogenized for 2 min in a Waring blender with 30 ml. of 0.033 molar phosphate buffer containing 0.1 per cent thioglycollic acid, adjusted to pH 6.8. Samples of

the homogenate (50 ml.) were subjected to ultrasonic treatment with a Kerry Vibrason cell disruptor (probe diameter 0.9 cm, output 50 W) for 10 min⁴. During the treatment the fungal suspension was kept at 4° C. The crude homogenate was then clarified by a method based on that of Kitano et al.5. To 1 volume of homogenate was added an equal volume of 2.5 molar potassium phosphate buffer pH 6.8 and 0.8 volumes of a mixture of 2-butoxyethanol and 2-ethoxyethanol (1:2). After mixing the components gently and centrifuging at 1,000g for 5 min the gelatinous interphase was resuspended in 10 ml. of 0.033 molar phosphate buffer pH 6.8. After centrifugation at 5,000g for 10 min the supernatant was centrifuged at 105,000g for 60 min. Resuspension of the pellet in 0.25 ml. of phosphate buffer was followed by centrifugation at 5,000g for 10 min to give a preparation which was negatively stained with a drop of 2 per cent phosphotungstic acid adjusted to pH 5.5 and examined with a Siemens Elmiskop 1 electron microscope.

Altogether nine samples of P. fulva apothecia of various origins were studied. Some of them contained a few spherical particles, diameter 25 mμ, resembling mushroom virus 1 (refs. 3 and 6). In five samples, however, there were rigid rods, strikingly virus-like (Fig. 1). They had a clear central cavity and tended to aggregate. Most particles were 350 × 17 mµ in size. The apothecia of the samples with rods did not differ visibly from those without. No cytological observations were made, however.

Their morphology and their occurrence only in some samples and even then in varying concentrations suggest that the particles represent a virus. Infection experiments could not be carried out because it has not yet been possible to cultivate apothecia of P. fulva1.

In some samples of cultivated mushrooms similar rodshaped particles were observed although in minute concentrations (Fig. 2). Attempts are being made to infect cultivated mushrooms with the rods. The known mushroom viruses are easily transmitted by hyphal anastomosis. but inoculation of mushroom cultures with cell-free virus preparations has been successful only in a few cases3.6. For the rod-shaped virus-like particles this might be even more difficult, for their concentration in cultivated mushrooms is much lower.

Inoculation of purified preparations from apothecia of P. fulva, containing large numbers of rods, onto Nicotiana glutinosa L., N. tabacum L. var. White Burley, Phaseolus

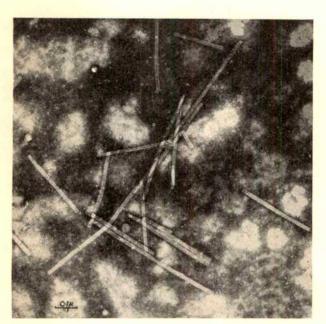


Fig. 1. Rod-shaped particles from Plicaria fulva R. Schneider,

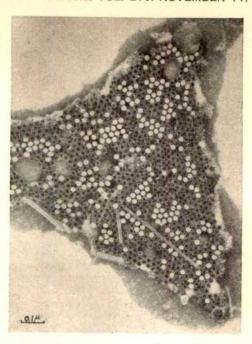


Fig. 2. Some rod-shaped particles among 'spherical' mushroom virus from Agaricus bisporus (Lange) Sing.

vulgaris L. var. Bataaf, Gomphrena globosa L. and Chenopodium amaranticolor Coste and Reyn did not induce any visible reaction in these plants, thus eliminating the possibility of contamination of the fungal material with a strain of tobacco mosaic virus.

Electron micrographs were prepared by the Service Institute for Applied Mechanics and Technical Physics in Agriculture, Wageningen.

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Morphogenesis of Shoot Primordia in Cultured Stem Tissue of a Garden Rose

Skoog and Miller1 reported that auxin and kinetin can be used in growth media for tobacco stem tissue cultures to control the morphogenesis of shoots and roots. Other authors have reported on shoot formation in tissue cultures of other species, for example, convolvulus2,3 and endive4.

A climbing hybrid tea rose, tentatively identified as 'The Doctor', which grows in a border of Twyford Laboratories in London, has served as the source of many tissue cultures grown in these laboratories. A fast growing callus can be started and maintained on a 15 per cent coconut milk medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations from 0.05 to 5.0 mg/l. For some time I used such callus for studies on the effect of auxin and kinetin, but no organogenesis was ever observed. In August 1965, however, explants of stem were inoculated onto synthetic media containing 2 mg/l. of naphthyl acetic acid (NAA) and 0.2 mg/l. kinetin or

0.2 mg/l. NAA and 2 mg/l. kinetin. The callus which developed was cultured on media with 1 mg/l. of kinetin or 1 mg/l. kinetin plus 1 g/l. of soya peptone. Three cultures of those treated developed one or more shoot primordia. They were subcultured a number of times onto various media in an attempt to make the primordia develop into normal looking shoots, but this has failed, although one culture has a bud with recognizable trifoliate leaves. In general, however, the primordia consist of clusters of small petaloid organs (1-2 mm in diameter) either colourless or green. Although there has been little development of the primordia, their proliferation has been considerable: for example, from one culture originally with one primordium there are now (after five subcultures) twenty cultures, each with several primordia. Table 1 shows the history of this line of cultures with the media

Table 1. HISTORY OF SUBCULTURES OF ROSE CALLUS WITH SHOOT PRIMORDIA

subculture	mg/l.)	with primordia
Explant	2 NAA 0 2 K	None
1	1 K	1
2	0-1 IAA	1
3	0-5 NAA	2
4	0.5 NAA or 0.5 NAA+1 K	7
5	0·5 NAA 0·2 K 20 GA	20

IAA, β-indolylacetic acid; GA, gibberellic acid; K, kinetin.

The medium given last, with 20 mg/l. of gibberellic acid, is the most successful with regard to both proliferation and development of the primordia.

It has been comparatively easy to keep the cultures producing a primordia despite changes of medium, and it can be concluded that the constitution of the culture is fairly stable. Why then is it so difficult to produce such a culture? It seems probable that the development of such a culture requires conditions which rarely occur, but which sometimes arise in the interaction between explant cells and the medium. An interesting question is whether a primordium, once present, can influence the culture to produce further primordia. One approach to this problem would be to culture shoot tips or axillary buds and see if a callus can be obtained with desired properties.

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Photosynthetic Oxygen Evolution by Isolated Chloroplasts in the Presence of Carbon Cycle Intermediates

RECENTLY developed techniques have led to the isolation of intact chloroplasts which will assimilate carbon dioxide at rates approaching those achieved by the intact plant¹⁻⁶. This has made it possible to measure the associated oxygen evolution, polarographically, in aerobic conditions? Normally, oxygen evolution parallels carbon dioxide fixation^{7,8}. On illumination, both start slowly and accelerate gradually until a maximum rate is reached after several minutes. This induction phase is thought to reflect corresponding changes in the concentration of carbon cycle intermediates as these increase autocatalytically to a steady state level^{2-4,7-11}. If 3-phosphoglycerate (PGA) is provided as a substrate the initial lag is virtually

eliminated? and the kinetics then approximate to those observed in the Hill reaction¹² in which an artificial hydrogen acceptor reacts more directly with the photochemical system. This was held? to be consistent with the view¹³ that the 3-phosphoglycerate is the immediate precursor of the hydrogen acceptor (1,3-diphosphoglycerate) in the carbon cycle. If this is correct, it would follow that the oxygen evolution observed when PGA is added as a substrate? should not be dependent on carbon dioxide-bicarbonate (Fig. 1). Conversely, any stimulation of oxygen evolution by triose phosphate should cease in the absence of carbon dioxide. Experiments which substantiate these conclusions are reported here.

Chloroplasts, with intact envelopes, were isolated from spinach as before^{1,8,11} but in a solution of sorbitol (0.33 molar), magnesium chloride (0.1 per cent w/v) and sodium pyrophosphate^{5,8,11,14} (0.01 molar) at pH 6.5. They were then resuspended and assayed in a similar solution except that the pyrophosphate was replaced by Hepes solutions (0.05 molar) at pH 7.6, the magnesium chloride was 0.601 molar and a small quantity of manganese chloride (0.002 molar) and EDTA (0.002 molar) were also added. Oxygen evolution was measured at 20° C in rapidly stirred mixtures using two Rank electrodes' and a twin channel recorder. High intensity white light (about 6,000 ft. candles) passed through 15 cm of water as a heat filter, was provided by two quartz-iodine slide-projectors. Illumination was started after 4 min of dark equilibration.

Fig. 2 shows induction phenomena in oxygen evolution by isolated chloroplasts in reaction mixtures containing no added orthophosphate and in which the inorganic phosphate requirement^{5,6,8,11,14} is met by small quantities

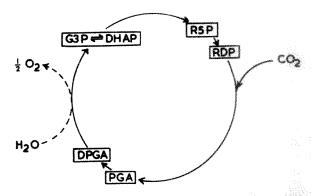


Fig. 1. In the photosynthetic carbon cycle¹⁸, reduction of added PGA could occur in the absence of carbon dioxide. Conversely, triose phosphates such as DHAP and G3P would normally be converted to PGA only in the presence of carbon dioxide.

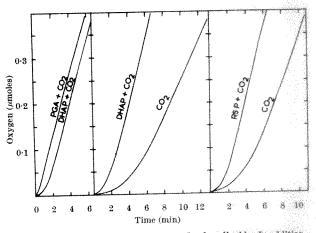


Fig. 2. Oxygen evolution in the presence of carbon dioxide. In addition to chloroplasts (100 μg of chlorophyl) in resuspending medium (see text) each mixture (1-5 ml.) contained bicarbonate (15 μ moles) and 5 μ moles of PGA, R5P and DHAP as indicated.

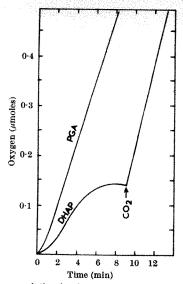


Fig. 3. Oxygen evolution in the absence of added carbon dioxide. Mixtures as for Fig. 2 but initially without bicarbonate which was later added as indicated (15 μ moles) to the mixture containing DHAP. Similar results were obtained when G3P was substituted for DHAP.

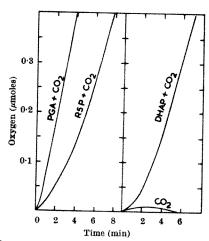


Fig. 4. Suppression of oxygen evolution by high orthophosphate in the presence of carbon dioxide alone. In addition to chloroplasts (200 μg of chlorophyll) in resuspending medium (see text) each mixture contained bicarbonate (15 μ moles), sodium orthophosphate (10 μ moles, pH 7·6) and 5 μ moles of PGA, R5P and DHAP as indicated.

of pyrophosphate carried over from the grinding medium. The traces are presented in pairs which were recorded simultaneously to minimize such differences as may develop as the chloroplasts "age" after separation from the parent tissue. It can be seen that there was a lag of 5-6 min with carbon dioxide as the sole added substrate, but that this could be considerably shortened by the addition of ribose-5-phosphate (R5P) or dihydroxyacetone With PGA, the lag was barely phosphate (DHAP). discernible (compare ref. 7). Each reaction mixture represented in Fig. 2 contained added bicarbonate. When the bicarbonate was omitted (Fig. 3) the kinetics of oxygen evolution in the presence of PGA were essentially unchanged. With DHAP (or G3P), however, oxygen evolution started but soon ceased, presumably as the supply of endogenous carbon dioxide became exhausted. At this point evolution could be restarted by the addition of bicarbonate. Similar results have been obtained with mixtures containing R5P (ref. 7) and also in the absence of added substrate⁸. It follows that, although this result shows that DHAP, unlike PGA, cannot sustain oxygen evolution in the absence of added bicarbonate, it does not provide definitive evidence of an actual contribution of

DHAP to oxygen evolution. Such evidence may be obtained by taking advantage of the fact that the lags vary according to the orthophosphate concentrations, 11, 14. Fig. 4 shows the responses obtained in reaction mixtures containing 10 µmoles of added orthophosphate (6.6×10^{-3}) molar). Oxygen evolution in the presence of PGA started as before without an appreciable lag. With R5P and DHAP the lags were marginally longer. With carbon dioxide alone the lag was so prolonged (compare refs. 8, 11 and 14) that oxygen evolution was almost entirely suppressed during the period of measurement. The mechanism of suppression is still uncertain but orthophosphate is a known competitive inhibitor of ribulose diphosphate carboxylase¹⁵. As such it would not be expected to affect the oxygen evolution observed in the presence of substrate concentrations of 3PGA, and its effect on carbon dioxide fixation might well be reversed by those intermediates¹³ which could facilitate a build-up of ribulose diphosphate. This suppression, by high orthophosphate, of the oxygen evolution obtained when carbon dioxide is the only added substrate^{8,11} was used to advantage in the experiment illustrated in Fig. 5. In the same way as before, evolution was allowed to start in the absence of added bicarbonate. When net evolution had ceased, 10 µmoles of orthophosphate was added to each mixture, followed by carbon dioxide and DHAP as indicated. In these conditions it can be seen that evolution was not resumed until both DHAP and carbon dioxide were present. Conversely, the fact that the oxygen evolution which occurs in the presence of added PGA' will start and continue in mixtures containing very little carbon dioxide is again evident in Fig. 6. Illumination was started in the absence of added

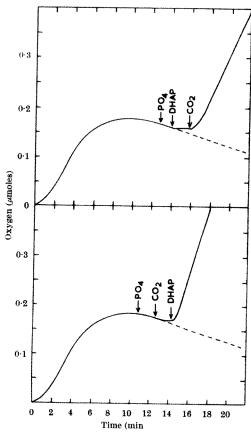


Fig. 5. Carbon dioxide dependent oxygen evolution induced by DHAP. Initially each mixture contained only chloroplasts (100 µg of chlorophyll) in resuspending medium (see text). When net evolution had ceased, sufficient orthophosphate (Fig. 4) was added to suppress the oxygen evolution which would otherwise have followed the addition of bicarbonate (Fig. 6). Oxygen evolution was then only resumed when both DHAP (5 µmoles) and bicarbonate (15 µmoles) were added. Similar results were obtained when G3P was substituted for DHAP.

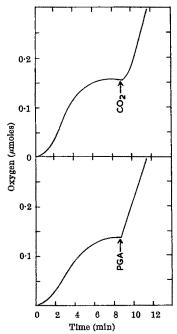


Fig. 6. Oxygen evolution in the presence of PGA in carbon dioxide deficient mixtures. Initially each mixture contained only chloroplasts (100 µg of chlorophyll) in resuspending medium (see text). When net evolution had ceased it could be restarted either by bicarbonate (15 μmoles) or PGA (5 μmoles).

bicarbonate. When net evolution had ceased it could then be restarted either by the addition of carbon dioxide-bicarbonate (compare ref. 7) or PGA alone.

The results suggest that 3-phosphoglycerate can replace carbon dioxide as a substrate in initiating oxygen evolution whereas the triose phosphates are only effective when carbon dioxide is also present. This is consistent with the Benson-Calvin cycle¹³ (Fig. 1) and provides further evidence (see also refs. 16 and 17) of its operation in isolated chloroplasts.

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MICROBIOLOGY

Isolation in Pure Culture of Human Oral Organisms capable of producing Neuraminidase

Previous reports¹⁻³ have indicated that most, if not all, of the carbohydrate components of the mixture of glycoproteins as they exist as saliva in the human mouth are labile and are released by induced enzymes of bacterial origin. One of these enzymes—neuraminidase or sialidase is of particular interest because the loss of sialic acid from saliva causes some of the glycoprotein components to precipitate out of solution, and this reaction may be an important factor responsible for the formation of dental plaque on the teeth. The loss of the other carbohydrate components (three hexoses, two hexosamines and fucose) must influence the calcium binding capacities of the residual proteins and this reaction must also be of significance in the formation of both dental plaque and calculus. Dental plaque has already been shown to bind more calcium than does an equivalent amount of salivary protein. Neuraminidase is also of interest in other fields, where it has been shown, for example, to be the receptor destroying enzyme of myxovirus to erythrocytes and its presence could also be responsible for markedly changing the physical characteristics of mucins throughout the body during certain disease processes.

Few bacteria, particularly those of the genera usually found in the mouth, have as yet been recognized in pure culture as able to produce neuraminidase. enzyme and the other equivalent extracellular glycosidases, however, are regularly produced by the mixed oral flora as it exists in saliva and have been found in the mouths of everyone tested to date3.

Bacterial neuraminidase has been shown to be an induced enzyme which requires the necessary substrate together with other essential materials in trace quantities for its effective elaboration^{6,7}. Because the mixed oral flora very obviously thrive in the mouth and collectively produce neuraminidase, saliva itself seemed to be an extremely suitable medium containing all the necessary ingredients for its elaboration.

Saliva was collected from members of the laboratory staff and from dental students by paraffin wax stimulation. All samples were pooled within 15 min of collection and were placed in a water bath at 60° C for 1 h. This procedure was found both to sterilize the saliva and to retain most of its sialic acid in a bound form. Before the saliva could be used to prepare saliva-agar plates, however, it was found to be essential to remove the contaminating wax because this formed an impervious and almost undetectable layer on the surface of the solid culture medium and made effective inoculation with a standard bacteriological loop almost impossible. The wax particles were removed by suction from the surface of cooled (0°-4° C) and centrifuged (1,000 RCF, 10 min) samples of the saliva using a Pasteur pipette. A fungicide ('Nystatin'. 2,000 U/ml. of saliva) was then added and the saliva reheated at 60° C for a further hour, after which 15 per cent by volume of 5-8 per cent agar (60° C) was added. Saliva-agar plates prepared from this material by this method were found to be bacteriologically sterile. When these plates were inoculated with freshly collected human saliva and incubated overnight at 37° C, many bacterial colonies were produced both aerobically and in an atmosphere of 95 per cent nitrogen and 5 per cent carbon dioxide. Most of the organisms were found to be Gram positive cocci, but some Gram negative cocci and many Gram positive and negative rods were also present. The number of colonies growing on this medium was found to be of the same order as on blood-agar under the same conditions of growth. The individual colonies of bacteria could be subcultured onto further saliva-agar plates and,

when samples of these pure cultures were added to 1 ml. of sterile saliva and incubated overnight, they were found to remove most of the sialic acid, as measured by the method of Warren⁸.

It therefore seems that those organisms capable of growing on sterile saliva-agar are also capable of producing neuraminidase. This is confirmed by further subculturing with sterile liquid saliva when the specific loss of sialic acid can be easily assayed by chemical methods. The marked loss in reactivity of the incubated saliva to the periodic acid-Schiff reaction⁹ indicated that at least some of the other glycoprotein carbohydrates had also been lost, but at the present time this has been confirmed for fucose only. The most surprising aspect of this work from our point of view is that most of the oral bacteria, of which there are many different genera present10, seem to be able to produce neuraminidase and presumably the other complementary extracellular glycosidases when presented with an environment suitable for their elab-

A more precise identification of the organisms involved and an attempt to produce a more easily available synthetic medium for demonstrating the presence of these enzymes is now being undertaken.

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DNA Base Composition of Toxoplasma gondii grown in vivo

THE nature and taxonomy of the parasite Toxoplasma gondii are uncertain. A relationship to Besnoitia and Sarcocystis has been suggested and classification with Sporozoa has been proposed. The problems have been discussed in detail4.

Phylogenetic relations of protozoa may be reflected in their DNA base compositions, and information on DNA base composition may be particularly useful where relationships among Sporozoa are being examined because of their apparently polyphyletic origins4. This study was undertaken to provide such information for Toxoplasma.

Toxoplasma (RH strain) were obtained from albino mice infected by the method of Blaker⁶. Dying animals were killed by spinal dislocation, and peritoneal exudates collected in heparinized buffered saline (0.5 mg of heparin [75 USP units]/ml. of 0·1 molar phosphate buffer, pH 7·0, made isotonic by the addition of 3.5 g of sodium chloride/l.). Cells were washed once in the same buffer, resuspended in saline-EDTA7 and leucocytes removed by filtration8 through a sintered glass filter (Corning, medium coarse porosity, 15-25µ maximum pore size; a medium porosity filter, $10-15\mu$, did not allow Toxoplasma to pass). The procedure also removes some Toxoplasma but has no effect on

the viability of parasites as shown by mouse inoculation. Microscopic inspection of filtrates showed Toxoplasma and very few erythrocytes; leucocytes were rarely observed. Cells were lysed with sodium dodecyl sulphate and the DNA partially purified7. The DNA base composition was determined by density gradient centrifugation.

The DNA base compositions of organisms representing several subclasses of protozoa have been reported and most cluster in groups which reflect their taxonomic position: Phytomastigophora^{5,10,11} range from 66 to 58 per cent guanine plus cytosine (*Euglena*, 46 per cent); Zoomastigophora^{5,12,13}, 59–50 per cent; Holotricha^{5,10,14}, 35-29 per cent (Tetrahymena patula, 22 per cent); and Rhizopoda⁵, 22 per cent.

DNA from Toxoplasma has a density of 1.712 g/cm³ (Escherichia coli DNA taken as primary reference, 1.710 g/cm3), and a guanine plus cytosine content of about 53 per cent. This value is close to that reported 15 for the Haemosporidium, Anaplasma (about 50 per cent guanine plus cytosine), and also falls within the range of values reported for flagellates. A conclusive statement on the position of Toxoplasma cannot be made, however, until additional data for the Sporozoa become available for comparison.

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Uncoating and Development of Vaccinia Virus in Miniature Cells induced with an Extract from Marine Algae

Dissection of individual cells by microsurgery into nucleate and anucleate cytoplasm provides an effective means for the delineation of nuclear participation during virus infection¹. Recently, we described² the formation of aberrant cells and mitotic anomalies after treatment of tissue cultures with a biologically active concentrate derived from marine algae. The induced abnormalities included mixed populations of micronucleate and miniature cells. The sequence of their formation was recorded by time-lapse, phase cinematography. Micronucleation presumably occurred by a process similar to that obtained with colchicine3. Following metaphase, miniature cells were formed by multiple cytokinesis. Three to eight or more discrete daughter cells were usually produced from each mother cell thus affected. Each miniature cell contained one or more nuclear-like structures and hence only part of the total chromosomal complement of the mother cell. Occasionally, cytoplasmic fragments without nuclear material were noted which were, however, usually shortlived in our experimental conditions. This communication describes the development of vaccinia virus in preformed populations of miniature cells.

The following procedures were used. Stock cultures of the McCoy cell line4 were trypsinized and suspended in 30 ml. of nutrient medium (mixture 199 supplemented with $10\,\mathrm{per}$ cent calf serum, $0{\cdot}1$ mg streptomycin/ml. and $100\,\upsilon$ of penicillin/ml.) at a concentration of 300,000 cells/ml. in each 300 ml. flask. A lyophilized preparation of algal extract was added at a predetermined final concentration as described elsewhere². Cultures were placed in a rotary shaker water bath at 37° C for about 22 h. Samples (2 ml.) were transferred to Leighton tubes with coverslips. After incubation for 6 h, unattached cells and debris were decanted and nutrient medium (without algal extract and only 1 per cent calf serum) was replaced for 16 h. Coverslips were stained with acridine orange and examined by fluorescence microscopy5. In most experiments, miniature cells, micronucleate cells and arrested metaphase configurations represented approximately 30 per cent, 40 per cent and 5 per cent of the total cell population, respectively. The remaining 25 per cent were polykaryons and giant cells with deformed nuclei. Only occasionally were "normal" mononucleate cells seen.

Such atypical cell populations on coverslips were challenged with a large dose of vaccinia virus (strain WR). Preparations were examined 4-6 h after challenge for cytochemical evidence of "factory areas" (viral inclusions) as recently described. Within the limits of resolution of the cytochemical staining technique used, typical "factory areas" associated with vaccinia development were noted in most of the micronucleate and miniature cells. The relatively few cells which did not contain visible inclusions were either too small or could have been in metaphase arrest at the time of virus challenge and hence did not synthesize appreciable amounts of messenger RNA for 'uncoating'' proteins.

Joklik, has suggested that the "uncoating" of vaccinia virus is mediated by proteins coded by the host cell genome. In view of the varied and incomplete chromosome composition of each miniature cell, our observations suggest that the "uncoating" of vaccinia is not dependent on a specific segment of the host cell genome, although duplication of gene function on different chromosomes cannot be discounted at this time. Rather, our findings complement recent biochemical observations concerned with the synthesis of messenger RNA by the "coated" viral genome10.

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BIOCHEMISTRY

Amino-acids and Amino-sugars in Calcified Tissues of Portunid Crabs

THE different parts of the exoskeleton of portunid crabs show a wide range of hardness and rigidity. The most rigid structure is the dactylus of the chela; most flexible and soft are the unmineralized joints between the limbs. The propodus, the carapace and the pleopods represent intermediate stages of rigidity. Because the extent of mineralization largely determines the flexibility and hardness of the exoskeleton we were interested to identify the important factors in this calcification process.

Previous work on mineralization in biological systems¹⁻⁷ has shown that the proteinaceous matrix in calcified tissues provides a set of very specific templates. Most essential in nucleating a mineral phase seems to be the availability of free carboxyl and amino groups provided by certain acidic and basic amino-acids. In the light of these results, we decided to determine the amino-acid and amino-sugar composition of representative regions in the exoskeleton and to relate these data to the calcification phenomena.

The animals selected were four specimens of Callinectes sapidus, one of Ovalipes ocellatus and one of Carcinilles maenas, all intermoult males. The regions sampled were the soft uncalcified joint membrane between the carpus and merus of the cheliped, the flexible paddle of the pleopod, the cardiac and gastric regions of the carapace and the propodus and dactylus of the cheliped. samples were freed of extraneous tissues and subjected to decalcification, hydrolysis and ion exchange chromatography8. Data on calcium, magnesium and strontium obtained by atomic absorption spectrophotometry, and on phosphate by colorimetry 10 were used as a measure of the degree of calcification of the individual organic matrix. The analytical results are summarized in Tables 1 and 2.

About 80 per cent of the weight of the samples could be accounted for after the chemical analysis if the inorganic salts are assumed to be carbonates and glucosamine to be an acetylglucosamine polymer. The missing portion partly represents humin and partly water; chitin is known to retain up to 10 per cent water even when dried to constant weight at 105° C (ref. 11).

The inter-relationships within and between the aminoacids, the amino-sugars and calcium were explored using factor analysis^{12,13}. This technique showed that 83 per cent of the total variance of the data was explained by only four groups of covariant compounds. A covariant group consisting of aspartic acid, tyrosine, serine, phenylalanine, glycine and threonine was strongly negatively correlated with a group consisting of lysine, proline, methionine, calcium and the ratio of the amino-sugars to total protein. A third group consisting of glutamic acid, valine, alanine, leucine and isoleucine was independent of the first two groups and a fourth group, similarly independent, consisted of arginine, histidine and hydroxylysine.

We believe that the association of the first two groups is related to the calcification process. For the independent group consisting of glutamic acid and isoleucine, although the pleopod, propodus and carapace contain increasing quantities of these amino-acids, different individuals have different relative amounts, as indicated in Fig. 1. Because of the difference in individuals, it seems most likely to be an environmental factor (for example, water temperature, pH, Eh, salinity or diet).

The increase in lysine and OH-lysine with progressing calcification agrees with the idea 1-3 that both amino-acids may provide nucleation sites for crystal growth. Inasmuch as chitin may also contain free amino groups11,14, the higher yields of glucosamine in the most mineralized regions of the exoskeleton may also be linked to calcification. Should dicarboxylic acids be essential in pro-

	Lubic			AMINO ACID	b, andoona		.,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,					
Callinectes sapidus				Ovalipes ocellatus			Carcinides					
Residues/1,000 amino-acids	Joint membrane	Pleopod	Carapace	Propodus	Dactylus	Joint membrane	Pleopod	Carapace	Propodus	Joint membrane	Carapace	Propodus
Aspartic acid	116	91	87	77	72	99	87	91	75	100	95	87
Threonine	71	59	55	54	48	57	46	59	54	66	53	56
Serine	59	104	81	73	64	73	111	109	74	70	99	90
Glutamic acid	118	84	101	88	95	111	90	92	92	106	112	88
Proline	101	115	109	144	165	104	82	98	115	90	87	92
Glycine	122	127	108	101	103	124	155	130	103	130	120	114
Alanine	67	108	125	124	115	67	73	130	107	74	98	107
Cystine	1	3	2	2	4	0.3	6	1	7	3	6	10
Valine	47	64	81	77	77	39	60	77	60	47	62	63
Methionine	8	5	12	12	10	5	6	11	4	5	. 6	4
Isoleucine	39	28 39	30	27	28	35	24	33	27	39	29	28 55
Leucine	49	39	52	49	50	46	42	54	45	45	48	55
Tyrosine	81	41	27	25	16	26	40	24	18	32	33 36	21 27
Phenylalanine		44 3	39	39	32	39	40	27	21	46		27
OH-Lysine	2	3	3	5	4	0.4	0.4	2	3	0	0.5	- 9
Lysine	37	27	84	46	55	38	35	31	87	28	42	107
Histidine	21	21	17	20	23	42	36	18	49	37	22	25
Arginine	71	34	28	36	34	95	67	_13	60	_81	50	18
Glucosamine	561	749	1,297	1,227	1,527	1,113	1,150	1,738	3,870	775	2,290	5,430
Weight %*												
Protein	54	22	13	13	7	36	22	1.9	0-8	44	2.2	0.7
Chitin	46	42	24	20	19	64	43	5.7	4.1	56	8.3	_6∙0
Mineral	Ó	36	62	67	74	0	35	92	95	0	89	93

Table 1. DISTRIBUTION OF AMINO-ACIDS, GLUCOSAMINE AND MINERAL MATTER IN CUTICLES OF PORTUNIO CRABS

Adjusted to 100 per cent.

Table 2. ELEMENT COMPOSITION IN VARIOUS REGIONS OF THE EXOSKELETON OF Callinectes

Weight per cent	Joint membrane	Pleopod	Carapace	Propodus	Dactylus
Calcium Magnesium	0·05 0·03	13·5 0·87	23·4 1·01	25·2 1·19	27·7 1·26
Strontium Phosphorus		0·27	0·23 0·9	0·22 1·3	0.26 1.2

viding negative sites for the fixation of calcium, their effect is masked by other factors.

Crustacean cuticles are hardened by both tanning and mineralization processes. Usually an increase in tanning is accompanied by a reduction in mineral deposition and vice versa. Thus at least two distinct protein matrices are contained in the rigid structures of the exoskeleton. This phenomenon is analogous to the occurrence of mineralized proteins and the periostracum in molluse shells. A study of the amino-acid composition in tanned proteins of gastropods and cephalopods is helpful in the interpretation of the first covariant group consisting of aspartic acid, threonine, serine, glycine, tyrosine and phenylalanine. Essentially, the same amino-acids characterize the periostracum of these two classes of molluses15. This similarity is further underlined by the great abundance of amino-sugars in the tanned shell proteins. The association of aspartic acid with the tanned proteins and of proline with the mineralized tissues does not necessarily imply that the former is not involved in the actual calcification process whereas the latter is. It may be that because of processes unrelated to calcification, the mineralized tissues contain less aspartic acid and more proline than their tanned counterparts. The proportions

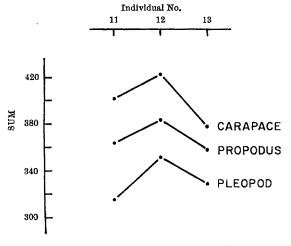


Fig. 1. SUM = glu + ala + val + leu + isoleu.

of tanning and mineralization vary from region to region as shown by representative values for the thickness of tanned and mineralized layers in Callinectes presented in Table 3. The strong negative correlation between the two groups of amino-acids is probably related to the changing ratio of tanned to mineralized cuticle in the various regions. The other two specimens included in this report, namely, Ovalipes ocellatus and Carcinus maenas, show essentially the same relationships we discussed before. The enrichment in lysine and amino-sugars with progressive calcification is even more pronounced than in Callinectes and may be a species characteristic.

Table 3. THICKNESS OF TANNED AND MINERALIZED REGIONS IN Callinectes Endocuticle Epicuticle and pigmented Region layer (mm) (mm) Pieopod Carapace Propodus (chela) Dactylus (chela) 0·25 0·45 0·75 1·30 0.06 0.08 0.07

The epicuticle and pigmented layers are tanned and calcified. The endo-cuticle is calcified.

Based on the amino-acid composition, the joint membrane differs in some aspects from the mineralized structures so far considered. For this reason, we excluded the data in our factor investigation. Particularly noteworthy is the abundance of proline and acidic, aromatic and basic amino-acids.

In relating the present data on mineralized tissues in portunid crabs with previous results largely inferred from electron micrographs¹⁶, we offer a tentative model of the calcification in this biological system. The cuticle of crustacea is a layered structure composed of minerals deposited in and between matted layers of chitin and protein fibrils. The fibrils chiefly lie in the plane of the layers, but some branch up and down to connect adjacent layers. The vertical fibrils line the walls of the numerous pore canals which run through the cuticle in a direction perpendicular to its surface. Organic materials form a diffuse matrix between the fibrils and in the pores. Mineralization proceeds along the fibrils but the resulting crystals are always small in size. Larger crystals are restricted to the interstices and lumen of the pore canals. It is inferred that the formation of multitudes of small mineral seeds in the fibrils represents the first crystallization stage. The deposition of large crystals comes later as nucleation sites in the matrix become gradually available. The larger size of the crystals in the second mineralization phase is simply a consequence of the fewer nucleation sites available along the organic templates. The individual layers are spaced further apart in the thick, highly calcified regions compared with the less mineralized regions. The proteinaceous matter is principally contained in the layers of matted fibrils, and consequently the most calcified regions have a greater proportion of mineral to

organic matter. This implies that with advancement of calcification, the organic matrix becomes a more effective template. In a sense, this is a duplication of what we observe in mollusc shells by going from primitive to very advanced forms¹⁵. With evolution, progressively less organic matrix is required for the nucleation of calcium carbonate. Whereas Nautilus, Haliotis or Mytilus may require a few per cent of organic matter for the deposition of their shell structure, some highly evolved gastropods such as Architectonica or Bulla get along with just 0.1 per

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Effect of Age on the Amino-acids associated with Vascular Heparan and Chondroitin Sulphates

THREE principal groups of mucopolysaccharides-hyaluronic acid, heparan sulphates, and chondroitin sulphates exist in the ground substance of the human arterial wall. Their relative proportions change with age1,2, blood pressure² and arterial site³ in such a way as to suggest a possible relationship with atherosclerosis.

In connective tissue, mucopolysaccharides are firmly bound to protein. Muir4 implicated serine in the chondroitin sulphate-protein link, and recent work by Anderson. Hoffman and Meyer⁵ has substantiated this. Rodén et al. have shown that serine is not linked directly to the repeating disaccharide of chondroitin sulphate; in the vital region of the linkage, the regularly repeating disaccharide is broken into two galactose moieties and one xylose before the O-serine linkage.

Evidence of a different type of mucopolysaccharideprotein link has been advanced by Seno et al.7, who have also shown that the amino-acids associated with keratan sulphate after exhaustive proteolytic digestion vary according to the anatomical source of the material.

We have examined the amino-acids associated with mucopolysaccharides from the aortae of young and old human subjects. Whole aortae were obtained from four young (male, 20 ± 2 yr) and four old $(80\pm2$ yr) human cadavers at necropsy. The adventitia was removed and the tissue diced. Mucopolysaccharides were isolated by papain digestion² followed by dialysis and precipitation of the mucopolysaccharides with cetylpyridinium chlorides. The complex of mucopolysaccharides and cetylpyridinium chloride was dissociated in 2 molar sodium chloride, and the sodium-mucopolysaccharide was precipitated in 90 per cent ethanol. The isolated material was subjected to this procedure of papain digestion, cetylpyridinium chloride precipitation and final alcohol precipitation, three times. The purified mucopolysaccharides were then fractionated by increasing sodium chloride gradient elution from a 4.5×45 cm column of 'Dowex 1-C1' (ref. 9), with uronic acid assay10 of the column effluent.

Chromatograms obtained from young and old aortae are shown in Fig. 1. The average yield of mucopolysaccharide was 10.2 mg/g of dry tissue from young aortae and 5.9 mg/g from old aortae. Heparan sulphates left the column in 0.9 molar sodium chloride and chondroitin sulphates in 1.6 molar sodium chloride. uronic acid and heparan sulphates were reduced in old aortae. The quantities of hyaluronic acid isolated were too small for amino-acid studies. The heparan and chondroitin sulphates were isolated from the relevant column effluent fractions by dialysis and lyophilization.

Samples of mucopolysaccharide (2-4 mg) were hydrolysed in vacuum-sealed tubes (6 normal hydrochloric acid, 110° C, 22 h) and dried in vacuo over potassium hydroxide. Amino-acid analysis was carried out in an auto analyser. Samples for hexosamine assay11 were hydrolysed in 4 normal hydrochloric acid (100° C, 8 h).

Four amino-acids—serine, glycine, glutamic acid and aspartic acid-were found in each mucopolysaccharide sample, together with traces of threonine and alanine. Analyses of typical samples are shown in Table 1. The molar ratios Ser: Gly: Glu: Asp did not differ significantly in samples from young and old aortae, but there was a difference in the total amino-acid associated with the mucopolysaccharides from young and old aortae, there

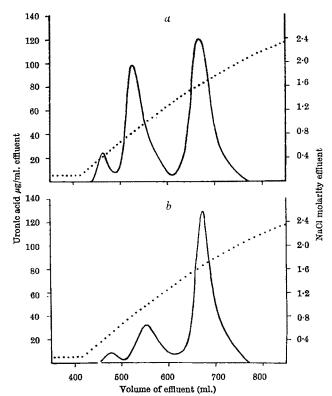


Fig. 1. Salt gradient, ion-exchange column chromatograms of muco-polysaccharides isolated from (a) a young human aorta (19 yr old male) and (b) an old human aorta (82 yr old male). (—) Uronic acid content of column effluent. (...) Sodium chloride molarity of column effluent.

being approximately twice as much amino-acid associated with heparan sulphates from old aortae, both on a weight for weight basis and in terms of mmoles of amino-acid/mole of hexosamine. This behaviour was similar in each of the four young and old heparan sulphate pairs examined, and was detectable, though in much lesser degree, in the chondroitin sulphate pairs.

Table 1. THE FOUR PRINCIPAL AMINO-ACIDS ASSOCIATED WITH HEPARAN AND CHONDROITIN SULPHATES

	Amino-acids (mmoles/mole of hexosamine)					
Sample	Ser	Gly	Glu	Asp		
Young CS	25·3	24·4	20·5	15·7		
Old CS	33·6	28·6	23·3	17·1		
Young HS	20·5	24·9	15·6	14·6		
Old HS	45·0	47·2	45·0	36·9		

HS, Heparan sulphates; CS, chondroitin sulphates; young, 19 yr old male; old, 82 yr old male.

Contamination with protein is unlikely to account for these results because the mucopolysaccharides were subjected to exhaustive proteolytic digestion and repeated precipitation; the mucopolysaccharide fractions isolated by column chromatography migrated as single homogeneous peaks on electrophoresis and in the ultracentrifuge; and only four amino-acids were present in measurable amounts in each mucopolysaccharide sample. digestion of the mucopolysaccharides with DNase did not affect the results, nor did fractionation of the mucopolysaccharides by the varying solubility of their cetylpyridinium chloride complexes in salt solutions9 instead of by ion-exchange column chromatography.

If, as seems likely, these amino-acids constitute minor peptide chains attached to the major mucopolysaccharide chains, the age effect may be caused by an increase in the amino-acids attached to the mucopolysaccharide chain, or by a decrease in the length of the mucopolysaccharide chain with increasing age. Preliminary studies in the ultracentrifuge reveal no striking difference in the molecular weights of young and old aortic mucopolysaccharides, and so it is likely that the change is in the minor amino-acid region of the molecule. The fact that the relative proportions of the four amino-acids remain constant (within the limits of experimental error) despite the increase in their total amount, with age, is slightly in favour of an increase in the number of aminoacid chains attached to the mucopolysaccharide molecules, rather than an increase in the length of the individual amino-acid chains, with increasing age.

One possible interpretation of these results is that they indicate an increasing degree of protein-mucopolysaccharide linkage with increasing age. Such a phenomenon could be of profound importance in influencing the molecular sieving effect of vascular ground substance, and may be of relevance to the development of arterial

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Alkaline Phosphatase Relationships in Drosophila

ELECTROPHORETIC and genetic studies of the alkaline phosphatases in D. melanogaster have demonstrated that most of these enzymes are probably tissue specific, and are controlled by at least two separate genetic loci1,2. It was recently reported that a gene locus on chromosome III previously found to control electrophoretic mobility and enzyme activity of a larval skin phosphatase band also controls mobility and activity of the pupal phosphatase band, and it was suggested that these two phosphatases share a common molecular unit, controlled by a single gene locus3. The experiments reported here suggest that pupal phosphatase band is modified larval skin phosphatase which has been altered by exposure to larval gut enzymes in early pupation.

Methods for homogenization of flies, starch gel electrophoresis and staining for phosphatase have been described before2. It was initially decided to purify the phosphatases, and as a first step, extractions of fly homogenates with n-butanol were carried out using standard procedures. When butanol extracts of homogenates of 3 day old larvae were subjected to electrophoresis, however, it was found that the larval skin phosphatase band was transformed into a more electronegative band migrating at the same rate as the pupal band. Butanol treatment did not affect the mobility of larval gut bands or the pupal band, and if butanol extracts were made from dissected larval skins, rather than whole larvae, there was no change in mobility. Butanol seems to transform larval skin bands to pupa-like bands indirectly, probably by extracting some transforming substance from larval gut. When larval guts were dissected and homogenized separately, and then combined with a larval skin homogenate, the transformation of larval skin band to pupa like bands occurred in the absence of butanolization (Fig. 1), and butanol extracts of these mixtures produced no further changes. Boiling the larval gut homogenate destroyed the transforming factor. This factor does not seem to be a phosphatase. Larvae homogenized in 0.25 molar sucrose were centrifuged at 105,000g for 1 h. The skin transforming factor was found in the supernatant alone, but all phosphatase bands were present in both the supernatant and precipitate.

Because it seemed likely that the skin transforming factor is a larval gut enzyme, larval skin homogenates and gut homogenates were separately incubated with individual purified enzymes from various sources. zymes were added in concentrations of 0.75 mg/ml. in buffers of appropriate pH at 25° C and at 37° C. Samples of treated and control homogenates were examined electrophoretically. The results are shown in Table 1.

Table 1. TRANSFORMING ACTIVITY OF VARIOUS ENZYMES

Skin transforming activity

$p\mathbf{H}$	No trypsin inhibitor	Trypsin inhibitor added
7.5	0	
7.5	0	
6.0	0	
8.0	++++	0
8.0	+	0
8.0	+++	0
6.0	++	++
7.5	0	
7.5	0	
9.1	0	
8.0	++ to +++	. 0
	7·5 7·5 6·0 8·0 8·0 8·0 6·0 7·5 7·5 9·1	pH inhibitor 7:5 0 7:5 0 6:0 0 8:0 ++++ 8:0 + 8:0 +++ 6:0 ++ 7:5 0 7:5 0 9:1 0

Scoring was based on the rapidity and completeness of transformation.

Bovine trypsin was the most active enzyme in transforming larval skin phosphatase bands to bands with mobility identical to pupal bands (Fig. 1). The transformation was complete after 30 min of incubation at either 25° C or 37° C. Treatment of larval gut homogenates with trypsin resulted in the loss of midgut bands, and the same treatment of whole adult homogenates similarly

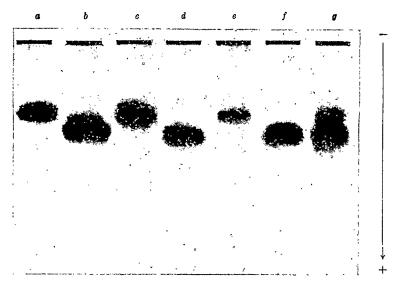


Fig. 1. a, Larval skin; b, larval skin and trypsin; c, larval skin and trypsin inhibitor; d, larval skin and larval gut: e, larval skin and larval gut and trypsin inhibitor; f, larval skin and papain; g, larva and pupa.

resulted in the loss of midgut bands, but there was no effect on the mobility or activity of other bands. Although porcine lipase had transforming activity, the preparation used was found to contain about 4 γ of trypsin/mg of protein. The addition of soybean trypsin inhibitor abolished the transforming activity of this lipase, trypsin, α-chymotrypsin, and also of larval gut homogenate (Fig. 1), which suggests that the skin transforming factor in the larval gut is trypsin or a trypsin-like enzyme. The activity of papain even in the presence of trypsin inhibitor suggests that transforming activity may be possible by means of differing mechanisms.

To compare further the properties of these phosphatase bands, untreated larval homogenates, butanolized larval homogenates, and untreated pupal and adult homogenates were each incubated for I h at 60° C. Samples were withdrawn at 10 min intervals and submitted to electrophoresis. Larval skin bands, transformed skin bands and pupal bands were resistant to this treatment, but all other bands were absent after 10 min of heating. In addition, when these homogenates were treated with ammonium sulphate solutions of varying concentrations, it was found that larval skin bands, transformed bands and pupal bands all precipitated in the 35-60 per cent range, while other phosphatase bands precipitated at different ranges of ammonium sulphate concentration.

The pupal phosphatase band activity comes solely from the yellow body, a structure that is said to be derived from sloughed larval gut⁵. In normal development, the gut bands seen in young larvae disappear in older third instars. The skin band, which has strong activity in third instar larvae, disappears in prepupae. The pupal band appears as the skin band is lost, and persists to the first few hours of adult life. These findings may be explained as follows: gut phosphatase is destroyed during the histolysis of gut occurring in mature larvae. Larval skin phosphatase, perhaps accompanying skin cells, is trapped in the yellow body during histolysis, is then exposed to the activity of trypsin-like enzymes released from gut cells, and is modified but not destroyed. This modified band is lost when the yellow body is passed out of the adult gut. The nature of the modification of larval skin phosphatase is unknown. Neuraminidase, however, is known to affect the electrophoretic mobility of alkaline phosphatase and other enzymes⁶, presumably by splitting off sialic acid residues, and perhaps a comparable reaction occurs with trypsin.

The relationship of alkaline phosphatases from different tissues in Drosophila and most other organisms is still

These experiments emphasize the dangers obscure. encountered in the interpretation of isozyme patterns, even when electrophoretic analysis is accompanied by genetic studies.

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Glial \alpha-Glycerophosphate Dehydrogenase and Central Myelination

A PREVIOUS study of oxidative enzyme activity in the macroglia of adult cat optic nerve¹ has shown that α-glycerophosphate dehydrogenase (GPDH) activity which is not linked to NAD is localized exclusively in astrocytes. This suggests active involvement of these cells in lipid metabolism. Astrocytes are distributed in association with myelinated nerve fibres in the postlaminar part of the optic nerve, with myelinated and non-myelinated fibres in the lamina cribrosa and with nonmyelinated fibres in the prelaminar part of the nerve. Astrocytes alone of the macroglia cells are associated with the myelinated fibres within the lamina cribrosa³. For these reasons it has been suggested that astrocytes may support the myelin sheath of the adult central nervous system.

In this investigation, the techniques previously applied to the demonstration of GPDH activity in cat optic nerve1 were used to demonstrate enzyme activity in kitten optic nerve at six developmental stages: 6-7, 10-12, 15-16, 23, 28-29 and 38 days after birth. Myelination was studied at the same time using both cryostat sectioned material and optic nerves fixed in calcium formol and sectioned on a freezing microtome. The stains were sudan black, Luxol fast blue and osmium tetroxide.

Myelin sheaths stainable with sudan black and Luxol fast blue extended to within 4 mm of the lamina cribrosa 6-7 days after birth: in the third week they had reached to within 2 mm of the lamina, and at 23 days and thereafter as far as the posterior border of the lamina. There was thus evidence of centrifugal advance of myelination. Osmiophilia, however, was absent from the nerve fibre bundles during the first four developmental stages examined. In optic nerves from 28-29 day old kittens, the bundles of fibres were intensely osmiophilic to a level approximately 0.5 mm behind the lamina cribrosa: at 38 days and in the adult cat osmiophilia extended to the posterior border of the lamina. Osmium tetroxide seems to be a reliable indicator of unsaturated bonds in the fatty acids of myelin3, and so it is suggested that the relatively late appearance of osmiophilia indicates a difference in the unsaturated fatty acid content of "early" and "late" myelin.

In material from kittens of the first three age groups studied, stellate glioblasts present throughout the optic nerve showed intense GPDH activity. At 23 days and 28–29 days substantial GPDH activity was found in the glial cell columns within the prelaminar part of the optic nerve and the lamina cribrosa, and in glioblasts in the post-laminar part of the optic nerve within about 0.5 mm of the posterior border of the lamina. More posteriorly GPDH activity was virtually absent, only an occasional stellate cell showing very weak staining. In contrast, at the 38 day stage, post-laminar astrocytes throughout the optic nerve exhibited intense GPDH activity and, as before, this was also present in the glial cell columns at the head of the optic nerve. The situation characteristic of the adult cat optic nerve was thus established.

There seem to be three alternative explanations for the presence of an intense GPDH activity in glioblasts and astrocytes: first, that the breakdown of lipids provides a source of energy5; second, that the a-glycerophosphate cycle operates with the rapid production of energy and the oxidation of reduced NAD; third, that carbohydrates are being used in the biosynthesis of phosphatidic acids and thus phospholipids. To be effective in the rapid production of energy, however, the GPDH system probably needs to operate in conjunction with the citric acid cycle as a complex multienzyme system⁶ and such a system is unlikely to operate in the neonatal optic nerve of cat in which activity of enzymes of the citric acid is virtually undemonstrable in the first 2 weeks?. The idea of the GPDH in glioblasts and astrocytes being concerned in the biosynthesis of phospholipids is attractive because these cells also show an intense reaction for the presence of enzymes of the phosphogluconate oxidative pathway⁴ which is one of the principal sources of reduced NADP, utilized in the biosynthesis of fatty acids.

Furthermore, such considerations accord well with the histological context of glioblasts and their association with advancing myelination as demonstrated by sudan black and Luxol fast blue staining: for they are the only cells present in the neonatal kitten, and so at least some of them must be concerned with the local deposition of myelin and thus with phospholipid synthesis. In the adult cat, laminar astrocytes are the only macroglial cells present in a region of finely myelinated nerve fibres2: presumably these are the sheath supporting cells of the region and are concerned with some slow phospholipid turnover. It seems reasonable to assume a similar sheath supporting role for post-laminar astrocytes which are similar in morphology² and histochemical profile¹. Within the model, however, are the prelaminar glioblasts and astrocytes which have similar histochemical profiles1 but are isolating and insulating axons which remain unmyelinated: it may be that isolation and insulation require synthetic activity of a similar kind

whether or not these processes entail the formation and support of a myelin sheath.

The absence of GPDH activity from glioblasts further than 0.5 mm behind the lamina cribrosa in the fourth and fifth week is intriguing: it suggests that the maturation of myelin from the "early" non-osmiophilic type to the "late" osmiophilic type is not dependent on active lipid metabolism. At this stage, however, there are present oligodendroblasts which are developing their complement of citric acid cycle enzymes?: perhaps these cells provide the energy for the maturational change.

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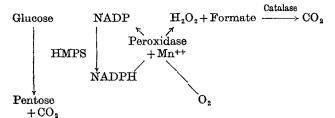
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Oxidation of NADPH by Polymorphonuclear Leucocytes during Phagocytosis

It is now known that the phagocytic process in polymorphonuclear leucocytes (PMN) is accompanied by marked increases in the rates of oxygen consumption, glucose oxidation by the hexosemonophosphate shunt (HMPS) and oxidation of formate to carbon dioxide1-3. The energy for the phagocytic process seems to come from glycolysis1. The rate of glucose oxidation by the shunt in leucocytes seems to be regulated by the intracellular concentration of NADP (refs. 3 and 4). A suitable hypothesis for the enzyme basis underlying the metabolic stimulation in PMN during phagocytosis should therefore include a mechanism for the reoxidation of NADPH. Attempts have been made to correlate the stimulated activity of the hexosemonophosphate shunt in phagocytosing leucocytes with increased oxidation of NADPH. The evidence indicates that oxidation of NADPH in phagocytosing leucocytes is not accomplished by the classical route of hydrogen transport by way of cytochrome reductase and cytochrome c (ref. 1). Evans and Karnovsky⁵ have presented evidence for the existence in PMN cell extracts of an NADP-linked lactate dehydrogenase which is activated at low pH. Results obtained by Iyer, Islam and Quastel² indicated that PMN possess an enzyme system capable of oxidizing NADPH and NADH by a reaction involving the formation of hydrogen peroxide. The enzyme, however, was found to be much more active towards NADPH than NADH and its activity was strongly enhanced by manganese ions. Later Roberts and Quastel⁶ reported that the NADPH oxidase in PMN was probably peroxidase. Based on the available evidence, a mechanism was proposed to account for the metabolic changes observed in PMN during phagocytosis. This is seen as follows.



Results described here suggest that the NADPH derived from accelerated HMPS activity in phagocytosing PMN is not oxidized by lactate dehydrogenase. This and other recent findings have implications in relation to the mechanism of NADPH oxidation in PMN.

Polymorphonuclear leucocytes were prepared by injecting guinea-pigs intraperitoneally with 20 ml. of 12 per cent sodium caseinate and collecting the peritoneal exudates after 16-18 h¹. Microscopic examination of this preparation showed the following differential count: 84 per cent neutrophils; 9 per cent monocytes; 6 per cent eosinophils; 1 per cent lymphocytes. The leucocytes were washed twice with 10-15 volumes of cold calcium-free Krebs-Ringer phosphate solution.

Phagocytosis experiments were carried out in 125 ml. Erlenmeyer flasks immersed in a temperature controlled water bath shaker at 37° C. Each flask usually contained 15 ml. of Krebs-Ringer phosphate solution free of calcium, 5 mmolar glucose, 500 mg (wet weight) of cells, and 50 mg of polystyrene latex spherules (1.3µ in diameter) where indicated. The incubation was carried out for 30 min. At the end of the experiment the cells and particles were removed by centrifugation and the resultant supernatant was used for the estimation of lactic acid and hydrogen peroxide. Lactate was isolated from the incubation medium by first removing the residual glucose by the copper-lime method, and then extracting the acidified aqueous phase for 24 h with diethyl ether. The ether extract was evaporated to dryness and the residue was dissolved in a small volume of water. The extracted lactic acid was further purified by ascending paper chromatography. The solvent system used was tert-butanol (10): formic acid (2): H_2O (15). After developing and drying the chromatograms, lactate was eluted from the paper and used for specific activity determinations. Lactic acid was assayed by the method of Barker and Summerson⁸. Radioactivity was measured in a Packard liquid scintillation counter with the solvent of Davidson and Feigelson⁹. Assays for hydrogen peroxide were carried out on incubation media by the periodate method10.

3-3H-glucose has been shown to be the substrate of choice for investigating the function of NADPH in reductive reactions¹¹. According to established schemes of glucose metabolism in the animal cell, tritium from the 3 position of glucose will be transferred to NADP in the 6-phosphogluconic dehydrogenase reaction yielding NADP³H. Because PMN have been shown to be devoid of transhydrogenase activity, NADH should not be labelled with tritium from the 3 position of glucose. It can also be seen that the tritium from the 6 position of glucose will not be transferred to either NADP or NAD, but that lactic acid derived from 6-3H-glucose will

be labelled with tritium at the β -position. Results summarized in Table 1 indicate that in the presence of 6-3H-glucose the specific activity of lactic acid synthesized by phagocytosing PMN is almost the same as that derived from resting cells. Experiment also showed that the magnitude of the incorporation of tritium label from the 3 position of glucose into lactate was almost 32 per cent higher in resting leucocytes than in phagocytosing PMN. If, according to the scheme proposed by Evans and Karnovsky, the oxidation of NADPH in phagocytosing PMN was accomplished by an NADPlinked lactate dehydrogenase, there should have been considerably more tritium incorporated from the 3 position

Table 1. Tritium labelling of lactic acid from 3-3h-glucose and 8-3h-glucose in resting and phagocytosing polymorphonuclear leucocytes

	Specific activity of lactate (c.p.m./\mumole)		
	Resting leucocytes	Phagocytosing leucocytes	
3-°H-Glucose 3-°H-Glucose+1 mmolar sodium azide 6-°H-Glucose	5,670 4,800 3,260	4,260 6,950 2,990	
m 10 (1 1) 0 0 ATT 1	3 2 2 2 3	00.000	

The specific activity of 3-3H-glucose and 6-3H-glucose was 39,000 c.p.m./µmole.

Table 2. EFFECT OF UPTAKE OF POLYSTYRENE PARTICLES ON PRODUCTION OF HYDROGEN PEROXIDE BY POLYMORPHONUCLEAR LEUCOCYTES

	μ moles H_xO_x produced by 50 mg (wet weight) cells*
Leucocytes	7·2
Leucocytes + particles	14·1

* Average of 54 experiments.

of glucose into lactic acid in phagocytosing PMN than in resting cells. This apparently is not the case. We were not able to determine the extent of incorporation of tritium into hydrogen peroxide because of the rapid equilibration of 3H2O2 with H2O. Results presented in Table 2, however, indicate that the rate of peroxide formation by phagocytosing PMN is nearly double that of resting leucocytes. The observation that the rate of oxidation of formate to carbon dioxide in phagocytosing PMN is also about twice that of resting leucocytes 12 suggests that the latter process which is catalysed by leucocyte catalase is dependent on the availability of hydrogen peroxide. Paul et al.13, using a fluorometric assay for hydrogen peroxide, have observed a two to four-fold increase in peroxide formation in PMN after particle ingestion. These investigators have also noted a correlation between production of hydrogen peroxide in PMN and intracellular killing of bacteria¹⁴.

The finding that incubation of phagocytosing PMN with 1 mmolar sodium azide results in a significant elevation of tritium incorporation into lactic acid from the 3 position of glucose may imply that when NADPH oxidation by peroxidase is inhibited more of the reduced nucleotide is oxidized by lactate dehydrogenase. This, then, would assure an adequate supply of NADP for operation of the hexose monophosphate shunt during phagocytosis. It may be that in resting leucocytes the production of NADP through action of peroxidase is adequate even when the enzyme is inhibited with azide and thus increased oxidation by lactate dehydrogenase is not observed.

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Relationship between Vitamin E and Certain Enzymes dependent on the Sulphydryl Group

VITAMIN E was discovered in the 1920s and its physiological role has still to be demonstrated1. Much of the interest in this vitamin has centered around its casual relationship in the events leading to a vitamin E deficient myopathy in several animal species²⁻⁴.

Ames and Risley⁵ suggested that essential sulphydryl groups of enzymes may be sites which are protected by the antioxidant action of vitamin E. This report examines enzymes which are unique and important to muscle and which depend on intact sulphydryl groups for their activities: myofibrillar ATPase, which provides energy for contraction⁶; and supernatant ATPase, which is the source of energy necessary for the accumulation of calcium ions by the sarcoplasmic reticulum during relaxation?.

English strain guinea-pigs were maintained on vitamin E sufficient and deficient diets as described before. Heart, masseter and gastrocnemius muscles were examined after 15 and 21 days.

The tissues were prepared by the procedure of Perry and Grey⁸. Myofibrillar and supernatant ATPase activities were determined by the method of Baird and Perry¹⁰ and expressed as activity/mg of non-collagen nitrogen (NCN)11. Sulphydryl groups were quantitated by a modification of the Ellman method12 and expressed as moles of sulphydryl groups/mg of non-collagen protein¹³. The sulphydryl inhibitor N-ethylmaleimide (NEM) was tested on heart supernatant fractions at a concentration of approximately 1 mole of NEM/mole of —SH in the supernatant.

All preparative procedures and assays were completed on the day animals were killed. Results of the ATPase and sulphydryl assays were analysed statistically by the F test. The effect of NEM on sulphydryl groups was analysed by Student's t test on paired observations. A probability of ≤0.05 was accepted as revealing a significant difference.

No significant differences were found in any category after 15 days. After 21 days, the E+ (vitamin E sufficient) gastrocnemius myofibrillar ATPase activity was significantly greater than the E- (vitamin E deficient) activity (Table 1). This was the only significant difference in the fibrillar fraction of the tissues examined. Heart and gastrocnemius supernatant ATPase of E+ animals was significantly greater than the corresponding fractions from E- animals.

Assay of tissue-free sulphydryl groups revealed no significant differences between the dietary groups. The average free sulphydryl group content of eleven E+ and E- supernatant fractions from each muscle was (moles ×108/mg of NCP): heart, 6.9; masseter, 5.8; gastrocnemius. 6.6. The average free sulphydryl group content of ten E+ and E- myofibrillar samples from each muscle was: heart, 5.1; masseter, 5.3; and gastrocnemius, 5.3. The inhibitory effect of NEM on E - heart supernatant sulphydryl groups was, however, significantly greater than its effect on the corresponding E+ fraction.

A point to emphasize is the investigation of animals in the initial stages of vitamin E deficient myopathy. The appearance of effects caused by decreased vitamin E in the heart and gastrocnemius muscles, but not in the masseters, suggests that these changes are related to the onset of the myopathy. The gastrocnemius muscle is a primary target of nutritional muscular dystrophy, while it is not yet clear to what extent the heart is affected. The masseter seems to have little if any involvement in short-term studies of the vitamin E deficiency syndrome of guinea-pigs10.

Localization of the effects of vitamin E deficiency in the sarcoplasmic reticulum would provide a possible explanation for some symptoms of the myopathy. A defect in the functional integrity of the reticulum, such

Table 1. MYOFIBRILLAR AND SUPERNATANT ATPASE ACTIVITY FROM TISSUES OF GUINEA-PIGS FED A VITAMIN E SUFFICIENT (E+) OR VITAMIN E DEFICIENT (E-) DIET FOR 21 DAYS

	(13/ DIE	I FOR 21 DAIS	
Myofibrillar	ATPase activity (μ	moles inorganic ph	osphate/mg NCN)
Diet group	Heart	Masseter	Gastrocnemius
E+	1.18 ± 0.12	2.03 ± 0.06	2·69 ± 0·06*
E-	(6) 1·22±0·15 (5)	(6) 1·93 <u>±</u> 0·17 (5)	2·26 ± 0·15 (5)
Supernatant	ATPase activity (µ	moles inorganic pl	osphate/mg NCN)
E+	1.59 ± 0.10*	1.77 ± 0.12	0.45 ± 0.01*
E-	1·09 ± 0·05	1·49±0·19	0-29 ± 0-06

 $(\overline{4}) \qquad (\overline{4}) \qquad (\overline{3})$ Values are means \pm standard errors. Parentheses indicate number of • P < 0.05 for differences between diet groups.

as could be caused by the labilization of sulphydryl groups essential for ATPase activity, would initially affect the calcium binding ability of the system, and have a secondary effect on the membrane structure. It seems that in mice with hereditary dystrophy, the ability of the membrane to release calcium can be enhanced by nitrate ions to a greater extent than in the normal controls14, suggesting that the properties of the calcium binding sites in the membrane have been altered by this dystrophy. Guineapigs fed a vitamin E deficient diet have a significant increase in serum creatine phosphokinase activity after 21 days15. This enzyme is of muscular origin16, and the permeability of the muscle cell membrane to this enzyme seems to have been altered.

The negative results of the sulphydryl assay on the muscle tissues studied imply that vitamin E has no direct or general effect in maintaining sulphydryl groups in the reduced state. Conversely, the effect of vitamin E deficiency on several enzyme systems dependent on sulphydryl, and the increased susceptibility to NEM of heart supernatant sulphydryl groups from vitamin E deficient animals, implies some indirect relation between tocopherol and sulphydryl groups. Possibly in the living system the active form of the vitamin can mask some sulphydryl groups by the reversible formation of a mercaptole or hemimercaptole¹⁷, or by substitution by the sulphydryl group at the 8a position of α-tocopherol, as reported by Goodhue and Risley¹⁸. Substituted α-tocopherones have not been isolated from biological materials. This may, however, be because they are easily reduced to α -tocopherol or hydrolysed to α -tocopherolquinone¹⁸.

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Possible Sources of the Carbonate Fraction of Egg Shell Calcium Carbonate

THE classical theory of the mechanism of production of the carbonate fraction of the calcium carbonate of the hen's egg shell is that of Gutowska and Mitchell¹. This theory makes use of the enzyme carbonic anhydrase which occurs in the glandular cells of the shell gland in large amounts. It can be represented thus

Blood Shell gland Lumen
$$2HCO_3^- \rightarrow 2HCO_3^- \rightarrow H_2CO_3 + CO_3^- \rightarrow CO_3^- \rightarrow Egg shell$$
 $\uparrow \downarrow \text{ carbonic anhydrasc}$
 $H_2O + CO_9$

There is little evidence in favour of this theory; in fact, the very low solubility product of calcium carbonate makes it unlikely that calcium and carbonate ions could be secreted together by the tubular glands of the shell gland. This would result in crystallization of calcium carbonate within the glands². Because of this lack of evidence the following experiment was designed to provide evidence

either for or against the theory.

White Leghorns in a stage of active shell formation (ten birds) or without an egg in the shell gland (controls: ten birds) were anaesthetized and the sciatic artery and vein and the inferior oviducal vein were cannulated³. Injections were made into a wing vein of either: 0·1 mc. of ¹⁴C-sodium bicarbonate (specific activity 57 mc. of carbon-14/g of sodium bicarbonate), or 0·2 mc. of ⁴⁵Ca-calcium chloride (specific activity 0·196 mc. of calcium-45/mg of calcium chloride) in 2·5 ml. of 0·9 per cent saline. Samples of blood were taken from the cannulae at 15 (⁴⁵Ca) or 30 (¹⁴C) min intervals. Activities of deproteinized (methanol) plasma were determined in a liquid scintillation spectrometer. The arterio-shell gland venous (AV) differences of both ⁴⁵Ca and ¹⁴C are given in Table 1.

Table 1. ARTERIO-SHELL GLAND VENOUS DIFFERENCES OF CALCIUM-45 OR CARBON-14 ACTIVITY OF PLASMA WITH RESPECT TO THE FUNCTIONAL STATE OF THE SHELL GLAND, AFTER INJECTION OF LABRLLED CALCIUM CHLORIDE OR SODIUM BICARBONATE

Activity/ml. of deproteinized plasma from sciatic artery (100) minus activity/ml, of deproteinized plasma from the uterine vein

Injections were of 0.2 mc, of $^{46}\text{Ca-calcium}$ chloride or 0.1 mc, of $^{14}\text{C-sodium}$ bicarbonate.

* t Test.

When the logarithm of the activity/ml. of plasma was plotted against time, the pattern of removal of calcium-45 during egg shell formation was considerably different from that in non-calcifying hens. On the other hand, the pattern of removal of carbon-14 from the blood was almost identical in both experimental and control birds.

Analysis of the egg shells from the experimental birds showed that although 45–50 per cent of the calcium-45 dose was deposited in the shell within 150 to 180 min, only 3–5 per cent of the carbon-14 was deposited within 180 to 220 min. When equivalent doses of ⁴⁷Ca-calcium chloride and ¹⁴C-sodium bicarbonate were injected simultaneously into the wing veins of five unanaesthetized birds, amounts similar to the former ones were recovered from the shells after periods of 120 min (38–45 per cent of the dose of calcium-47 and 1–3 per cent of the dose of carbon-14).

If Gutowska and Mitchell's theory is correct, when producing the carbonate fraction of the shell the gland must remove amounts of bicarbonate from the blood which are at least equivalent to the amounts of calcium which it

removes. From a simple calculation based on the quantities of both calcium and bicarbonate present in the blood, it is evident that an AV calcium-45 difference of about 60 per cent would imply an AV difference of at least 8 per cent in the content of labelled sodium bicarbonate if Gutowska and Mitchell are correct. Table 1 shows that there is no AV difference of carbon-14 during shell formation or during shell gland quiescence and the patterns of removal of carbon-14 from the blood indicate that the shell gland takes little or no part in this process.

Although no AV difference in the carbon-14 content of the plasma was detected during shell formation, up to 5 per cent of the dose was found in the shells. It is presumed that the carbon-14 activities in the shells were caused by ¹⁴C-carbon dioxide which originated from the ¹⁴C-sodium bicarbonate. This suggestion is supported by the fact that similar activities were determined in the egg shells of hens dosed with glucose labelled with carbon-14.

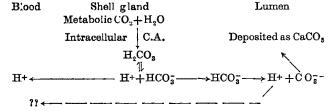
These results, together with the objections put forward by Diamantstein and Schlüns², suggest that Gutowska and Mitchell's theory is no longer tenable. If this is so, what alternative theories can be put forward? One possibility can be represented thus

Blood Shell gland Lumen
$$HCO_3^- \rightarrow Shell gland$$
 tubular glands $\rightarrow HCO_3^- \rightarrow H^+ + CO_3^-$

/ Deposited as CaCO₃

This suggestion, however, still relies on the removal of bicarbonate from the blood by the shell gland, although the calculated AV difference in ¹⁴C-sodium bicarbonate would only be 4 per cent. Moreover, it does not account for the carbonic anhydrase in the shell gland cells.

Another theory has been proposed by Diamantstein⁴, in which the shell gland derives the shell carbonate from its own metabolic carbon dioxide production, thus



This mechanism would account for the presence of the carbonic anhydrase in the glandular cells, for the drop in the shell gland venous pH which occurs during shell formation^{3,5} and possibly also for the fluctuations in the blood bicarbonate which occur during shell formation⁶. Neither of these alternative theories, however, explains how the bicarbonate ions in the lumen of the shell gland are deposited as calcium carbonate. When carbonate ion is formed from bicarbonate ion a proton will be produced, but nothing is yet known of how this transformation occurs and what is the fate of the proton. In these theories the proton is tentatively shown passing into the blood. Any transformation of bicarbonate to carbonate would have to take place in close proximity to the actual site of shell formation, for the carbonate would tend to crystallize out as calcium carbonate in situ. A possible explanation of carbonate formation put forward by Robinson and King? has been denied by Diamantstein, Bronsch and Schlünse.

The experimental results described here give considerable support to Diamantstein's hypothesis, although they do not provide proof of it.

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Stoichiometry and Coupling: Theories of Oxidative Phosphorylation

An integral and constant stoichiometric ratio is usually assumed to relate to coupled metabolic processes. In particular, the ratio of ATP production to oxygen consumption (P/O) is considered integral and constant in all discussions of oxidative phosphorylation. This view is maintained despite the fact that it conflicts with a great deal of experimental evidence¹. Although the discrepancies are often explained on the basis of technical difficulties, Slater² has pointed out that some uncoupling may represent physiological reactions, and that it is probably not possible completely to eliminate "uncoupled" reactions. We feel that despite the recognition of intrinsic uncoupling, its true significance has not been appreciated. Mechanisms and efficiency are still invariably assessed in terms of stoichiometry. We shall discuss some sources of non-stoichiometry, and then consider the implications of incomplete coupling, with particular reference to theories of oxidative phosphorylation.

Sources of non-stoichiometry or uncoupling can be divided into two categories: scalar sources and vectorial sources (processes respectively independent of and dependent on the symmetry of the system). Considering the scalar sources, a general scheme involving two coupled reactions is shown in Fig. 1. Here the oxidation of A and reduction of B are coupled to the phosphorylation of ADP. Oxidative phosphorylation is usually described in terms of such a scheme, with the belief that there is a stoichiometric relation between the oxidation and phosphorylation reactions. It is clear, however, that this need not necessarily be the case. For example, Fig. 2 shows the detailed scheme of reactions involved in a well known case of substrate level phosphorylation, the oxidation of acetaldehyde by NAD+ in micro-organisms3. In this system, deviations from stoichiometry can result from side reactions involving any of the reactants or products in

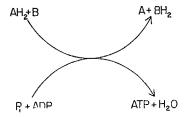


Fig. 1. General scheme for coupling of oxidation to phosphorylation.

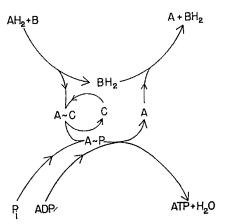


Fig. 2. Substrate level phosphorylation involving the oxidation of acetaldehyde by NAD+ (C is coenzyme A).

the two principal chains. These are trivial side reactions, easily recognized by lack of stoichiometry in the oxidation and phosphorylation reactions considered separately, and easily corrected for. The more interesting sources of decoupling arise from hydrolysis and other side reactions of the intermediate which links the two reactions, that is, $A \sim C$. Although the stoichiometry of the chief reactions is undisturbed, there is a deviation from the "theoretical ratio" relating them to one another.

In the example shown in Fig. 2, the coupling, although not perfect, is fairly tight as would be anticipated from the stability of the intermediates. But the situation may be very different in the case of respiratory chain oxidative phosphorylation. The "chemical" theories invoke mechanisms similar to that described in Fig. 2. The inability, however, to identify the intermediates (which may simply be molecules in an excited state) is usually explained by their supposed lack of stability. If the rates of breakdown of high energy intermediates are comparable with the rate of oxidative phosphorylation, there will be significant uncoupling.

In the foregoing scheme the coupling is effected by the transfer of bonds or groups at each step. Coupling may also occur by means of electron transfer. More generally it is the transfer of energy which is important, whether or not any chemical group is transferred. Intermolecular energy conversion between different excited molecules, involving changes in their electronic states only, is not uncommon4.

Considering the vectorial sources of non-stoichiometry or uncoupling, many reactions which are encountered in bioenergetics take place in a structural environment, an important example being oxidative phosphorylation in mitochondria. In such systems, compartmentalization of the reactants and products, and orientation of the enzymes, introduce the possibility that vectorial forces (for example, concentration gradients, potential gradients) may influence the coupling between reactions. In oxidative phosphorylation this can occur in at least two ways. First, if the reactions are coupled to each other through a high energy intermediate as in Fig. 2, and if they are both coupled to a transport process through the same intermediate, the electrochemical potential gradient of the transported species will influence the rates of oxidation and phosphorylation, and the P/O ratio. Second, the coupling may not be effected through a high energy intermediate, but directly by a transport process, for example, of a proton as suggested by the "chemiosmotic" hypothesis. In both cases, the extent of leakage (or exchange) of the transported species will influence the ratio of reaction rates. (In the case of an active transport mechanism involving a "pump", for example for protons, uncoupling may occur as a consequence of either scalar or vectorial processes. No specific molecular mechanisms are known, and so it is unnecessary to speculate on the origin of uncoupling; unpublished work of Essig, Caplan and Kedem.

We see from the considerations given here that the general way of describing oxidative phosphorylation⁶

$$SH_2 + \frac{1}{2}O_2 + nADP + nP_1 \rightarrow S + nATP + (n+1)H_2O$$

may be misleading, in that n need be neither integral nor constant in different conditions. Specifically, n will vary if the rate of oxidation varies although physical characteristics of the system such as the permeability of any leakage pathway and the concentrations of enzymes and cofactors remain constant. (In terms of the linear phenomenological equations of non-equilibrium thermodynamics, n will vary with changes in the forces and the flows even though the phenomenological coefficients and hence the "degree of coupling" may remain constant7.)

These considerations are important in the current controversy between two important theories of oxidative phosphorylation, the chemical and the chemiosmotic hypotheses. Arguments for and against each of these are usually based on stoichiometric considerations^{8,9}. example, the experimental P/O ratio discussed previously, and the ratios H^+/O , Ca^{2+}/H^+ and $\sim P/K^+$ are all supposed to reflect integral numbers, despite the fact that oxidation rates, pH, osmolality and ion concentrations all greatly affect the observed values^{1,10-13}. The emphasis placed on stoichiometry can be appreciated from the following recent quotations. Mitchell and Moyle's state that "The observed \rightarrow H⁺/O and \rightarrow H⁺/2e⁻ quotients. are in accord with the chemiosmotic conception of the folding of the respiratory chain into three proton-translocating o/r loops. The observations would be difficult to reconcile with the orthodox chemical coupling conception . . .". On the other hand, Slater14 states that "The stoichiometry of proton extrusion by mitochondria and of proton uptake by chloroplasts . . . (is) difficult to reconcile with the chemiosmotic hypothesis". although the proponents of each theory disagree in their interpretation of the data, they are agreed on the central importance of stoichiometry in deciding the issue. The foregoing considerations suggest, however, that constant stoichiometric ratios would not be expected in either case, and thus a choice cannot be made on this basis.

The assumption of complete coupling can lead to misinterpretation of experimental data in terms of the postulates of either model. For example, the number of

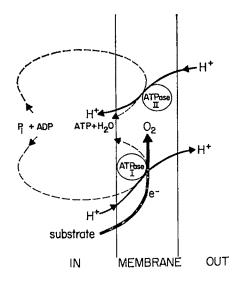


Fig. 3 A model for the coupling of oxidation, phosphorylation and proton translocation (see text).

proton translocating o/r loops in the chemiosmotic theory, or the corresponding number of phosphorylation sites in the chemical theory, may be underestimated.

Proponents of the two theories discuss ratios in terms of complete coupling and assume on this basis that their respective models are incompatible. This is not necessarily the case. A combination of the two is possible, as shown in Fig. 3. In this scheme, electron transport through the respiratory chain is coupled chemically to ATP synthesis by ATPase I, and also drives the translocation of H⁺. In addition, ATPase II couples the back flow of H+ to ATP synthesis. Either mechanism may be predominant.

An experimental evaluation of the various mechanisms would require measurement of the rates of hydrogen translocation, phosphorylation and oxygen uptake as functions of the forces. These are: the electrochemical potential difference of H+ across the membrane; the phosphate potential"; and the substrate/product ratio. The conventional presentation of data in the form of stoichiometric ratios without reference to the rates of reaction and the driving forces is inadequate.

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PATHOLOGY

Interaction of some Polyvinylpyridine Oxides with Polysilicic Acid and its relationship to their Ability to inhibit Silica Fibrosis

FINELY divided silica produces fibrotic lesions when it is inhaled or injected into the lung. The mechanism by which silica exerts its pathogenic effects has not been established but it is probable either that it functions by absorbing some cell constituent onto surface silanol groups or that, after particles have been ingested by phagocytes. monosilicic acid which is produced when silica dissolves in the cytoplasm exerts the cytotoxic action. In either case silanol groups are involved.

Poly-2-vinylpyridine 1-oxide will inhibit the fibrogenic action which quartz usually exerts when it is injected into animals or inhaled and evidence of any interaction

between this polymer and silanol groups may be of interest in explaining its chemotherapeutic action. In a preliminary communication², it has been shown that monosilieic acid in concentrations below 0.01 molar produces a bathochromic shift in the ultraviolet absorption spectrum of poly-2-vinylpyridine 1-oxide. A similar shift is not observed when monosilicic acid is added to 2-ethylpyridine 1-oxide. A slight shift in the spectrum of poly-4-vinylpyridine-1-oxide in the presence of monosilicic acid indicates that there is also some interaction between the 4-isomer and monosilicic acid. These deductions have been confirmed by viscosity measurements. The interaction presumably involves hydrogen-bond formation between the N-oxide group and the silanol group.

Nash, Allison and Harington³ have shown that there is also interaction between poly-2-vinylpyridine 1-oxide and polysilicic acid because a precipitate is formed when solutions of the two are mixed, and this again indicates hydrogen bonding. The implied correlation between hydrogen bonding and chemotherapeutic activity has been examined by synthesizing a number of other pyridine oxide polymers, and the ability of these and of poly-Nvinylpyrrolidone to precipitate polysilicic acid has been observed. The ability of each to counteract the cytotoxic effects of silica was measured by adding each polymer to a culture of peritoneal macrophages to which silica powder had been added and comparing the survival times of the cells. (These tests were carried out by Dr Beck and Miss Sack in the Institut für Lufthygiene, Düsseldorf.) The results are summarized in Table 1. There is apparently no correlation between the ability of a polymer to inhibit the cytotoxic effect of quartz and its ability to precipitate polysilicic acid.

Table 1	
Precipitation with polysilicic acid†	Protective action against silica
· + +	Highly active Some activity
+	Inactive
+	Highly active
-	Inactive
-	Inactive
-	Active Inactive
	Precipitation with polysilicic acid† . + + + +

^{* 2} per cent w/v aqueous solutions. † 0.02 molar aqueous solution.

Further studies (unpublished) on the effect of monosilicic acid on the viscosity of solutions of poly-2-vinylpyridine 1-oxide and poly-4-vinylpyridine 1-oxide indicate that, while the complex formed by the first polymer is stable up to 60° C, that formed by the second is much less stable above 20° C. It seems that loose interchain crosslinks and links between proximate parts of a randomly coiled polymer chain are formed by monosilicic acid with poly-4-vinylpyridine oxide but that monosilicic acid is more intimately bound to poly-2-vinylpyridine oxide. Interaction between oxygen and alkyl group in 2-methyl pyridine oxide has been demonstrated. The type of bonding is probably present in poly-2-vinylpyridine 1-oxide because this polymer has a lower pK_a value than the 4-isomer. A model shows that the polymer structure would then be very compact with the oxygen atoms aligned so that monosilicic acid could become attached to the polymer by two or possibly three hydroxyl groups. Cross-linking would be less likely with poly-2-vinylpyridine oxide than with poly-4-vinylpyridine oxide.

It seems probable that the ability of poly-2-vinyl-pyridine oxide to exert a protective action against silica depends not merely on its ability to form a hydrogen bonded complex, for most N-oxides will form such complexes, but on the stability of the complex which is formed.

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Carcinogens in Chinese Incense Smoke

CONSIDERATION of the high incidence of naso-pharyngeal cancer among the Chinese¹ led us to search for carcinogenic constituents in condensates from burning Chinese incense. Using thin-layer and column chromatography, several polycyclic aromatic hydrocarbons, including 3,4-benzopyrene, have been detected by ultraviolet absorption spectra. The 3,4-benzopyrene content was estimated fluorimetrically to be of the order 0.4 µg/stick of incense. The free radical content of the tar condensates was estimated by Dr A. Horsfield of Varian Associates Ltd. to be approximately 1.3×10^{15} stable electrons/g (comparable with the figure $1 \times 10^{16}/g$ obtained for cigarette tar³).

A search for nitrosamines in the condensate by the polarographic method4 gave negative results, but the method of Preussmann et al.5, and the Griess reagent, gave a salmon pink spot, R_F 0.35, on thin-layer chromatography (hexane: ether: dichloromethane, 4:3:2).

The same colour was, however, obtained by spraying the plate with sulphanilic acid (1 per cent in 30 per cent acetic acid) without the need of previous irradiation and of the second component of the Griess reagent, 1-naphthyl-

Nitrosamines do not give a colour with the sulphanilic acid reagent, but aromatic aldehydes give chiefly yellow colours. Furfuraldehyde which gives a slowly developing red colour similar to that of the unknown constituent of incense condensate has, however, a different R_F on thinlayer chromatography.

The colours given by aromatic aldehydes with sulphanilic acid should be borne in mind when applying the Griess reagent for the detection of nitrosamines according to the procedure of Preussmann et al.5.

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CYTOLOGY

Establishment of a Line of Cells from the Silkworm Bombyx mori

Following the establishment of lines of cells from tissues of the moth *Antheraea eucalypti* Scott¹ and the mosquito *Aedes aegypti* L.², a line of cells from the silkworm *Bombyx mori* L. has now been established.

The method of culturing the cells and the medium were the same as for the A. eucalypti cells. All the cultures were incubated at 29°-30° C. The cultures were set up in 1964 and consisted either of six whole ovaries torn apart or of dissociated cells from six ovaries suspended in 3·0 ml. of medium in Petri dishes. The ovaries were obtained from larvae which had started to spin their cocoons. Of the six cultures of whole ovaries, five died after 6 months and one survived for 12 months after it had been set up.

In the cultures of dissociated cells, most cells became attached to the substrate within 12 h. During the next 4-5 days many cells died, but by the tenth day the cultures were extremely healthy and dividing cells were common. Some cells formed sheets, very like syncytia, which attached firmly to the substrate. Short lengths of undissociated ovariole showed muscle contractions at 12 days. The sheaths surrounding these pieces of ovariole formed long processes that contracted regularly (Fig. 1). At the end of 6 months only two of the cultures had survived. Three months later, cells in one of these cultures rapidly increased in number. Most cells did not attach



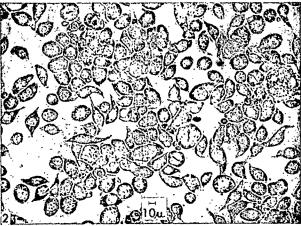


Fig. 1. Protoplasmic extensions which have formed a network, in a culture of silkworm ovarian cells. The network was contractile, 8 months in culture.

Fig. 2. Cells from the established line of silkworm cells 29 months in culture.

to the substrate. Two weeks later a subculture was made by transferring the cells to a new culture vessel along with half the medium. The remaining culture died 2 months later. The culture which had "transformed" and the subcultures continued to proliferate and further subcultures were made at 2–3 week intervals for 3 months. Since this time subcultures have been made at 6 day intervals.

The most common type of cell in the line is spindle-shaped, $12-25\mu$ wide and $50-70\mu$ long. The only other type of cell significant in number is slightly spindle-shaped to about $18-30\mu$ in diameter (Fig. 2). Many of these latter cells form small sheets on the surface of the culture vessel. The network of contracting tissue persisted after the "transformation". but by 16 months only single cells remained.

The generation time of the cells, measured 2 yr after the culture was set up, was 48 h. The cells have been adapted to grow in medium containing 1 per cent bovine plasma albumin and 1 per cent heat treated haemolymph from Antheraea pernyi (Guer.). The cells have been deep frozen in medium containing 10 per cent glycerol and have proved to be still viable after 10 months at -180° C.

Although the diploid number of chromosomes in the silkworm is 56, most cells examined 15 and 18 months after the line was established contained many more than 100 chromosomes.

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Metaphase Arresting Compounds in Embryos

COLCHICINE, demecolcin (N-desacetyl-N-methylcolchicine) and vinblastine are all metaphase arresting agents¹. The first two compounds have been used extensively to determine various parameters of cellular dynamics, such as the mitotic index and the turnover time. In adults good agreement has been found between data obtained using colchicine and isotopes². Vinblastine is not used to examine these cell parameters, probably because it has been shown to affect DNA synthesis³. The effect of these compounds on embryonic rats in utero has been examined. In this study, we found that the mitotic index obtained using the different compounds differed profoundly.

Mitotic indices were obtained by counting one thousand cells in sections of the lining of the cerebral ventricles of twelve day embryo rats after administering the metaphase arresting agent intraperitoneally to the mother. The animals were killed 4 h after the administration of the drug. The number of mitotic figures found was expressed as cells in division per hundred cells over a period of 4 h. Nine embryos from three litters were examined at each dose level. In the absence of any metaphase arresting agent 7.4 per cent of the cells of the inner surface of cerebral ventricles were found in various stages in division.

Wegner et al.⁴ using ³H-thymidine reported that the generation time in different ectodermal and endodermal tissues in the rat embryo varied between 12 and 18 h. They also noted that the source of variation was in the G1 period, the S, G2 and M periods being fairly constant. The mitotic time was 1 h. We used this figure in this experiment and a generation time of 13.5 h is found. If the generation time is 13.5 h, then some 30 per cent of the cells should be found blocked in division after 4 h treatment with the arresting agent. The data pre-

sented in Table 1 are not only inconsistent with one dose and one drug but are at variance with the mitotic index determined from the generation time.

Table 1. REFECT OF METAPHASE ARRESTING AGENTS ON THE MITOTIC INDEX IN 12 DAY EMBRYO RATS

Drug	Mitotic ind 1 mg/kg	ex after 4 h 8 mg/kg
Colchicine Demecolcin	* 20 ±2†%	7·0±0·8% 6·6±0·7%
Vinblastine	6.8±0.4%	5.3 ± 0.8%

Metaphase arrest not seen in these embryos.
 † Standard deviation.

In the untreated embryos, 68-72 per cent of cells found in division were in metaphase and 15-17 per cent in anaphase. In the treated embryos 95-97 per cent of the cells found in division were in metaphase, but only 1-3 per cent were found in anaphase. This indicates that the cells were blocked in metaphase, and it can therefore be assumed that the unexpectedly low yield of mitotic figures was not caused by a lack of penetration of the drug through the placenta. Another cause for the small number of division stages might be the destruction of the arrested metaphase figures. This would not, however, be expected to occur during the relatively short duration of these experiments and no indication of such destruction was seen in the sections.

We examined the effect of demecolcin on embryonic mitotic indices and we came to the conclusion that the drug was causing some inhibition during interphase, and demecolcin has been reported to inhibit DNA synthesis in embryos in uteros. The same effect seems to be present with the other metaphase inhibitors, colchieine and

Whatever may be the cause of the differing mitotic indices produced by these different agents, the indices found in mammalian embryos in utero using these metaphase arresting agents do not correlate with the isotopic data of Wegner et al.4, nor do they reflect the mitotic activity of the untreated tissue.

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APPLIED SCIENCE

Formation of Thin Polymer Films in a Glow Discharge

GLOW discharge polymerization of organic materials has already been described in detail. The process involves introducing vapours of relatively volatile monomers into a low pressure chamber (1 to 5 torr) and subjecting these to an A.C. discharge maintained between two parallel electrodes. Ions and electrons, produced in the glow, bombard the electrodes and the molecules in the gas phase; these interactions initiate complex chemical reactions which result in the formation of thin continuous coatings on the electrode surfaces. Film formation actually takes place by positive ion bombardment of monomer molecules adsorbed onto the electrodes. For a given monomer, the

composition of the films varies with the ratio of adsorbed molecules to the number of ions striking the surface and also with the degree of contamination produced by breakdown products formed in the gas phase.

This article is concerned with a modified glow discharge process—based on the mechanism referred to briefly here for depositing polymer coatings from non-volatile starting materials. Furthermore, the process is easily controlled and produces high quality coatings free of gas phase contaminants.

The apparatus used in this work is shown in Fig. 1a; steel samples about $60 \text{ cm} \times 7 \text{ cm} \times 0.025 \text{ cm}$ were shaped into a drum and placed in a 'Teflon' holder which could be rotated (Figs. 1b and 1c). Organic material (with V.P. ≤10-2 torr at 20° C) was evaporated from a heated boat onto the rotating drum at its lowest point. The deposited material was then subjected to ionic bombardment by initiating and maintaining a glow discharge between the rotating sample and a stationary electrode. This electrode was located above the highest point of the drum and about 1 to 4 cm away. The discharge was maintained in argon, precautions being taken to avoid contamination of the glow with organic material; for example, argon gas was injected into the system through the stationary electrode and diffusion of hot vapours away from the evaporation area was minimized by means of cold baffles.

Coatings up to 10 were built up on the rotating drum by successive evaporation and bombardment of layers 10-100 Å thick. Any attempt to deposit and bombard thicker layers resulted in films containing a large percentage of unreacted material, even when the number of ions striking the surface was in excess of that required to allow every deposited molecule to react. It was concluded that the maximum layer which could be processed was a function of the penetration of the ions formed in the discharge.

Coatings were readily produced from materials of widely differing structures, for example, butyl phthalate, acrylamide, uncured epoxy and polyester resins, silicones, liquid paraffin and linseed oil. Film properties were readily controlled—compared with conventional glow discharge polymerization—by varying the discharge In addition, the final current and evaporation rate. coatings were completely free of gas phase contaminants because the discharge was maintained in argon.

This technique could be attractive for depositing very thin coatings (<100 Å) on a moving substrate, but for coatings of 1μ and above it is clearly unsuitable because of the large number of deposition-bombardment cycles required. A possible method of depositing the thicker coatings would be to combine the two stages and run them concurrently—a technique similar in many respects to conventional glow discharge polymerization. This would however, re-introduce gas phase reactions which might result in excessive contamination.

A more feasible approach², with the exciting possibility of being developed into a high speed, solventless strip coating process, involves the use of more penetrating radiation. A high speed, low pressure (about 10-2 torr) technique has been developed by Williams and Moore for vapour depositing thin uniform films of a wide range of organic materials (for example, low vapour pressure monomers, uncured thermo-setting resins, natural resins, silicones, etc., and mixtures of such materials) without effecting any thermal degradation or fractionation during evaporation. It has been shown that these films can be either polymerized or cross-linked or both while still at reduced pressure by bombarding for a very short time with low energy electrons (<20 kV) at current densities up to 1 m.amp/cm². Attempts to produce coatings with similar properties to the present lacquers on tin-plate have been very promising. This method could be an extremely attractive lacquering technique; the vacuum required is moderate, the electron energies low and hence

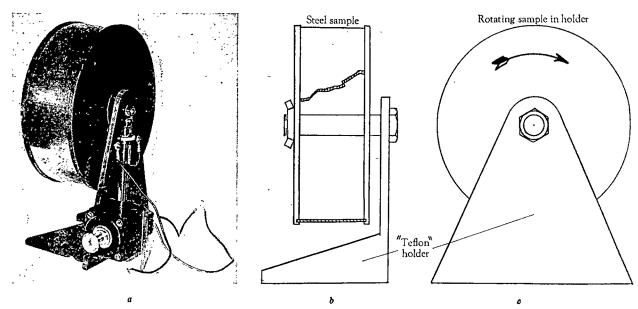


Fig. 1. a, b and c, Views of the apparatus used showing the rotating steel drum on which the polymer was formed and the 'Tefion' holder.

relatively inexpensive to produce, and because solvents and heating ovens are eliminated it has considerable advantages over the present process, especially for high speed applications.

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GENERAL

Diesel Exhaust Particulates

Although the experimental techniques are available, limited effort has been made to characterize physically and chemically the particulate emissions from air pollution sources with respect to particle size, total surface area and chemical composition. If the potential to affect human health, property or atmospheric reactions or visibility of the particulate pollution, present in urban atmospheres, is to be evaluated, the nature of the contribution from each source will have to be more fully understood than at present. In this study, we focused on the characterization of particulates in a diesel engine exhaust.

Quantitative information available on diesel exhaust composition has, until recently, primarily pertained to gaseous constituents¹⁻⁵. Other studies were concerned with visible smoke and odour^{6,7}. Several investigations were made of the reactions and kinetics in the formation and combustion of smoke in hydrocarbon flames with controlled laboratory combustion chambers⁶⁻¹⁰. Others studied the relationship between smoke production, work parameters and fuel properties¹¹⁻¹³.

The engine used was a single cylinder, four cycle diesel. On acquisition the engine was completely overhauled and reconditioned; at the beginning of these tests it had accumulated approximately 500 h of prior running time. Although not of usual industrial size, the engine was not atypical in general design, speed, exhaust temperature, and carbon dioxide and oxygen emissions. Load was provided by driving a 5 kW generator, the output of which was dissipated through adjustable immersion

heaters. Engine specifications and fuel properties are shown in Table 1.

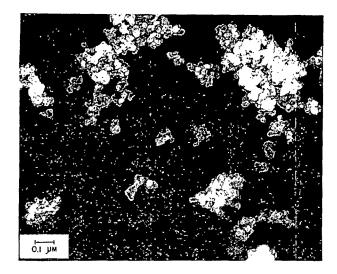
Table 1. ENGINE AND FUEL SPECIFICATIONS							
Test engine description	Test engine description						
Туре	Four-cycle, valve in head Diesel, American Marc, Inc. (Model AC1)						
Number of cylinders Bore Stroke Piston displacement Compression ratio Speed range Minimum working Maximum working Maximum rated brake horsepower (2,500 r.p.m.)	1 3.500 in. 3.625 in. 34.8 cu. in. 20:1 1,200 r.p.m. 2,500 r.p.m. 9						
Chemical and physical properties	of fuel*						
Flash point, minimum Pour point, maximum Cetane number, minimum Gravity, deg. API Distillation temperature 90 per cent point, maximum End point, maximum Distillation recovery, per cent by volume minimum	125° F - 5° F 45 38 650° F 700° F 97·0						

* New York Central specification number 1,370-C, Grade 2.

All sampling for particulates was performed in the exhaust pipe approximately four inches from the exhaust valve. A vacuum pump was used to withdraw centreline samples at isokinetic flow. Air samples were metered with orifices, were not diluted, and were obtained at as high a temperature as possible in order to avoid moisture condensation. Engine speed was monitored with a stroboscope. Fuel consumption was determined from reservoir fuel volume changes during a test run. The fuel to air ratio was calculated after the carbon dioxide and oxygen content were determined by Orsat analysis.

Samples for electron microscopy were collected on carbon filmed glass slides in an oscillating thermal precipitator 14 . Electron photomicrographs were obtained after transferring the carbon films to the grids. Resulting micrographs were sized with a Zeiss TGZ 3 particle size analyser. From the number sized, photographic area and magnification, the number of particles per unit area of sample was calculated. The number concentration per unit volume of exhaust was obtained from the air sample volume and the area of particle deposit.

Samples for specific surface area and bulk density determinations were collected on 'Millipore GS' membrane filter. Specific surface area measurements were performed according to Brunauer, Emmett and Teller surface adsorp-



a

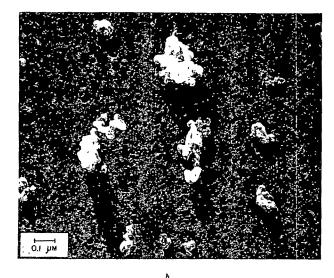


Fig. 1. Electron photomicrographs of diesel engine exhaust particulate (a) unshadowed and (b) shadowed with platinum, $(\times c.~58,000.)$ Sampled using a thermal precipitator 4 in. from the cylinder at engine operating conditions 1400 I.

tion theory¹⁵ and techniques¹⁶. Gravity-standing bulk density was determined by weighing the amount of material in a container of known volume. Weight concentrations of particulates (mg/m3) were determined by filtering through pre-weighed glass wool packed alundum thimbles and measuring the weight gain.

The free acidity of a sample of particulates, successively gathered and removed from a 'Millipore GS' filter, was measured (in the absence of carbon dioxide) by titrating with 0.01 normal sodium hydroxide in the presence of 85 per cent ethyl alcohol with mixed cresol red-thymol blue indicator. A twenty hour leaching period was used.

Table 2 summarizes our results. Operating conditions can be described as follows: 1400 I, idle speed, no load, low temperature; 1800 C, intermediate speed, no load, moderate temperature; 1800 L, reasonable load, resultant speed and temperature. The results show that the engine is a principal source of particles. Thus we found approximately 107 particles/cm3 released, while 103/cm3 is representative of the ambient air concentration of 0.1μ diameter particles17. A dilution factor of 104 would be required to reduce particle numbers to ambient concentrations.

Table 2. CHARACTERISTICS OF DIESEL EXHAUST PARTICULATE—SUMMARY

No. obser- vations	Operating condition .	1400 <i>I</i>	. 1800 <i>C</i>	1800 N 1/2*
7	Weight concentration, mg/m ³ Rate, mg/min	75·3 47·8	72·5 55·6	33·3 28·6
_	mg/cm³ fuel	6.3	5.4	1.3
7	Number concentration, 10° particles/cm³	199	185	682
	Rate, 1019 particles/min	12.6	14.2	58-3
	1012 particles/cm3 fuel	1.6	1.4	2.6
1	Specific surface, m ² /g	28.1	37.5	50.0
	Specific surface concentration, m2/m2	2.1	2.7	1.7
	Rate, m²/min	1.3	2.1	1.4
	m²/cm³ fuel	0.18	Õ·19	0.06
16	Bulk density, g/cm ²	0.12	0.12	·0·10
	Particle projected area diameter			
	Arithmetic mean size, A	860	860	830
	Geometric mean, A	62.0	60.5	21.0
3	Free acidity, micromole/min	5	8	5
-	micromole/cm³ fuel	ŏ.8	ŏ·8	ŏ·2
* 3.6	b.h.p.	0.0	V 0	0.77

Platinum shadowed and unshadowed photomicrographs offered a startling contrast (Fig. 1). Under shadowing conditions an entire unidentified second family of material was made visible which was not included in sizing and counting data reported. The particle height to diameter ratio was about 1:4, an aspect ratio produced by hydrocarbon oils.

Spectrographic analysis of the inorganic portion of particulates, which comprised 55-60 per cent of total particulates 18, demonstrated that about 98 per cent by weight was carbon. The chief ingredients of the non-carbon fraction were silicon, iron and zinc. Traces of aluminium, nickel, calcium, barium, as well as other metals were detected. Emission rates of iron and zinc at the 1400 I operating condition were 3.6 μg/m³ and 2.6 μg/m³, respectively. A dilution factor of approximately four would reduce these emissions to average ambient air concentrations.

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BOOK REVIEWS

PEOPLE AND THEIR LAND

Population Growth and Land Use

By Colin Clark. Pp. 406. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1967.) 70s. net. STUDENTS concerned with problems of population growth and the result of population pressure on land use will be familiar with most of the arguments, data and methods in Colin Clark's new book, though previously they would have had to search far and wide for some of the material. Much of the book's value lies in the way the author has brought together such a mass of material, strung it on a tenuous thesis and compressed it to a mere 400 pages. A measure of the range of interests which the book covers is that it begins with measuring human fecundity and ends with land values in Chicago; yet there is a line of enquiry, sometimes more implicit than explicit, which firmly links them, and this has been central in Dr Clark's research during the past twenty years. He is always concerned with the human condition, the necessity to refine our tools of analysis and the need to see problems in economic terms. The reader is led successively through the intricacies of the reproductive capacity of human beings, problems of survival and growth, history of population growth, the relationship between this and food and fertility, sociology of reproduction, economics of growth, locational analysis and finally urban land values. The author acknowledges his debts to many fields of study and makes only modest claims for this volume as a "preliminary attempt to survey the range of material"; but there is no doubting his deep appreciation of the problems and the need to find immediate answers.

In addition to providing so many statistical methods of collating data Clark can be provocative and stimulating. His ideas about world hunger and his estimates of how it could be met make an interesting chapter. He is particularly critical of the FAO for giving a desperately pessimistic slant to world hunger by setting too high a nutritional standard. He says, "if the F.A.O. were disbanded and a fraction of its revenue devoted to the universities and private institutions where real research is being done the world would be better off". He has convincing arguments for modifying the standard, but his estimate of what the world can support is probably too generalized a picture, and in any case depends on either a redistribution of population or a system of exchange so radical that it remains an ideal. His world total of 47 billion by western standards is probably optimistic, but at least his arguments for the possibilities of meeting growing needs rather than of limiting population are challenging.

Many of Clark's views are controversial in spite of being asserted as fact. Whether medical improvements began to make discernible changes as early as 1750 is debatable. It was surely the end of the nineteenth century before the effects of medicine were radical and massive enough to affect population changes very markedly. The increased food production and the opening up of new food producing areas in the nineteenth century surely played a part in the earlier changes. Nor would everyone accept the constant implication that population increases were the prime cause of technological innovation—often it may well have been the reverse.

The book is most interesting when views such as these are being argued, though often one would wish Clark to

justify them more fully. The erudition and scope of the references are impressive—as is the absence of reference to British workers in these fields—the pace is sometimes furious, but one could wish for a more coherent statement of the central theme. The final paragraph hangs tantalizingly in mid-air, for the book has no conclusion. The greatest value of the volume is the way in which it brings together such a diverse mass of data and techniques concerning man and his demands on the land. Students of economics, sociology and geography will be indebted to Clark for this service.

EARTH DISCUSSED

Debate About the Earth

Approach to Geophysics through Analysis of Continental Drift. By H. Takeuchi, S. Uyeda and H. Kanamori. Translated by Keiko Kanamori. Pp. 253. (San Francisco: Freeman, Cooper and Co., 1967.) \$4.50.

This is a surprising book in several ways. Perhaps the pleasantest surprise is its simple readability. The authors have spared no pains to simplify every concept and argument. Comparable care has obviously been given to the design of the illustrations. An easier and more effective introduction to the current and amazing interpretation of the history of the Earth would be difficult to imagine.

The book is primarily a history of the theory of continental drift. But it is much more than that. geophysicists who wrote it found it necessary to digress rather deeply into the fundamentals of the Earth's magnetism and its thermal history. Necessary, too, were considerations of the origin of the Earth, its internal structure, the sources of its heat and the inferred mechanics of the thermal convection within its solid mantle. Into these fundamentals are woven the diverse pieces of pertinent evidence derived from palaeontology, oceanography, palaeoclimatology, and the exciting postwar discoveries by the geophysicists that bear on the most important properties of the Earth. In all this, the authors are commendably objective. Yet, to use one of their phrases, "the discerning reader" will sense their conviction that extensive drift of the continents since the Jurassic is the only satisfactory interpretation. In the light of what has since been learned about the migration of north-south belts of magnetic anomalies east and west from the mid-Atlantic ridge and the extraordinarily high heat flow and the young deposits of metals in the deepest part of the Red Sea trench, their belief seems to have been well founded.

Many readers will derive satisfaction from the way the authors show the necessary interdependence of geophysics and such disciplines of Earth science as oceanography, palaeontology, glaciology, climatology and rock magnetism. It seems, however, and perhaps only because I am a geologist, that a more important role might have been ascribed to geology. It is not very sure-footed, for example, to match mountain ranges end to end without the kind of understanding of them that comes from years of systematic mapping. Likewise, the essentiality of the understanding that petrology brings to the process of discriminating between suitable and questionable rocks for magnetic studies might have been enlarged on without unduly lengthening the text. But these are quibbles. The book makes the significant point, and makes it well, that ideas and evidence from many disciplines are essential to the solution of problems in Earth history.

Fascinating and valuable also are the ways these geophysicists have shown how a theory may be born and what the originator had in the way of facts to spark the idea. They also show what the originators lacked and, in the perspective of later knowledge, what they missed or misinterpreted.

Quite apart from their authoritative review of the Earth's history, the authors have captured much of the fun, the excitement, the doubts, the frustrations and the satisfactions of scientists who devote their lives to unravelling one aspect or another of the Earth's fascinating Having so subtly woven this human interest into the history and development of one of the boldest theories ever to come true, they should not be surprised to find, in the years to come, that they have drawn into the fraternity of Earth scientists many able young minds.

Debate About the Earth will be stimulating to a broad spectrum of students, and it will be useful to the professional geologist as a means of catching up with important and startling developments in the fundamentals of his science. The authors obviously enjoyed a happy relationship with the publishers. W. H. Bradley

NEW PHYSICS SUMMER SCHOOL

Recent Developments in Particle Physics

Edited by Michael Moravcsik. (Proceedings of the First Pacific International Summer School in Physics, August 1965.) (Nuclear Physics, Vol. 3.) Pp. viii+263. (New York: Gordon and Breach Science Publishers, 1966. Distributed in the UK by Blackie and Son, Ltd.)

THE University of Hawaii at Honolulu has now joined the ever expanding host of institutions which sponsor a vacation school or institute in some advanced area of research. They should not find much difficulty in recruiting the lecturers, but the student body is likely to remain confined to the Pacific seaboard of the United States. Thus the printed version of the lecture notes will be all the more valuable in Europe.

Proceedings of the first school in 1965 contain notes on "Particles and Cross Sections in a Theory of Local Observables", by R. Haag; "Spontaneous Symmetry Breaking and Related Problems", by H. Miyazawa; Breaking and Related Problems', by H. Miyazawa; "The Algebraic Description of Hadron Matter and its Observational Implications", by Y. Neeman; "Lectures on Bootstraps", by F. Zachariasen; "The Bootstrap Theory of Symmetry Breaking", by S. Frautschi; and "The Non-Dynamical Structure of Particle Reactions", by M. Moravcsik. These are all self-contained, concise expositions, a few overtaken by more recent developments. The most valuable are those by Haag, Miyazawa and Moravcsik. Zachariasen gave the same lectures later that year in Vienna and they have appeared in print before. By the way of preface there is a most amusing and yet convincing apologia for this kind of school by the director, M. Moravcsik.

The volume is a paperback and the price seems excessive. S. ZIENAU

SEMICONDUCTOR SURFACES

Electrical Properties of Semiconductor Surfaces By Daniel R. Frankl. (International Series of Monographs on Semiconductors, Vol. 7.) Pp. xv+310. (Oxford, London and New York: Pergamon Press Ltd., 1967.) 84s. net.

THE extent to which the electrical properties of a semiconductor are dependent on the physical and chemical conditions of its surface has been the subject of much experimental study during the past twenty years, largely prompted by the technical requirement to control and stabilize the performance of semiconductor devices. There have been corresponding advances in theoretical understanding, and much previously empirical knowledge has now been rationalized, leading to useful insight and prediction.

The literature of research on semiconductor surfaces is widely scattered except for the proceedings of topical conferences and symposia, so that the appearance of this up to date text is specially welcome. The book succeeds in its aim of being largely self contained by presenting a thorough treatment of the theoretical background necessary to interpret the experimental data. The first two chapters discuss the equilibrium distributions of electrons and ions in bulk intrinsic and extrinsic semiconductors and in their surface space-charge layers. The next chapter deals with non-equilibrium conditions arising from illumination or carrier injection and considers both the final steady state and the relaxation spectrum. The fourth chapter treats field effects in great detail as befits the experimental importance of the topic, and the related theory is competently and critically presented.

The second half of the book is devoted to surface preparations and to the surface states resulting from them. There is shown to be little relation between observed surface states and those derived from the idealized theoretical models of Tamm and of Shockley. Experimental data are presented in considerable detail together with descriptions of methods of measurement and interpretation. Germanium and silicon receive by far the greatest attention as would be expected from the extensive study already devoted to their bulk properties which is a prerequisite for an understanding of the surface data. The available fragmentary data on GaAs, InSb, ZnO, CdS and PbS are also collected.

This book conveys the impression throughout of being well organized and critically written. It will be useful to research workers both as a text and a reference. bibliography is copious and continues through 1966. The style is eminently readable, but it can only be a matter of conjecture whether the excessive use of commas should be attributed to the author or to an enthusiastic copy editor. Misprints are few and when they occur in the equations they are readily corrected by the alert reader.

R. E. Burgess

RIGOUR AND THE CALCULUS

Differential and Integral Calculus

By Friedhelm Erwe. Translated by B. Fishel. Pp. x+494. (Edinburgh and London: Oliver and Boyd, Ltd., 1967.) 57s. 6d. net.

THERE are many books on the differential and integral calculus. What justifies the appearance of another one? The present book differs from many in the rigour with which the subject matter is developed. It is not a collection of recipes and formulae, deduced largely by an appeal to intuition or experience of the physical world. Instead, it is "pure" from the start, and it is a long way to the introduction of the calculus. Continuity and differentiation are introduced only after ninety pages of preliminary material about real numbers and infinite processes. After that there is a careful development of the usual topics covered in a good course on the differential calculus. This is followed by a fairly easy chapter on the elementary functions. Integration is not the next topic to be treated. Instead, the author proceeds to a rigorous discussion of the differential calculus in the case of two or more variables. This discussion includes a long section on curves, and the definition used for a curve "admits entities which defy the possibility of geometrical visualization". The section closes with a statement of the Jordan curve theorem, and it is something of an anticlimax for us to learn that "its rigorous proof goes very deep and because of its length we shall have to omit it". Of course, the reason is that the proof lies buried in topology, and that is another story.

The last two chapters deal with integration. The first, relating to functions of one variable, covers the usual topics. But the second contains some new material, including a discussion of the parts of algebraic topology which enter into the formulation of the main results on the integration of differential forms. The book, oddly enough, concludes with a down to earth section on "the calculation of content, surface area, centroid and second moments".

From this brief survey it is evident that the book is not for the applied mathematician or the engineer. But it is an exciting book for the student of pure mathematics, and its method of approach is most valuable for anyone who hopes to do postgraduate research in mathematical analysis. L. S. GODDARD

ADVANCES IN POLYMER SCIENCE

Stereoregular Polymers and Stereospecific Polymer-

Edited by Giulio Natta and Ferdinando Danusso. (The Contributions of Giulio Natta and his School of Polymer Chemistry.) Vol. 1: Pp. xxii+1-466. Vol. 2: Pp. xx+467-888. (Oxford, Lendon and New York: Pergamon Press, Ltd., 1967.) 315s. net per set of two volumes.

Heteroatom Ring Systems and Polymers By H. R. Allcock. Pp. xi+401. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 132s.

THE announcement on December 11, 1954, by Professor Giulio Natta and his co-workers of the synthesis of a new class of polymers, obtained by the polymerization of alpha-olefins and with an exceptional regularity of structure, opened up an immense field of research which has brought remarkable fruits to both polymer science and to industry. In recent years the interest in the subject of stereoregular polymers and stereospecific polymerization has increased steadily and development of this subject continues. It is thus of some significance to have collected together in a single work the papers of those researchers who made these discoveries. Apart from its historical interest, this collection is very useful to scientists working in this field. Many of the original papers, which were published in Italian or in a language other than English, are translated in Stereoregular Polymers and Stereospecific Polymerizations. The first two volumes comprise the 170 papers, in chronological order, submitted for publication, from the discovery of stereoregular polymers (March 1954 to 1959 inclusive). All the important papers and reviews are unabridged but the others are reported as abstracts.

Among the papers will be found the first announcement of the discovery and the description of the first syntheses and industrial production of stereoregular polymers of alpha-olefins and diolefins. Other papers describe the kinetics and reaction mechanisms, the study of the structure of stereoregular molecules, of their properties in solution and in the solid state, and the industrial application of the polymers. The new polymers described in the pericd covered by these volumes were predominantly hydrocarbons. It is the editors' intention to publish the corresponding works on non-hydrocarbon monomers and polytactic polymers in subsequent volumes.

Another branch of chemistry in which there has been striking advances in recent years is that of heteroatom ring systems and polymers, particularly those in which the skeletal systems consist of silicon, phosphorus, sulphur, aluminium or carbon, bonded to oxygen, sulphur or nitrogen. The impetus to this subject has been provided by the requirements of aerospace technology for unusual polymeric materials, and in particular for materials of high thermal stability. The series of compounds such as

the siloxanes, phosphazenes, borazenes and s-triazines have received considerable attention, and in the second book, Heteroatom Ring Systems and Polymers, an attempt has been made to present a unified picture of the very wide and diverse subject of heteroatom systems. The first part of the book (first four chapters) is devoted to the general and theoretical background of the subject. The second part (fifth to seventh chapters) deals with the fundamental chemistry of these compounds and the final section (eighth chapter) is concerned with the high polymer chemistry of hetero-compounds.

The book is not devoted predominantly to the "inorganic concept" and this particular aspect of the subject is considered as part of the more general chemistry of these compounds. The general approach of the authors is a comparative one and the different heteroatom systems are compared with respect to bonding theory, aromaticity, ring-polymer equilibration, synthesis, polymerization, reactions and polymer chemistry. References up to the early part of 1966 are included in the body of the book and some important papers which appeared later are reviewed in an appendix. A second appendix contains a valuable compilation of bond angles and bond length for heteroatom compounds.

The book is a most excellent introduction and review of a new and important branch of chemistry and will be of value to the inorganic, organic and polymer chemist.

C. E. H. BAWN

CYCLOBUTADIENE AND RELATIVES

Cyclobutadiene and Related Compounds

By M. P. Cava and M. J. Mitchell. With a Chapter on Theory by H. E. Simmons and A. G. Anastassiou. (Organic Chemistry: a Series of Monographs, Vol. 10.) Pp. xiii+503. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 176s.

EVER since 1865 when Kekulé put forward the cyclic structure for benzene, chemists have been interested in the preparation of homologues of this compound in order to find out whether or not they possess an aromatic character. The next higher homologue of benzene, cyclo-octatetraene, was synthesized by Willstätter in 1911, but the lower homologue, cyclobutadiene, proved to be much more elusive. Despite the discouraging predictions of molecular orbital calculations an ever increasing number of chemists are making contributions to the field of cyclobutadienes and related compounds. Cyclobutadienes have been postulated, with varying degrees of probability, as transient intermediates in several reactions and some very elegant and sophisticated methods have been used to try to prove their existence. The most compelling evidence for the successful synthesis of cyclobutadiene itself was provided by Watts, Fitzpatrick and Pettit in 1965.

The history of attempts to prepare benzocyclobutadiene and biphenylene is also of great interest. Finkelstein was the first to prepare benzocyclobutadiene (as a transient intermediate). He also made the first derivative of biphenylene, namely 5-bromobenzo[a]biphenylene. Actually Finkelstein himself did not elucidate the structure of the biphenylene but his work was repeated and enormously extended by Cava and his co-workers. therefore very appropriate that Cava and Mitchell should write an account of cyclobutadiene and related compounds.

The quest for stable derivatives of cyclobutadiene has led to the preparation of many novel types of small ring compounds and to the discovery of many new reactions. According to the preface: "The aim of this monograph is to present in readily accessible form all of the information available on four-membered carbocyclic compounds having only trigonally hybridized carbon atoms in the ring". Thus there are chapters on cyclobutadiene: the cyclobutadiene-metal complexes; cyclobutadiene divalent ions; cyclobutadienequinone; methylene analogues of cyclobutadienequinone; benzocyclobutadiene; benzocyclobutadienequinone; methylene analogues of 1,2-benzocyclobutadienequinone; higher aromatic analogues of benzocyclobutadiene; biphenylene; benzobiphenylenes; and theoretical aspects of the cyclobutadiene problem. The monograph includes all relevant material published up to January 1, 1964, and there is an appendix containing abstracts of papers published in 1964 and 1965. In addition to published work much information has also been extracted from unpublished theses. The text is excellently written and is profusely illustrated with formulae, diagrams and tables.

The authors have admirably succeeded in their task and the book will be of great interest and value to workers on carbocyclic and on aromatic polycyclic compounds. It will be an essential work of reference for specialists in the field of four membered unsaturated rings and their benzo derivatives.

J. F. W. McOmie

CHEMISTRY OF ALKALOIDS

An Introduction to the Alkaloids

By G. A. Swan. Pp. viii + 326. (Oxford and Edinburgh: Blackwell Scientific Publications, 1967.) 63s. net.

THE problems facing an author who wishes to write an introduction to the alkaloids "suitable for advanced undergraduate and postgraduate students or for others wishing to begin a study of the subject" are daunting indeed. To make a reasonably comprehensive selection from the many classes of alkaloid, to include sufficient detail and thus avoid a sense of remoteness but at the same time to give a satisfactory general picture—these are just a few of the difficulties to be overcome. The present author has tackled these and other problems in a straightforward way and has produced a book which is instructive, up to date, comprehensive and extremely readable. Apart from an introductory chapter and a final chapter on biogenesis, the main body of the book is devoted to a description of the major classes of alkaloid, illustrated with carefully selected examples. The historical background is often described and accounts of classical degradative and synthetic work are complemented by numerous illustrations of the application of modern physical techniques such as ultraviolet, infrared, nuclear magnetic resonance and mass spectroscopy to problems of structure elucidation.

Aspects of the pharmacology of the alkaloids are also included and give added interest to the purely chemical sections.

The final chapter on biogenesis is up to date and very clearly written. The author has shown (for example, in the section on indole alkaloid biogenesis) how biogenetic studies can help to clarify and rationalize the relationships between alkaloids of apparently diverse structural types.

In a book of this nature, it is inevitable that some errors should have crept in—these, however, are very few in number and are usually of minor importance (for example, the formula for chaksine, page 275, which lacks a methyl group). Nowadays, however, when so many publications of this type show obvious signs of having been written in great haste and with little thought, it is a real pleasure to come across one in which the author has combined experience, careful planning, accuracy and style, to produce a book which fully accomplishes the intentions quoted at the beginning of this review.

A book of this kind has been long needed and this volume can be thoroughly recommended (especially in view of the very reasonable price) to everyone interested in alkaloid chemistry, specialists and non-specialists alike.

D. H. G. GROUT

WORLD KEY TO ANGIOSPERMS

Key to the Families of Flowering Plants of the World By J. Hutchinson. Revised and enlarged for use as a supplement to The Genera of Flowering Plants. Pp. vii+117. (Oxford: Clarendon Press; London: Oxford University Press, 1967.) 30s. net; paperback edition 18s. However controversial Dr John Hutchinson's well known system of classification of flowering plants might be, the key to families included in the two editions of his Families of Flowering Plants has proved to be of outstanding value to botanists throughout the world. This latest book is simply a revised and rearranged version of the family key included in the 1959 second edition. The author intends it to serve as a complement to the most ambitious yet of his publications, The Genera of Flowering Plants, of which two volumes have so far been published in 1964 and 1967, respectively. There is no doubt that it will be widely used in the field and herbarium as a working key quite independently from the Genera or any other works. As the author says in the preface, "by means of this key it should be possible to determine the family of most of the flowering plants to be met with on a day's march in any part of the world, from the north and south poles to the equator or from Greenland's icy mountains to India's coral strand".

The book consists almost entirely of the keys. A short glossary is appended and there are two pages of diagrams illustrating terms used in the keys. There are separate keys to the Dicotyledons and Monocotyledons. The Dicotyledons are divided into thirty-two artificial groups, a larger number than in the *Families*, because the main ones are subdivided into smaller units so as to ease the task of identification. Many of these smaller groups are made on the basis of the presence of opposite or alternate leaves. Although the keys are deliberately artificial, Hutchinson cannot refrain from adding that alternate leaves are usually a more primitive character and opposite leaves more advanced.

A disastrous error occurs on page 102 when about half the key to group 1 of the Monocotyledons (about twentytwo lines to judge by the key on page 528 of Vol. 2 of The Families of Flowering Plants) is omitted, so that ten families (Typhaceae, Sparganiaceae, Lemnaceae, and so on) cannot be run down.

Mechanically the keys are good and the contents usually clear. They are not, however, always dichotomous: three or four contrasting leads are common—quite unnecessarily so in most cases—and are often confusing. There are few misprints but seldom significant, except perhaps "Leaves compound of 1-foliolate, pellucid-punctate" on page 14. Another that will baffle foreign readers is "often healthy' plants" in the key to Ericaceae. The punctuation is careless in many places and should be revised in any reprint that is issued.

The keys as a whole represent an intellectual achievement of a very high order. It is difficult to imagine any competing versions being offered in the future, when, in any case, alternative methods of preparation and presentation of keys using electronic data processing will be available. Even today, it would be valuable to have the information given in Hutchinson's key available as a set of punched cards.

The brief glossary is somewhat scrappy and the definitions are often over-simplified: for example, "monoecious, unisexual flowers on the same plant"; "embryo, rudimentary plant still enclosed in the seed"; "pollen, the powdery contents of an anther (the male element of the flower)". It is difficult to believe that any serious user of the key will need to consult such an elementary list. It could well be omitted in a further edition.

Hutchinson's Keys will be a deservedly popular work, and both he and his publishers are to be commended for making it available as a separate and relatively inexpensive book.

V. H. HEYWOOD

OBITUARIES

Sir John Cockcroft

JOHN DOUGLAS COCKCROFT, who died on September 18, was born in 1897 at Todmorden, the son of a cotton manufacturer. He was one of five sons, three of whom entered the family business. Of strong physique, he played games well and loved hill walking and sports of all kinds. He showed an early interest in mathematics and physics during his education at Todmorden Secondary School. He won a scholarship to the University of Manchester at the age of 17 and began to study mathematics there, but the year was 1914 and for the next four years he was on active service in the Royal Corps of Signals and later in the Royal Artillery.

In 1919, after the war, Cockcroft returned to Todmorden and decided to become an electrical engineer. He became a student of Miles Walker at the Manchester College of Technology and a college apprentice at Metropolitan-Vickers, Ltd. Rutherford was at that time working at the University of Manchester but he was shortly to leave for Cambridge. In his years at Manchester, Rutherford had established the nuclear theory of the atom through his studies of a-particle scattering and had gathered around him a brilliant band of young colleagues-Geiger, Marsden, Nuttall, Mosely, Bohr, Chadwick, Robinson, Andrade, Darwin-giving to Manchester the excitement and challenge of entering a new world. Rutherford had demonstrated for the first time that certain light atomic nuclei could be disrupted by the impact of a-particles from radioactive substances; this moment, when Ra-C α-particles were shown to disrupt nitrogen nuclei and eject fast hydrogen nuclei, could perhaps be regarded as the birth of the new and vast subject of nuclear physics. It is not surprising, in retrospect, that the attraction of Rutherford's work drew Cockcroft to Cambridge, first to resume his mathematics and then to join Rutherford at the Cavendish Laboratory. There, in 1924, he was put to work in the laboratory attic by Chadwick, to practise α -particle and proton counting, to produce what then passed for a high vacuum and to make gold leaf electroscopes to measure γ-ray intensities.

After this apprenticeship Cockcroft was diverted to make use of his electrical engineering in helping Kapitza install an alternator which was to be shortcircuited through coils, designed by Cockcroft, to generate magnetic fields of up to 300,000 gauss. Rutherford, in his presidential address to the Royal Society on November 30, 1927, urged "the development of sources of atoms and electrons with an energy far transcending that of α and β particles from radioactive matter" use in scattering and disrupting studies of target nuclei. Allibone started work on electron acceleration and his success in operating accelerating tubes at 300 kV led Cockcroft to think seriously of accelerating protons with similar objectives in view. In November 1928, Gamow paid a timely visit to the Cavendish Laboratory and in a lecture expounded his quantum theory of atomic disintegration according to which a finite probability existed of a-particles penetrating the potential barrier around a Cockeroft calculated that, according to this theory, 300 kV protons should produce about 2×10^6 d.p.m./min/m.amp of protons impinging on boron. He decided to try accelerating protons at a steady voltage of 300 kV and, with E. T. S. Walton, installed a 250 kV transformer, built a voltage doubling rectifier circuit for 300 kV and used this to drive an accelerating tube with a high voltage canal tube as a proton source.

A wide range of targets from lithium to lead were examined under the proton beam but no gamma radiation (arising from proton capture) was observed. enforced move to another laboratory at Cambridge prevented a search for α-particle emission and led them to build a new apparatus for 500-600 kV using a voltage quadrupling circuit of a modified Greinacher type. This took six months and the main problem was that of retaining a high enough vacuum. The low vapour pressure greases and 'Plasticine' and the oil diffusion pumps developed by C. R. Burch at Metropolitan-Vickers played a vital part in the work. After long proving tests and running-in experiments, a lithium target was bombarded with 2 m.amp of protons and they observed large numbers of bright scintillations on a zinc sulphide screen placed outside the apparatus. made it plain that α-particles, and not protons, were responsible. The scintillations increased fortyfold as the accelerating voltage was increased from 126 to 250 kV. This was the first occasion on which nuclear reactions were achieved using artificially accelerated particles, and, according to Cockcroft's account, "there was a certain amount of fuss made by the Press after Rutherford made the announcement of the discovery at a Royal Society meeting and Rutherford told the Press that the idea of obtaining power from nuclei was 'all moonshine' '

The scale of the Cockeroft and Walton experiment, which Cockcroft's electrical engineering knowledge and expertise made possible, took nuclear physics into a region in which the work was beyond the scope of the individual physicist. Their work, together with that of Lawrence at Berkeley, began an age of accelerating machines in nuclear physics typified by the Brookhaven Cosmotron, the Berkeley Bevatron, the CERN and Dubna machines and the Rutherford High Energy Laboratory NIMROD. The work of Cockeroft and Walton, the first in the field to be successful, marks in retrospect a watershed between classical and modern nuclear physics. For their work Cockcroft and Walton were jointly awarded the Nobel Prize for Physics in 1951. In the same year as the Cockcroft-Walton experiment, Chadwick, using the simple and elegant methods of the age that was now to end, proved the existence of the neutron; this event was also of great significance and led directly to the studies of neutron-nuclei interactions that gave rise to the discovery of nuclear fission in 1939.

In 1934, after Kapitza's return to Russia, Cockeroft

took over the direction of the Royal Society Mond Laboratory and he participated in the various developments at the Cavendish Laboratory which led, in 1936. to the construction of a new high voltage laboratory under the leadership of Dee. In particular, Cockcroft was responsible for the design and construction of the 37 in. Cavendish cyclotron, one of the first two in Britain and which operated in 1939 just before the war. Early in 1938, however, Cockcroft was told about the secret developments which had taken place in radar methods of detecting the presence and position of aircraft. informant was Tizard who, together with many others already involved, saw that in the event of war the effectiveness of radar would depend on there being large numbers of trained experts. Cockcroft, who was a leading member of the Cavendish Laboratory, had just the right background in science and electrical engineering to appreciate not only the problems but the way in which they could be solved.

Cockcroft paid several visits to Bawdsey Manor on the Suffolk coast where the Air Ministry and War Office radar research teams were working, and saw one of the chain of long range stations that was already working routinely. He also saw equipment for airborne ship detection, gun laying and other applications of radar. He quickly fostered contacts between the Cavendish and Bawdsey Research Stations; Dee, Feather and Ratcliffe were initiated and Lewis, who was already working on pulse techniques, began to spend some of his time at Bawdsey. During the following months Cockcroft spread a secret net not only at Cambridge but in other universities, and recruited in an informal way more than eighty young academic scientists. They were sworn to secrecy and agreed to go out to the various stations around our coasts to learn the details of the circuits so that they could help to keep the equipment up to the peak of performance. In August 1939 the teams of scientists set out and Cockcroft, leading one of the parties, went to the radar station at Rve.



The expected air attacks on the United Kingdom did not materialize and after a few weeks Cockcroft and his party were sent to Bawdsey Manor to help Butement with an experiment using a 200 cm coast defence set in spotting a submarine allocated by Admiral Sir James Shortly after this successful experiment, enemy submarines began to use the channel between the Shetlands and the Orkneys. Somerville appealed to Watson-Watt for some radar equipment to help in intercepting them. Watson-Watt consulted with Cockcroft, who agreed that he and his team, which included Ratcliffe, Ashmead and Firtel, should organize and take part in the construction (with the help of Messrs Pye and Metropolitan-Vickers), the siting, the installation and the running of several stations.

The anti-submarine stations were constructed and became operational during the winter of 1939-40; this was a remarkable achievement in view of the difficulties of transport to the island sites and the wild weather which hindered the installation and testing. The desired detection of submarines was achieved, but the ability of

the 200 cm radar to see low flying aircraft heading for Scapa Flow proved even more important.

The use of 200 cm sets to detect low flying aircraft was already known but none had been installed in the main radar chain because all the effort had been concentrated on the 13 m early warning sets. In the autumn of 1939 much of the mine laying in coastal waters was being carried out by enemy aircraft at minimum height. There was a desperate need for two installations to cover the Thames, and Cockcroft, already committed to making equipment for the Hebrides, was asked to provide two extra stations. Pye Radio made the receivers and aerials, and assisted in the installation; gun laying radar transmitters were modified at Metropolitan-Vickers to operate on 200 cm wavelength and by the beginning of December 1939 the first station became operational.

From the beginning of radar development it had been clear that many problems would be solved, particularly concerning airborne installations, if sufficient power could be generated at wavelengths of a few centimetres, enabling narrow beams to be produced by small aerial systems. In 1939 Brundrett, who was then chairman of the Inter-Services Committee for Co-ordination of Valve Development, formalized the requirement by placing a contract with Professor Oliphant at the University of Birmingham. The cavity magnetron, produced by Randall and Boot, was so successful that it was judged the most significant item when Tizard, with Cockcroft as his deputy, disclosed it to the United States on the famous mission in 1940. Cockcroft had followed the development and early application work using the cavity magnetron, and on his return from the mission was appointed chief superintendent of the Air Defence Research and Development Establishment at Christchurch, where he supervised the work on 10 cm coastal defence equipment.

While Cockeroft was in charge at ADRDE he fostered many new ideas; for example, Bateman and Shine devised the anti-aircraft proximity fuse which was so effectively used against the VI weapon and in the Pacific war; and Eastwood, Oxford and Chick developed searchlight control by radar. Throughout the radar war, Cockeroft was always willing to take on the heaviest loads of the moment and there is no doubt that he played a vital part.

The early discussions on the possibility of developing an atomic bomb, including the deliberations of the famous MAUD Committee and Cockcroft's involvement in them, have been admirably described by Mrs Gowing1. 1944 he was sent to Canada to become director of the Anglo-Canadian Atomic Energy Laboratory in Montreal. It had been agreed at an earlier stage that, in the circumstances of the war, only the United States could provide the tremendous scientific and manufacturing resources required to make fissile material. Extraordinary efforts were made to achieve secrecy and inevitably key decisions were made in America by Americans. For a time, relations between the United States and Britain on the atomic bomb project became strained and the Anglo-Canadian team in Montreal began to feel isolated. Cockcroft's presence in Montreal and the support he received from Chadwick in Washington gradually effected an improvement in relations with the Americans and under his calm but energetic guidance the laboratory, with its mixed British, Canadian and French staff, acquired a real sense of purpose. The design and development of the 10 MW heavy water reactor were vigorously pursued, a site was selected on the Ottawa River, near Chalk River, Ontario, and construction began in 1946. Chemical processes were also developed for recovery of uranium from the irradiated fuel as well as for separation of uranium-233 from irradiated thorium oxide rods in the reflector. Cockeroft and many of the staff moved from Montreal to the new town site at Deep River towards the end of 1945. By the time he left Canada in the autumn of 1946 the reactor was almost completed, the laboratories were fully

occupied and the town had become a settled community. The NRX heavy water reactor later proved to be one of the most valuable research reactors in the world and became the prototype for further successful developments in Canada.

Simultaneously with the development of the programme in Canada, much thought and effort was devoted to British requirements. It was decided that the British production programme, like that of the United States, should be based on graphite moderated reactors, and the essential design features of a graphite moderated research reactor to be built at Harwell were worked out in Montreal. During this period the pressure on Cockcroft was extremely heavy. He was constantly travelling between Chalk River and Montreal, London and Washington, and besides all the problems of three widely spaced laboratories, he had to contend with a very high rate of staff turnover when people left at the end of the war and new recruits had to be brought in to replace them.

Cockcroft was appointed director of the new Atomic Energy Research Establishment at Harwell while he was in Canada. He was by now thoroughly familiar with the problems of atomic energy and was able to push the work ahead without hesitation. From the beginning he ahead without hesitation. insisted on the minimum of formality and red tape and secured from the Government a large measure of autonomy for the Establishment. Developments at Harwell have already been described2,3 and only a brief account of those

intensively active years need be given here.

The first task of the Establishment was to provide the information required to enable the Industrial Group to build and operate the atomic energy production plants at Under Cockcroft's guidance, progress was rapid; GLEEP and BEPO, the first reactors to be built in western Europe, began operating in 1947 and 1948, respectively, and the first particle accelerator, a 5 MV Van de Graaff machine, came into service in 1947. Research and development on the chemical separation process to be used at Windscale depended heavily on the Chalk River Laboratory until the new radiochemical laboratories opened at Harwell in 1949. By 1952, the initial development work had been successfully completed although much remained to be done, especially in connexion with the new diffusion plant at Capenhurst. Cockcroft then turned his attention to the development of civil nuclear power. He arranged meetings between representatives of the power industry, the Industrial Group and Harwell staff known as the Harwell Power Conferences which laid the foundations for future cooperation with industry. An early outcome of the work was the design and feasibility study of a carbon dioxide cooled natural uranium power reactor known as PIPPA. This study was taken up by Hinton and Owen in the Industrial Group and, after a substantial design and engineering development effort, the Calder stations were built at Calder Hall in Cumberland. This was the first large scale nuclear power station in the world and was the prototype for the MAGNOX series of nuclear stations subsequently built for the Central Electricity Generating Board. The first step in the development of another type of power reactor, the fast breeder, was taken when a zero energy experiment ZEPHYR began to operate at Harwell early in 1954. This was followed by ZEUS, which furnished the reactor physics data for the fast reactor later built at Dounreay.

It was by no means clear in the 1950s that the main reactor types had been identified which would prove to be most economic, and so several different systems were studied at Harwell in this period. These included the pressurized water, homogeneous aqueous, liquid metal and fused salt systems which were being examined in the United States, and two new schemes, one based on gaseous uranium hexafluoride and the other on a high temperature system based on graphite impregnated with fissile material. Work on pressurized water was continued in connexion with marine applications, but the other systems were dropped except for the high temperature (HTR) system.

In 1958 the idea of a joint European reactor project was considered and Cockcroft suggested that the HTR should be adopted. The OECD High Temperature Reactor Project (DRAGON) was started in 1959 at Winfrith as a result of this initiative, and has proved very successful.

Always eager to help universities to benefit from the technology and developments in government laboratories, Cockcroft entered into an agreement with two colleges of the University of London to build a large proton linear accelerator at Harwell "outside the wire" so that security restrictions need not affect collaboration between Harwell and university physicists as equal partners. This idea led to the creation of the National Institute for Research in Nuclear Science in 1957—now the Rutherford High Energy Laboratory of the Science Research Council. This was Cockcroft's own creation and the fulfilment of a dream of what he used to call a "British Brookhaven". He actively promoted international co-operation and was closely concerned in the formation and early growth of CERN, the European nuclear physics laboratory in Geneva, and he was the British representative on the council for many years.

Cockeroft also saw the potential of radioactive isotopes made in reactors for use in industry, agriculture, medicine and research, and he promoted the rapid development of techniques for producing and using them. In a remarkably short space of time a substantial international

market was developed.

The breadth and vision displayed by Cockcroft in developing Harwell from an RAF airfield into one of the great scientific institutions in the world were allied with a deep personal interest in everything that was going on. He was always insistent that basic work, on which future technology would depend, should be vigorously developed and the results published freely in the scientific literature. The basic studies ranged from solid state chemistry and physics to radiation chemistry, neutron physics and nuclear physics. In particular, he saw very early that nuclear fusion might well be a practicable power source in future years and his personal interest in the work led eventually to the setting up of the Culham Laboratory.

Harwell staff were always astonished to discover Cockcroft asking about the progress of work that he had glimpsed in a laboratory visit months or years before. The little black book, in which he noted everything requiring action or that he needed to remember, his microscopic handwriting, and the crisp terse notes, were all regarded affectionately as characteristic of this wise and modest man.

One of Cockeroft's most abiding convictions was that advancement in science, and indeed in human affairs, could only come through international understanding and co-operation. He was tireless in the arduous task of establishing international communications in the field of nuclear science and technology and in encouraging the growth of co-operation between individuals and laboratories in different parts of the world. The series of United Nations conferences at Geneva on the Peaceful Uses of Atomic Energy, and perhaps especially the first. owed a great deal to him.

In 1959, Cockcroft returned to Cambridge as Master of the new college named in honour of Winston Churchill. He accepted with enthusiasm the challenge of creating something new and fine and enduring while at the same time maintaining many of his other active interests. His life was a full one to the end.

Many colleagues have shared in the writing of this obituary. PENNEY

¹ Gowing, M., Britain and Atomic Energy, 1939-45 (Macmillan, 1964).

² Cockcroft, J. D., Nature, 215, 1228 (1967). ³ Spence, R., Nature, 214, 343 (1967).

University News:

London

PROFESSOR R. C. TRESS, professor of political economy in the University of Bristol, has been appointed master of Birkbeck College in succession to Dr F. K. Hare.

Massachusetts Institute of Technology

Professor N. Levinson has been appointed head of the Department of Mathematics in succession to Professor W. T. Martin.

Lagos

PROFESSOR KURT SALOMON has been appointed professor and chairman of the newly created Department of Radiation Biology and Radiation Therapy in the College of Medicine.

Announcements

PROFESSOR D. GABOR has been awarded the Cristoforo Colombo Prize for 1967 by the International Institute of Communications in Genoa.

ERRATUM. Professor R. E. Davies, of the University of Pennsylvania (School of Veterinary Medicine), has written to the Editor to complain that the words "Professor A. V. Hill's Further Challenge to Biochemists" were omitted from the title of his article, "ATP, Activation, and the Heat of Shortening of Muscle", published in Nature, 214, 148 (1967). This change was made in the Nature office because the statement following the title began with the words "Prof. A. V. Hill has challenged biochemists to find whether the heat of shortening of muscle . . .". Professor Davies wishes it to be known that he considers the omitted words "by far the most important part of the title" because he wishes "to honour him [Professor A. V. Hill] by using his name in the title".

The Editor reserves the right to make changes to titles either so as to keep their length within reasonable bounds or so as to make them easier to understand, and if there is any danger of a change of meaning, authors are, of course, informed. The Editor is at a loss to know why Professor Davies has argued so strongly in favour of his original words.

CORRESPONDENCE

First AGR for Scotland

SR,—May I comment on the statements attributed to me in your article "First AGR for Scotland" (*Nature*, 216, 213; 1967)?

1. It was not I, but other witnesses, who claimed in evidence to the Select Committee on Science and Technology that replication could save 10 per cent in the cost of a second station. My views are made clear from Mr Lubbock's question (minutes 381-V, paragraph 515) to me: "I notice that you do not think very much of replication; you do not agree with the figure which has been given to us of a 10 per cent reduction for a Chinese copy of an existing nuclear station".

2. Our views on replication and improvements in design are explained at length in paragraphs 18-23 of our memorandum. The circumstances at the time of the Hunterston tender led us to adopt a policy of replication,

in line with paragraph 19.

3. In comparing Hinkley Point 'B' with Dungeness 'B', I claimed that improvement of design through competition, and not replication, brought down the price by more than 10 per cent (paragraph 516).

4. The construction-cost of Hinkley Point 'B' has been published by CEGB as £92m (£94m including gas turbines). It is misleading to compare these costs with the figure of

£87.5m announced by SSEB for Hunterston, because site conditions and the extent of supply are different.

5. Such comparisons are extremely difficult to make with any accuracy. The best estimate we have been able to make of the relative prices of Hinkley and Hunterston after adjustments for the differences in the two contracts shows a reduction of about 7 per cent for replication. There would be no such reduction for a third station.

6. We now know that our price for Hunterston was the most competitive. It follows that Hinkley, at only 7 per cent higher, must also have been very competitive. It could not have been, as alleged by some irresponsible commentators at the time, £10-£13m too high, and a "national scandal".

Yours faithfully,

S. A. GHALIB (Managing Director)

The Nuclear Power Group Limited, Radbroke Hall, Knutsford, Cheshire.

Assessing the AGR

SIR,—The terms in which you have commented on the latest Annual Report of the Kjeller Laboratory of the Norwegian Institutt for Atomenergi prompts us to seek to add to the views we have already expressed to you.

The survey was an attempt made during 1966 to prepare an economic comparison of various reactor systems. Although it represents the AGR as having slightly higher generating costs than other systems it concludes that "there is no significant difference in the power costs of the thermal reactor types for large stations or high yearly load". Examination of the generating costs given in the report shows that the scatter between reactor systems for any given reactor size is small indeed—mainly under 5 per cent. Even under conditions of competitive tender for plants much more alike than those studied in the report, offers can span a price range several times greater than this. In this study the figures are just buyers' estimates based on a variety of uncertainties:

The AGR figures are based on data supplied by the UKAEA but even so are not a proper substitute for a tender price.

The BWR figures derive from a General Electrical Company of the United States price list issued in 1964 and replaced several times since, prices having increased 20–30 per cent up to the end of 1966.

The PWR costs are based on the same BWR price list.

The BHWR costs are obtained from a computerized projection.

The CANDU costs depend on a scaling assumption applied to Canadian data.

The report emphasizes that the comparison between the various systems will be kept up to date as new data on them are obtained. In view of the comments made at the recent IAEA Symposium in London on the dangers of drawing conclusions from generalized comparisons of reactor costs, this is clearly a wise policy. In this connexion it is relevant to note that, through the medium of the British Nuclear Export Executive, we are discussing the potential in Norway for nuclear power station designs based on British and Norwegian technology with a Norwegian group comprising the Institutt for Atomenergi, Norsk Hydro, NVE and Noratom.

Yours faithfully,

E. H. UNDERWOOD
Director of Public Relations.

United Kingdom Atomic Energy Authority, London.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 13

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Electron Microscopy Group of the Institute of Physics and the Physical Society, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 10 a.m.—Colloquium on "Electron Probe Instrumentation".

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, SW1), at 5 p.m.—Dr M. Elliott: "Structural Requirements for Pyrethrin-like Activity".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr A. R. W. Broadway and Mr G. Thomas: "Single-Unit P.A.M. Induction Frequency Convertors".

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "The Transport of Solids by Gases".

Tuesday, November 14

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 2 p.m.—Colloquium on "Design of High Frequency Transistors with Special Reference to High Power".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, NW1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 5.30 p.m.—Mr R. G. Blake, Mr P. M. Piggott and Mr R. J. Smale: "Optimization of the Control of a Large Plant by Hybrid Computation".

University of London (at Imperial College of Science and Technology, London, SW7), at 5,30 p.m.—Professor J. T. Stuart: "Hydrodynamic Stability of Fluid Flows" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr G. F. Joplin: "The Effects of Y-90 Pituitary Implantation in Man". (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

University of London (at the London School of Hygiene and Tropical Medicine. Keppel Street, Gower Street, London, WC1), at 5.30 p.m.—Dr Arthur Engel: "Perspectives in Health Planning. I, Health Planning in a Changing Society". (First of four Heath Clark Lectures.)*

ROYAL STATISTICAL SOCIETY, GENERAL APPLICATIONS SECTION (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, WCI), at 6 p.m.—Mr F. E. Whitehead and Mr P. G. Gray: "The 1966 Sample Census".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at the Woolwich Polytechnic, Wellington Street, London, SE18), at 6.30 p.m.—Mr D. Wilkinson: "Satellite Communications".

Tuesday, November 14-Thursday, November 16

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS and the INSTITUTION OF ELECTRICAL ENGINEERS (at the National Physical Laboratory, Teddington, Middlesex)—Conference on "R.F. Measurements and Standards".

PLASTICS INSTITUTE (at the Kensington Close Hotel, London, W8)—Conference on "Advances in Packaging with Plastics".

Wednesday, November 15

BRITISH INSTITUTE OF RADIOLOGY, and the HOSPITAL PHYSICISTS' ASSOCIATION (at the Middlesex Hospital Medical School, Cleveland Street, London, W1), at 10 a.m.—Meeting on "The Design of Counting Systems for Dynamic Studies and Uptake Measurements".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Atomic Energy Research Establishment, Harwell, Didcot, Berks), at 10.15 a.m.—Meeting on "Some Aspects of Inorganic Trace Element Analysis".

ROYAL INSTITUTION, HISTORY OF SCIENCE DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Mr D. Chilton: "X-rays—a Case History in the Immediate Application of a Scientific Discovery".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (in the Main Lecture Theatre of the Physics Department, Imperial College, London, SW7), at 2.30 p.m.—Symposium on "The Carbonyl Group".

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, SW1), at 3 p.m.—Professor Dr Hatsuzo Tani: "Manoeuvring Mammoth Ships".

ROYAL STATISTICAL SOCIETY (in the Botany Theatre, University College London, Gower Street, London, WC1), at 3 p.m. and 5.15 p.m.—Discussion Meeting on "British Official Statistics", Part 1: "Social and Medical Statistics".

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, SW3), at 5 p.m.—Dr F. J. Prime: "The Atmosphere".*

Society of Instrument Technology (at the A.E.I. Cinema, 33 Grosvenor Place, London, SW1), at 5.30 p.m.—"The Heat Motor".

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, WC1), at 6 p.m.—Professor R. W. Ritchie Russell and Professor P. N. Campbell: "Nucleic Acid Metabolism in Neurology—Clinical and Biochemical Aspects".*

Institute of Information Scientists (at Knightway House, 20 Soho Square, London, W1), at 6.15 p.m.—Mr A. G. A. Pickford: "An Objective Method for the Generation of an Information Retrieval Language".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (Joint meeting with the Food Group, at the School of Pharmacy, Brunswick Square, London, WC1), at 6.15 p.m.—Dr K. Kaindl: "Irradiation of Fruit and Fruit Julees".

INSTITUTE OF FUEL (at the Central Mess, Duke of York's Headquarters, Sloane Square, London, SW3), at 6.30 p.m.—Mr T. Beswick: "Developments in Nuclear Power Generation".

Wednesday, November 15-Thursday, November 16

POWDER METALLURGY JOINT GROUP of the IRON AND STEEL INSTITUTE and the INSTITUTE OF METALS (at Church House, Great Smith Street, London, SW1)—Tenth Anniversary Meeting.

Thursday, November 16

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 1.20 p.m.—Dr R. C. Fisher: "Parasitic Insects".*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m.—Professor George Porter, FRS: "Molecules" (Civil Service Lecture).

SOCIETY FOR ANALYTICAL CHEMISTRY, AUTOMATIC METHODS GROUP (at the Wellcome Building, Euston Road, London, NWI), at 3 p.m.—Second Annual General Meeting, followed by a meeting on "Automatic Methods in the Fermentation Industry".

ROYAL SOCIETY (at 6 Carlton House Terrace, London, SW1), at 4.30 p.m.—Mr P. V. E. McClintock and Mr H. M. Rosenberg: "The Interactions of Spins and Lattice Vibrations at Low Temperatures"; Mr D. M. Edward and Mr E. P. Wohlfarth: "Magnetic Isotherms in the Band Model of Ferramagnetism".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W1), at 5 p.m.—Professor V. H. Heywood: "Scanning Electron Microscopy and the Use of Micro-Characters in Taxonomy (with special reference to the Classification of the Umbelliferae)"; Dr Patrick Echlin: "The 1 se of Scanning Reflection Electron Microscopy in the Study of Plant Material".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W1), at 5 p.m.—Annual General Meeting, followed by Professor G. Higman: "Odd Characterizations of Simple Groups". (Presidential Address.)

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W1), at 5.30 p.m.—Mr J. E. Warren: "Optimization of Production—Storage-Export Systems".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Are Component Specifications being Improved?" opened by Mr R. H. W. Burkett, Mr J. D. Hinchcliff and Mr D. S. Girling.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr G. S. Wilton, Mr T. S. Ormiston and Mr R. A. Alian: "Modern Transmission Line Maintenance".

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr B. Creamer: "Paneth Cells". (Tenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5.30 p.m.—Dr Arthur Engel: "Perspectives in Health Planning. II, Statistics and Health Planning". (Second of four Heath Clark Lectures.)*

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Cleveland Street, London, W1), at 5.30 p.m.—Professor Bo Holmstedt: "The Use of Gas Chromatography and Mass Spectrometry in Studies of Drugs and Metabolites".*

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, SW1), at 6 p.m.—Mr S. G. Campbell: "Developments in the Manufacture and Application of Road and Airfield Marking".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Hendon College of Technology, The Burroughs, London, NW4), at 6.30 p.m.—Dr S. Jones: "Automatic Control in British Railways".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at the School of Pharmacy, University of London, Brunswick Square, London, WC1), at 7 p.m.—Meeting on "The 'Sainsbury' Report".

ROYAL TELEVISION SOGIETY (in the Conference Suite, 70 Brompton Road, London, SW3), at 7 p.m.—Mr Quentin Lawrence: "Whither Television? a Director's Look at Television Engineering".

BRITISH INSTITUTE OF RADIOLOGY (at 32 Welbeck Street, London, W.1), at 6 p.m.—Discussion Group Meeting. 7.30 p.m.—Professor W. V. Mayneord: "Radiation Carcinogenesis" (Mackenzie Davidson Memorial Lecture).

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1), at 7.30 p.m.—Laboratory Meeting. Chairman: Professor P. C. C. Garnham, FRS.

Friday, November 17

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Dr F. Wilkinson: "Photochemistry of Duroquinone".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr E. Cohen: "Hall Effect Transducers".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr L. Solymar: "Josephson Junctions".

SOCIETY FOR ANALYTICAL CHEMISTRY, THERMAL ANALYSIS GROUP (at the Geological Society of London, Burlington House, Piccadilly, London, W1), at 6 p.m.—Third Annual General Meeting followed by Dr R. C. Mackenzie: "Differential Thermal Analysis—Whence and Whither?"

ROYAL INSTITUTION (at 21 Albemaric Street, London, W1), at 9 p.m.—Mr Huw Wheldon: "Television and the Arts".

Saturday, November 18

BIOGHEMICAL SOCIETY (at the Medical Research Council Laboratories, Carshalton, Surrey)—476th Meeting. Colloquium on "Toxic Substances and Brain Metabolism", and an Ordinary Meeting for the presentation of communications.

Monday, November 20

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr W. D. Humpage and Dr T. N. Saha: "Digital Computer Methods in Dynamic Response Analysis Turbogenerator Units".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WCI), at 5.30 p.m.—Dr Arthur Engel: "Perspectives in Health Planning. III, Mass Screening for Asymptomatic Disease as a Public Health Measure". (Third of four Heath Clark London 1988) Clark Lectures).*

SOCIETY FOR ANALYTICAL CHEMISTRY, RADIOCHEMICAL METHODS GROUP (at the Pharmaceutical Society, 17 Bloomsbury Square, London, WC1), at 6 p.m.—Annual General Meeting, followed by Dr P. G. Jeffrey: "The Application of Isotope Neutron Sources in Analytical Chemistry"; Dr K. Ansell: "Isotopic Radiation Sources for X-ray Analysis"; Mr J. Johnston: "Bremsstrahlung Methods in Industry".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

TEMPORARY ASSISTANT LECTURER IN PSYCHOLOGY—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (November 15).

CHIEF LABORATORY TECHNICIAN in the ZOOLOGY SECTION of the DEPARTMENT OF BIOLOGICAL SCHENGES—The Staff Officer, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire (November 17).

LECTURER (medically or scientifically qualified candidate) in the DEPARTMENT OF CHEMICAL PATHOLOGY—The Registrar and Secretary, The University, Leeds, 2 (November 18).

CHAIR OF APPLIED MATHEMATICS—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (November 21).

ASSISTANT LECTURER (preferably with qualifications to undertake teaching and research in one of the following fields: physiology with special reference to cellular biology; population genetics) in Zoology at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, November 24).

DEMONSTRATOR (nonours graduate in an appropriate biological subject) in Histology in the Department of Zoology—Professor J. M. Dodd, Department of Zoology, The University, Leeds, 2 (November 25).

HEAD (with high academic qualifications and considerable research experience) of the Envomology Section—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (November 25).

LECTURER IN STATISTICS in the DEPARTMENT OF SPATISTICS, School of Mathematics—The Registrar, The University, Newcastle upon Tyne, 2 (November 27).

LECTURER of ASSISTANT LECTURER (with postgraduate experience in the field of higher plant genetics, and preferably some interest in plant breeding) in Genetics, the Facility of Strathchyde, George Street, Glasgow, CI (November 30).

LECTURER (physiciat, preferably with theoretical or experimental research interests in lasers or plasma physics) in the DEPA

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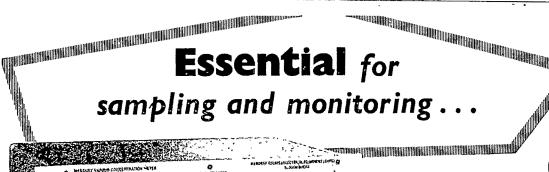
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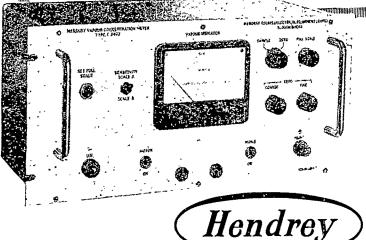
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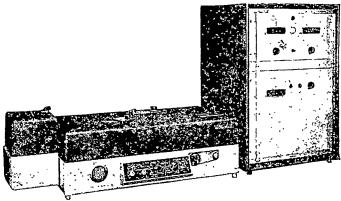
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No More Coal

THE White Paper on fuel policy on which the Ministry of Power has been brooding for the best part of a year has finally appeared, and the delay is to a large extent justified by the quality of the document (see page 628). The ministry has done its best to explain the steps which have led to its new policy as well as the principles on which it will try to hold the ring between the four competitors in the fuel market-coal, oil, natural gas and nuclear power. The ministry has also done a good deal to make an honest woman of the coal industry, which is something to be pleased about. It does not follow, of course, that the ministry will be able to forget about fuel policy for the next few years. The new document is not all that persuasive, and there are bound in any case to be continuing rows about the rate at which the coal industry can be allowed to decline. But at least it is now possible to know with quite exceptional clarity where the British Government stands on fuel policy.

For practical purposes, the new policy runs for the next five years or so. Because of the unavoidable delay between a decision to build a new pipeline or a new power station and the actual appearance of this machinery in service, it is, however, inevitable that the policy now laid down will have an important influence on the pattern of energy consumption in Britain in the middle seventies. The most striking decision is to attempt deliberately to exploit North Sea gas as quickly as possible. The calculation is that by 1975 natural gas will contribute the equivalent of 50 million tons of coal a year to the fuel economy, or 15 per cent of all the energy then consumed. By that time, nuclear power will generate the equivalent of 35 million tons of coal a year. The contributions of oil to the fuel economy will grow, but less quickly than in the past, and the Government has once more shrunk from an honest appraisal of the tax on fuel oil. But in circumstances like these, it is plain that something must decline. The ministry calculates that coal will contribute 120 million tons a year to the fuel economy in 1975-a third of all the energy consumed and two-thirds of the amount of coal produced in Britain in 1966. The ministry makes no secret of the fact that the use made of coal in 1970 is in part determined by the need not to allow the industry to decline more rapidly than is socially acceptable.

Curiously enough, the White Paper is least explicit on its most important decision—to make the fullest use of natural gas. To be sure, the ministry does explain that it has tried to balance its wish to win the greatest economic value from natural gas, which argues for slow exploitation where gas is relatively cheapest, and its wish to obtain the greatest benefit for the economy as a whole, which argues for rapid exploit-On the whole, the Government has plumped for the second course, which is understandable enough, particularly at a time of chronic economic crisis. The trouble is that the scale of investment in natural gas will not bring much price advantage in the decade ahead to those who actually pay for the privilege of burning gas. Without more details than are in the White Paper, there is at least a case for thinking that a middle course on gas would have been preferable. Possibly the ministry will be more forthcoming after it has struck a bargain with the oil companies about the price for natural gas. It may even be that the scale of exploitation is in part at least determined by the hope that oil companies will be more willing to settle for cheap gas if they can look forward to a rapid return on their investments.

The new policy on fuel says nothing new about the future of nuclear power. For practical purposes, there will be something like 10,000 megawatts of nuclear generating capacity at work in 1975, and most of this will have been begun two years from now. If it had not been for the decision to support coal at an artificially high level in the years immediately ahead, or if the British economy as a whole had grown more quickly, there might have had to be an acceleration of this programme. As things are, the interesting question is to know what should happen after 1975. The White Paper has a telling calculation of the natural rate of return on the extra capital involved in building nuclear power stations instead of power stations which burn coal. According to the price of coal, the rate of return from advanced gas cooled reactors is calculated at somewhere between 13 per cent and 33 per cent a year even when the electricity industry pays a royalty on the power stations to pay for past and future development. It would be hard to think of other fields in which the Government could secure such a splendid return on the investment of public funds. A boom in nuclear power in the late seventies is almost unavoidable.

The future of coal is necessarily the most controversial part of the new policy, if only because of the social and even political implications of the rapid decline now openly recognized. In the next five years the industry will lose 35,000 men a year, or 10 per cent of its strength each year. Mr Richard Marsh, the Minister of Power, is certain to be handled roughly by those

who would postpone this inevitable economic process, which is why it is a pity that he has not elected to be hung for a sheep and not just a lamb. It is also unfortunate that he has not included in his new statement some echo of the declaration in the House of Commons last July that in the seventies the competing fuel industries will have to find their own level by price competition in the open market.

But what should be the Government's policy on coal? This is where the ministry may have been too much distracted by the enthusiasm with which it has recently been converted to the doctrine of discounted cash flows as a means of comparing the value of alternative investments. The plain truth is that the market for coal in the 1980s will consist simply of two kinds of customers—the steel works which use coal as a chemical reducing agent and those electricity power stations which, for geographical reasons or simply because they happen to exist, can economically make use of the fuel. There may also, of course, be private persons who continue to burn coal in their houses more out of nostalgia for what will then be the past than as a means of keeping warm. In other words, with luck, Lord Robens' successor in the mid seventies may have to conjure with a natural demand for coal of between 50 and 75 million tons a year. In these circumstances the courageous policy is not so much to fix an acceptable rate of decline for the years immediately ahead as to plan deliberately for what should be a continuing reduction of the scale of the coal industry in the next two decades.

The question is important not merely because coal employs 370,000 miners but also because problems of industrial obsolescence will be increasingly frequent in the years ahead. It is also plain that the concentra-

The Future for Coal

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"This is a very complex subject," said Mr Richard Marsh, Minister of Power, warming to the task of presenting his Fuel Policy White Paper on November 14. Complex it may be, but this has not deterred the mining industry, and its supporters, from protesting very loudly about the rapid run-down of the mining industry. Mr Marsh was wise enough not to ignore their fears, but he did say that the manpower figures quoted for 1980—and inspired by the Coal Board—had "no validity whatsoever". "It's impossible to produce serious figures this far ahead." On the contrary, the coal mining industry would remain one of the biggest industries in Britain, producing 120 million tons in 1975, just over a third of Britain's total energy requirement. The claim that the ministry was "slashing the coal industry" could not be sustained in the light of the White Paper. Mr Marsh admitted that the figures would probably be wrong-"Every estimate of coal burning since the War has been too high"-but they were indications of the way things were going, and they could readily be adjusted if necessary.

What the White Paper does is to re-work the estimates made in a previous white paper in 1965. Since then, the emergence of nuclear power as a cheaper source of electricity than coal, and the discovery of

tion of the coal industry in particular parts of Britain means that social problems are not thinly spread about the country but, rather, concentrated painfully. How then to deal with them? The Government has accompanied its new policy for fuel with a number of schemes for softening the blows that will fall in mining communities in the years ahead. There are to be pensions for miners over 55 thrown out of work by the closing down of collieries, and other financial devices operated through the National Coal Board. should go some way to sweetening the bitter pill the miners will have to swallow, but they will of necessity only comfort the older part of the mining population. Indirectly, however, the industry as a whole will benefit from the way in which the Government is arranging that the industry's need of capital investment in the years ahead shall be provided almost entirely from public funds and not out of the industry's own revenue.

By themselves, however, these measures will only scratch the surface of the social problem of a rapidly declining coal industry. The other half of the Government's policy is to offer further incentives for industries to establish themselves in mining areas. But the Government should pay still more attention to the need for a vigorous retraining programme for people thrown out of work by technical change in industry. Given the scale on which the Ministry of Power now hopes to win economic benefit by a shift of emphasis in the fuel industries, it would not be unreasonable if several tens of millions of pounds a year were spent on retraining programmes for miners. For retraining is in its way an investment, just as is the building of new pipelines or new power stations. By comparison, increased doles are merely a palliative, necessary though they may occasionally be.

natural gas in the North Sea, have overturned the assumptions which it was then possible to make. Despite this, and an appendix to the White Paper which argues the case for nuclear power in terms which might almost convince Lord Robens, no increase is being made in the second nuclear power programme—it will still be 8,000 MW by 1975. By then, natural gas will represent 50 million tons of coal equivalent, and oil will be the major source of power, providing 145 million tons of coal equivalent.

Until 1970, Mr Marsh said, the gas and electricity industries had been persuaded to burn an extra 6 million tons of coal a year. The extra cost of this, £45 million, would be met by the Government and not the consumer. Foreign coal will continue to be banned, and oil will continue to be taxed. To alleviate hardship in the industry, the Government is proposing new legislation—the Coal Industry Bill—which will increase the amount the Government contributes towards the costs of redundancy or redeployment. Men between the ages of 55 and 65 who lose their jobs will get a high proportion of their previous wage for three years, or until they are 65. After this they will get their mineworker's pension immediately. There are also to be steps to encourage new industry to areas where pits

will be closed. The Board of Trade is establishing "super-development areas" within existing development areas. The attractions for industry will be even greater—a Board of Trade factory rent-free for five years, for example. Building grants and loans at moderate rates of interest will also be available. This will be costing a total of £100 million in the period up to 1971.

What emerges from all this? First, the coal industry will continue to contract, although the special arrangements will slow the process down in the difficult years up till 1970. Natural gas will go ahead fast, and oil will continue to expand, whatever obstacles are put in its way. Nuclear power will do no more than hold its own, at least until 1970. After that, Mr Marsh may be able to get back to the cheap fuel policy he set his heart on when he first became minister.

Nuclear Confusion

THERE is increasing impatience in industry, and in the Atomic Energy Authority, over the Government's reluctance to announce new plans for the organization of nuclear power in Britain. For the past six months it has been clear that some sort of reorganization is inevitable, but it is still doubtful what form it will take. Part of the responsibility for the delay must be laid at the door of the Select Committee on Science and Technology, which will publish on November 22 its report on the nuclear power industry. The Government is using the report as an excuse, if not for inaction, at least for declining to comment.

Reformers divide into two schools. One, led by the Atomic Energy Authority, favours the setting up of a central design authority, which would do research and design new stations. Individual parts of power stations would be put out to tender, but the formal structure of the consortia would be broken down. Overseas the AEA would tender for contracts, and would be responsible for the export work now done by the British Nuclear Export Executive. Only one consortium, Atomic Power Constructions Ltd, favours this solution.

The other two consortia, and the Central Electricity Generating Board, favour an arrangement which retains at least an element of commercial competition. (The AEA sometimes argues that competition from the USA is going to be more than enough to keep nuclear power in Britain on its toes.) It is generally accepted that the number of consortia would be reduced to two, possibly by a merger between Atomic Power Constructions and Nuclear Design and Construction. The two ministries involved, the Ministry of Technology and the Ministry of Power, agree that discussions have been going on between them, but decline to say what, if anything, has been decided. The Minister of Power, Mr Richard Marsh, did agree with the select committee that the question of the single design authority was crucial. "It is one of the biggest arguments involved in this particular issue."

But this was as long ago as June. Since then, little has been done to clarify the position. It is unlikely, though, that this silence can be taken as evidence of a division of opinion between the two ministries. Reports that the Government is preparing legislation to compel the formation of one central design authority are also heavily discounted in Whitehall. No mention

was made of such legislation in the Queen's Speech, and it would be very hard to find room for it in the parliamentary calendar. But if the idea of a single design authority has found favour, it is hard to see how it could be imposed except by legislation. The only alternative would be to force the consortia into line by an edict from the Central Electricity Generating Board, which buys the stations. If the buying policies of the CEGB were manipulated, then there is no doubt that a single design authority could emerge. The CEGB need only say that in future it would buy stations only from the AEA, and the consortia would soon be forced to knuckle under. Unfortunately for supporters of the idea, the CEGB has not always seen eye to eye with the AEA, and does not approve of the idea of a monopoly design authority. Thus if the Ministry of Technology is really wedded to the idea of a single design authority—and there is some evidence that it is not-it may have to force the idea through the CEGB.

Barriers to Collaboration

EXPORT efforts by British firms are often hindered by tariff barriers. One possible answer is to collaborate with foreign firms and either set up partly owned subsidiaries in the countries concerned or let the foreign companies make the product under licence. Although this sounds simple, Mr C. de Hoghton, author of a report called *Cross-Channel Collaboration* published by Political and Economic Planning (PEP), points out that there are many pitfalls because of different licensing laws, language barriers, the difficulty of finding the right company and the lack of comprehensive advice. Provided the company's outlook is realistic, however, it should not find collaboration with a European firm intrinsically more difficult than collaboration with a firm at home.

Mr de Hoghton points out that there is a profusion of sources of advice and assistance, but these sources are not used because firms are unaware of their existence. In some cases they offer only partial advice and help and they are distrusted, or the companies fail to recognize the need, in certain circumstances, for outside assistance. He recommends that an Institute of Industrial Licensing should be set up as a competently staffed clearing-house to assist firms in the profitable exploitation of their know-how abroad. A pilot scheme, limited to Western Europe, might be run by the British National Export Council.

Among the other recommendations Mr de Hoghton makes are that it should be standard drill for companies to comb through their product lines and their research and development to identify items which could be licensed abroad or exploited by other appropriate means in foreign countries. Both Government and private bodies should give greater publicity to the advantages of collaboration, and especially of collaboration with European firms. Consultants should concern themselves more actively with the whole range of issues raised by collaboration. Trade associations should consider what additional help they could give members who want to collaborate and should always become members of international European associations where they exist, seeking to be admitted as observers to those European associations limited to the EEC.

Few Bouquets for BEA

The committee of inquiry set up in July to examine the civil air transport industry in Britain was sent on its way last week by a report sharply critical of civil aviation in Britain. The report came from the Select Committee on the Nationalized Industries, which spent the last session of Parliament examining British European Airways. Judged by the opinion some people have of BEA, the report was comparatively mild, but there were distinct undertones of criticism. "Whether or not BEA have been slow to change, Your Committee are satisfied that they are changing now . . . It would be churlish indeed if Your Committee's only reaction to new developments were to ask why they were not introduced before. . . . They congratulate BEA on introducing the new methods, and accept them as indications that BEA's management is not at present static or lacking in ideas."

But BEA was not alone in being criticized. In particular, the committee commented on the division of function by which the Ministry of Technology is responsible for sponsoring new aircraft, while the Board of Trade administers civil aviation. As a result, the report says, the formal channels provide for BEA to have contact only with the Board of Trade, while the manufacturers have contact only with the Ministry of Technology. This tortuous link makes it harder for BEA to be heard when decisions are being made at the Ministry of Technology, particularly when the ministry is arguing the specification of the European airbus, which BEA will be expected to buy.

On the other hand, BEA should not have too much say in the design of sircraft. "Tailor-made" aircraft, like the Trident, were difficult to sell abroad—although the Viscount, another BEA aircraft, had been very successful. "Hawker-Siddeley, who agreed to change the specification of the Trident to suit BEA, had regretted it. This part of the report has an important bearing on BEA's current campaign to get approval for the building of the BAC 2-11. BAC first put the idea forward in February 1967, and immediately BEA put in an application for sanction to buy it. At the same time, the airbus negotiations were in progress with France and Germany. If BEA was forced to operate the Trident 3B, another possibility, it would need government compensation (a poor comment on an aircraft designed specifically for BEA's own needs), and if it was obliged to operate with VC 10s, it would need "cnormous" compensation. There the matter stands. BEA is still hopeful of getting the BAC 2-11, and the Government is still committed to the airbus, although Lufthansa, the West German national airline, seems to be having doubts.

The committee was rather more cheerful about BEA's financial record. In the five years from 1963-4, BEA will have reached its target set in 1963, and the committee welcomes this. As for licensing, the committee believes that the Air Transport Licensing Board, set up to grant licences to independent airlines, has become no more than a body from which to make appeals to the Board of Trade. The airlines themselves, the committee says, should be allowed to set their own fares, subject, if necessary, to a reference to the Prices and Incomes Board. Without its licensing or farefixing function, the ATLB would become only a consumers' council. The committee did not think that a

sufficient justification for its continued existence; it should be wound up.

Ceramic Mirror at Siding Spring

A £240,000 contract has been placed with Owens-Illinois of Toledo, Ohio, for the mirror blank for the 150 inch Anglo-Australian telescope which is to be constructed at Siding Spring Mountain in New South Wales. The order was placed by the Science Research Council, acting on joint Anglo-Australian behalf, and follows a decision taken at the first meeting of the Joint Policy Committee in August. The fifteen ton blank, to be delivered in about twelve months time, will be made of a glass ceramic material called 'Cervit'.

'Cervit' materials are produced from glasses of a special composition which are melted and formed in a manner similar to that used for conventional glasses. After these special glasses have been produced in the desired shape, they are subjected to a high temperature heat treatment during which the glass is gradually converted into a micro-crystalline ceramic material through the growth of crystals: the final product is homogeneous. By controlling nucleation and crystal growth, it is possible to produce very small and precisely controlled crystal sizes. Certain selected elements such as aluminium and lithium are incorporated into the crystal lattice, thus enabling the properties of the crystallized glass to be altered so that the expansion coefficient is reduced to very near zero and so that the change in expansion coefficient with temperature remains small over a wide temperature range. The telescopic mirror blank has adequate transparency for examination of defects within the blank and also has excellent polishing qualities. Furthermore, this mirror blank material has a higher thermal conductivity than fused silica and is more dense. The material is also considered stiffer than fused silica and should therefore display less sag across large edgesupported sections than the same shape made of fused

type is that it can be made in a mould in one piece.

Construction of the 150 inch telescope will take about six years. The capital and recurrent cost of the telescope, which is intended for the equal use of UK and Australian astronomers, will be shared equally by the two governments.

silica. The main advantage, however, of a blank of this

New Electrical Giant

MR ARNOLD WEINSTOCK has just managed to hang on to his reputation as one of Britain's more successful businessmen. Mr Weinstock is managing director of the General Electric Company, which last week took over Associated Electrical Industries after a brisk battle. But Mr Weinstock was twice forced to raise his offer, and AEI was able to put up an unexpectedly vigorous defence. After it was all over, things became very chummy, and Mr. Weinstock asked three AEI directors to join the board of the new company. Only Lord Beeching, called in by AEI as a sort of managerial deus ex machina, was left without work. But he is a deputy chairman of Imperial Chemical Industries, which should be enough to keep him happy until something better comes along.

It is tempting to see the takeover as a coming together of opposites—GEC sleek but technically suspect.

AEI engineers par excellence, clumsily managed. Neither company, of course, would accept more than one of these generalizations. The new company will be among the giants of the international electricity industry, although there are several in Europe larger. The Dutch Philips group, for example, is more than half as large again as GEC/AEI in terms of turnover. In the United States, there are five larger companies, headed by General Electric (USA)—no relation to GEC—with a staggering turnover of £7,239 million last year, against £445 million from GEC/AEI. In Britain, only the joint English Electric/Elliott Automation group comes anywhere near the new group—its turnover last year was £334 million.



Mr Weinstock; still cheerful.

There are good reasons for hoping that the size of the new group will bring dividends. Certainly, shareholders will be hoping so, for after the confident predictions of both sides during the battle anything less than complete success is going to feel like failure. Both companies are untidy conglomerates, spreading their activities over a wide range, although GEC opted out of the heavy end of the business several years ago. One of the problems will be deciding what to do with AEI's enormous cable interests, which make up 25 per cent of the company. In other fields the merging of the two companies will be more like a normal process of rationalization, at which Mr Weinstock has shown himself adept. Telecommunications, for example, is ripe for brisk reorganization, and it looks as if AEI's investment in rather dated Strowger electro-mechanical exchanges-which the GPO still needs-will fit in nicely with GEC's work on smaller electronic ex-In transistors and semiconductors GEC changes. has a minority interest in Associated Semiconductor Manufacturers (with Mullard) and AEI runs a joint company with Thorn. Apart from this, the companies have substantial interests in radar, automation and process control, transformers and switchgear and domestic appliances.

As befits companies in such advanced fields, both run extensive research activities (see *Nature*, 216, 8; 1967). These will no doubt feel the effects of rationalization too, but this week Mr Weinstock had nothing to say. The electronics and telecommunications interests may well offer the best chance of saving money by integrating research, but it is doubtful whether anything has been decided yet.

Small Change on Degrees

from our Oxford Correspondent

Last week, Congregation at Oxford resolved, by a narrow margin of votes, to change the university's somewhat anomalous mode of classifying BAs. At nearly all the other universities of Britain, degrees are divided into three classes, the best and worst 10 per cent of students receiving firsts and thirds, and the intermediate 80 per cent being subdivided into upper and lower divisions. At Oxford, four classes have been awarded. Last year, the proportion of firsts was the same as in other universities, at 10 per cent. But there were fewer seconds (60 per cent) and far more thirds (28 per cent). Fourths are rarely awarded, and Congregation voted unanimously to abolish the class.

So what distinguishes the Oxford system is the large size of the third class and the lack of subdivision of the second. The proposers of the resolution before Congregation claimed that some students who received thirds at Oxford, and thereby are financially penalized in certain professions, would have received lower second class degrees elsewhere. This may possibly be true, though account should be taken that few undergraduates drop out of Oxford, while at some universities large numbers of students leave who would otherwise go on to swell the numbers of the third class. But if it is true that an Oxford third could have been a London lower second, the remedy is simple: award more seconds.

The argument for the division of the second class is that those awarded a second vary in ability from the borderline firsts to those who narrowly managed to avoid thirds; that, in fact, the class designates everyone except the very best and the very worst, and is meaningless as a classification. But the nature of the normal curve is such that it is far more difficult to judge fairly who is just above the average than to distinguish the very best and very worst students from the rest. There is also the question of postgraduate studies. Whether or not a student can stay on for a postgraduate course depends on the availability of grants: if he has a first there is no problem; if he has a second, then the matter will rest on his tutor's recommendation and that of his head of department. So the really important classification is not a formal one. Those few employers, notably the Civil Service Commissioners, who draw a distinction between good and bad seconds, can always be informed of the nature of a candidate's degree by his tutor.

Nevertheless, by 101 votes to 99, with a number of abstentions, Congregation has decided to change the degree classes—a narrow vote on a decision that will alter little; still, Oxford may have cause to regret it. Flexibility, by which is meant indeterminacy, is adduced in defence of Oxford's various institutions quite as often as "democracy", and with as little justification in most cases. But in the degree arrangements, perhaps flexibility worked.

Planning Families

1968 has been designated Human Rights Year by the United Nations, and the International Planned Parenthood Federation (IPPF) has responded enthusiastically to the call. The federation, fortified by the belief that knowledge of contraception is a fundamental human right, has just announced that the estimated expenditure for 1968 is over £2 million, double the 1967 figure. Voluntary associations for family planning in fifty countries make up the federation, with support from the governments of Britain, Sweden, Denmark, Holland, Norway and the USA, as well as charitable During the coming year effort will be institutions. concentrated in areas where there are no government supported programmes, particularly in Latin America, where population is growing faster than in Asia, despite high abortion rates. If the present growth rate continues, the population of India will have trebled in under 50 years, while that of Brazil will have quad-

It is hoped that sufficient funds will be raised to make individual grants to twenty-one Latin American countries, the largest going to Brazil, to equip clinics and train personnel, as well as to provide education programmes which are essential if the schemes are to be successful. Mobile clinics are planned for various parts of the world, particularly in Kenya and West Africa, and—the nicest touch of all—a riverboat will take family planning to communities round the waterways of East Pakistan. The Indian offer of money for men willing to be sterilized has sometimes been described as bribery, but last week Lady Rama Rau, president of IPPF, said the benefit—only about £1—is given to make up for the lack of hospital facilities.

As well as work on a practical level, the IPPF finances a Basic Sciences Committee to discuss and evaluate the laboratory research which is in progress on the various aspects of human reproduction and its prevention. Professor A. S. Parkes, formerly professor of physiology of reproduction at Cambridge University, has been appointed chairman of this committee. Two new groups of specialists have been established to cover educational matters. One will investigate family planning in the teaching of doctors and nurses, and the other will advise on the best methods of bringing knowledge of family planning to organizations and individuals in different parts of the world.

Human Development

A CENTRE for Advanced Study in the Developmental Sciences is being established near Oxford, for the promotion of interdisciplinary communication and growth of knowledge in the behavioural sciences. Dr A. Ambrose, formerly at the Tavistock Institute and now director of the centre, has been largely responsible for the project from its beginnings, and has donated Minster Lovell Mill which will house the centre By providing a specialized library and facilities for study groups, individual study and short courses, the centre will allow specialists from all parts of the world and from all disciplines to meet and exchange ideas on human development and the factors which are likely to cause deviation from normal behaviour. No basic research will be carried out at Minster Lovell, but fellowships will be awarded for

research to be carried out wherever suitable material is available. Proceedings of study group discussions will be published to bring the cross-fertilization of ideas to a wider audience.

Considerable building and alteration work is being done at the mill to provide accommodation for $3\overline{0}$ people, as well as a library and conference rooms, but meanwhile the centre is beginning its scientific activities in accommodation provided by the Ciba Foundation in London. The first study group is being held this week on the functions of stimulation in early postnatal development, and is being attended by 15 people from all parts of the world. The centre, which will be controlled by a specially set up Developmental Sciences Trust, will be managed by an executive council consisting of Professor Brian Foss, Dr Ronald Mackeith, Mr John Marsh, Dr Christopher Ounsted and Dr Gordon Wolstenholme. The scientific advisory panel, constituted on an international basis, includes Dr J. Bowlby, Professor M. Abercrombie, Professor R. Hinde, Dr E. Leach, Professor J. Tanner and Professor N. Tinbergen. An appeal will be launched in the spring to raise £0.5 million which will be required to run the centre for five years.

Stopping Supertankers

As tankers get larger and larger, so does the problem of stopping them. Dr Hatsuko Tani, of the Tokyo University of Mercantile Marine, discussed the problem in a paper presented to the Institute of Navigation on November 15. Dr Tani discussed three ways of avoiding obstacles; putting the helm hard over, reversing the engines, or doing both at once. Until twenty years ago, he said, the distance needed for a ship to come to a stop in a straight line with the engines full astern was comparable with the forward distance travelled if the helm were put hard over. The distance in the first case is called the head reach, and in the second is called the turning advance. For a modern tanker weighing 100,000 tons, on the other hand, the head reach is between ten and fifteen times the length of the ship, while the turning advance is only five to seven times the length. This slightly surprising result comes about because the mass of the ship—and hence the inertial forces—increases with the cube of the length, while the total resistance of the hull underwater increases with the square of the length.

From the captain's point of view, it is vital to know how soon the ship can be stopped, both for everyday activities like picking up pilots, anchoring and berthing, or for emergencies like avoiding collisions or going aground. Dr Tani described theoretical studies which enable calculations of the head reach to be made. To make the problem even easier, he plotted the figures on a diagram. Comparison of the theoretical results with experience on two large supertankers and a medium size tanker (the Esso Suez) had shown that the calculations produced the right answer to a very fair degree of accuracy.

But this takes no account of the third possibility—that of reversing the engines and putting the helm hard across at the same time. The distance taken to stop in this case Dr Tani calls the stopping advance, and it depends on speed, as well as the other sailing characteristics of the ship. Turning advance, on the other hand, is virtually constant within the normal speed

range, and depends only on the rudder angle and not on the speed. At high speeds, therefore, the turning advance is smaller than the stopping advance; as speeds falls, the two become identical, and at low speed stopping advance is the shorter of the two. (This takes no account of the tendency of the ship to behave irrationally when the rudder is hard over and the propellers reversing. Occasionally under these conditions ships make sharp turns, often to starboard, either because of the unbalancing effect of the right-handed screw propeller, or, in Dr Tani's opinion, because the course stability of the ship becomes extremely poor.)

What conclusions can the captain draw from these unexpectedly complicated considerations? At high speed, a full rudder turn is probably the best course, without reversing the engines. At lower speed, reversing the engines and putting the helm hard over may be best. If the object to be avoided is approaching—as, for example, in a collision situation—the important criterion is to reduce speed in as short a time as possible; in this case, although the ship may be travelling above the critical speed, a crash-back procedure with engines in reverse is recommended.

Saturn Aloft

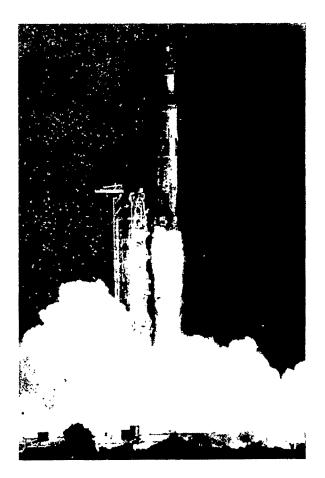
from a Correspondent

Last week's first Saturn V flight (called Apollo 4) may have helped NASA to catch up on the lagging timetable for landing two Americans on the Moon by 1970. By combining in one flight the first operational tests of several important components, time has been saved and many of the tactical obstacles to the first manned flight in the Apollo capsule cleared away. That flight is now fixed for autumn 1968, employing the less powerful Saturn I as booster for a low Earth orbit to check the capsule under "live" conditions. At least three further steps of graduated difficulty must follow before the lunar landing attempt.

There is no doubt of the technological achievement of the first Saturn V flight, however misplaced or outdated may now seem the goal of a manned Moon landing by 1970. The organization and co-ordination by themselves compare with an important military exercise—with far less margin for improvization. That the launching took place to the minute—0700 hours local time, October 8—marks the planning efficiency and the soundness of the new Moonport Complex 39 at its first launching.

The chief engineering tasks to be met were these. The unflown first stage (F1), presumed the biggest booster ever to take off, used conventional fuel, but to concentrate a thrust building up to 8.5 million pounds within 150 seconds depended on the fine adjustment and balance of the supply of 28,000 pounds-per-second of propellant for simultaneous burning in five matched The second stage, delivering one million pounds, had never flown before and used tricky liquid hydrogen as propellant in its five clustered $(\hat{J}-2)$ engines. To ensure smooth transfer of power between stages, eight solid fuel rockets used for stage separation also had to "settle" the super-cooled liquids in the bottom of their tanks to ensure instant feed under high vacuum and weightlessness. The third stage centred on a single liquid hydrogen J-2 engine, with fourteen other rockets for ancillary purposes. The critical and pre-

viously untried test for this stage was to restart the main engine after two Earth orbits to push the vehicle out to a distance (9,300 nautical miles) whence it could start a simulated lunar return and re-entry to prove the Apollo capsule's heat shield. This involved accelerating it downward by means of further rocket burns and high precision onboard control only partly prompted from the ground to ensure correct attitude, angle and so on, to narrow margins for the high speed collision with the atmosphere. A glancing blow, 7·13° (to local horizontal), on the heat protected blunt forward end of the spacecraft was called for, and achieved.



More Action on Aldabra

CONVINCED that Aldabra Atoll should be preserved entire without the air staging post which the Ministry of Defence wants, the Royal Society has made a further approach to the Secretary of State for Defence. Since its last approach in May, the society has reappraised the case in the light of the results of the preliminary ecological survey of the atoll and of recent modifications to the defence proposals. These proposals would limit any development, if and when a decision is taken, to the eastern end of the south island, instead of involving all the islands of the atoll as originally planned.

The Royal Society has repeated that any development on Aldabra will upset the integral ecosystem, which is the great scientific interest of this, the last remaining ecosystem of its kind in the Indian Ocean. Confining the air staging post to one part of one island will not save the ecosystem. It would not be possible

to put effective barriers between parts of the atoll, and in any case the eastern area of the south island is the most interesting part of Aldabra. Once development begins, the destruction of the ecosystem, which has been occurring very slowly ever since domestic animals and weeds were introduced by the settlers who arrived in 1888, will be accelerated, and the ecosystem as it is now—largely unaffected by man—will eventually be destroyed. In its new approach the Royal Society has stressed the great value of Aldabra as an integrated island ecosystem the study of which would give information valuable not only to biological science but also for the understanding of other ecosystems, including those of economic and social importance.

New Post

Following the discovery of natural gas, the Gas Council announced, in its annual report earlier this year, a reorientation of its research policy into two sectors, one to cover current problems and the other to investigate the full and economic use of natural gas. The council is now advertising for a distinguished scientist to direct all research activities, which means taking charge of the five research establishments and an annual expenditure of at least £2.5 million. The post is a new one and remuneration is a matter for discussion, but corresponding research posts in the National Coal Board and the Central Electricity Generating Board command salaries in the £7,000 to £9,500 range.

Safe Vaccine

A NEW method for preparing canine distemperhepatitis vaccine in established cell lines of canine kidney tissue has been developed by Dr L. Brown, director of the biological research division of Norden Laboratories Inc., Lincoln, Nebraska. Dr Brown described the method at a conference for the Division of Biologics Standards at the National Institute of Health, Bethesda, on November 6–8. The vaccine—containing two attenuated live vaccines and called 'Enduracell D-H', has been used to vaccinate approximately one million dogs since it was first introduced in 1963.

There are several advantages in using an established cell line for vaccine production. A cell line of this nature contains only one kind of cell and every cell is genetically related: each cell therefore has the same biochemical characters, the same growth rate, the same virus susceptibility and, when infected with a virus, will respond in the same way. As a result, vaccine production is very regular; it contains a uniform virus concentration and has consistent antigenic potency. It is important to use cells for vaccine production from the same species in which the vaccine will be used. This ensures that the virus maintains its maximum ability to invade cells of a given species, resulting in maximum immunity. It also reduces the danger of foreign protein reactions.

The DK line is produced from a master seed stock and every serial of vaccine is produced at the same passage level. This development of uniformly potent and safe vaccine is to be extended to the production of rabies vaccine for dogs, cats and cattle, and Dr Brown anticipates that it will soon be possible to prepare all vaccines in established cell lines.

Parliament in Britain

Oceanography

DR J. BRAY, Joint Parliamentary Secretary, Ministry of Technology, said that research was proceeding with the hope of increasing the range of optical detection in turbid water, and the National Research Development Corporation, in conjunction with the Ministry of Defence, had patented and was exploiting a non-optical imaging system for underwater viewing. The corporation had for some months been studying with a group of industrialists the requirements of a technologically based diving activity, and this group was now engaged in generating the necessary development work to produce a wide range of aids and equipment for submarine use. In common with the governments of other member countries of the United Nations, the Government was cooperating in a comprehensive survey of activities in marine science and technology, with a view to formulating proposals for an expanded programme of international cooperation in oceanography. (Written answer, November 7.)

Biological Warfare

THE Minister of Defence for Equipment, Mr R. Mason, said that the Government's policy was to publish as much as possible of the results of microbiological research at the Porton Laboratory. Last year forty papers by the staff of the Microbiological Reresearch at the Porton Laboratory. search Establishment were published and members of the staff gave lectures and attended national and international symposia. An annual report, in the form of abstracts of all original work published, was issued to about 300 libraries at universities, research institutions and industrial organizations, and copies were available on request. From December 1, 1967, all suitable unclassified departmental reports would be released to the National Lending Library for Science and Technology. Much of the work was helping the medical research centres. (Oral answer, November 8.) Weather

MR. M. Rees, Under-Secretary of State for Defence for the Royal Air Force, stated that over the next four years the British contribution to the World Weather Watch was expected to amount to some £2·5-£3 million, or about 7 per cent of the total cost. Britain had offered to act as a Regional Meteorological Centre and a Regional Telecommunications Hub, to continue to develop its research effort, to improve surface and upper air observations and to provide free training for overseas meteorologists. In addition, he said, Britain would make substantial amounts of equipment available for use for developing countries and would make an additional annual contribution of £30,000 to the World Meteorological Organization. (Written answer, November 8.)

Student Grants

MRS SHIRLEY WILLIAMS, for the Department of Education and Science, said that grants to students in the current academic year would cost an estimated £129 million. £9 million of this was for postgraduate awards and the remainder for first degree courses. Mr Patrick Gordon Walker said the raising of the school leaving age to 16 in 1971–2 would cost £44 million in the first year. It was too soon to tell, he said in answer to another question, whether the £0.5 million set aside for aid to overseas students would prove adequate. (Written answer, November 9.)

NEWS AND VIEWS

What Future for Biogenesis?

Those who work on the origin of life must necessarily make bricks without very much straw, which goes a long way to explain why this field of study is so often regarded with deep suspicion. Speculation is bound to be rife, and it has also frequently been wild. Some attempts to account for the origin of life on the Earth, however ingenious, have shared much with imaginative literature and little with theoretical inference of the kind which can be confronted with observational evidence of some kind or another. But if biogenesis has been plagued by too much speculation, exobiology, as it has been called, has suffered more grievously. It is still hard, for example, to be reconciled to the use of good public money on the solemn search for extraterrestrial life which rejoiced—the correct word—in the name of project OZMA. To be sure, there is no good reason to shrink from accepting as a working hypothesis that there are living things elsewhere than on the Earth. It would be a great surprise if one planet were unique in this respect. But, even if it is now necessary to suppose that there are living things elsewhere, it is surely unreasonably innocent—and even unimaginative -to think that a search of the radio sky would yield more than anecdotal information. In this and a host of other ways, biogenecists and exobiologists have earned themselves a bad name. This is a misfortune, for the hard core of these studies now deserves close and careful attention.

There has, in any case, always been a hard core, and it goes back at least to Lyell and his contemporaries. More recently, Haldane occupied a distinguished place. And now, in the past decade or so, a great many developments have conspired to stiffen speculation and to make it more absorbing. One way and another, problems of the origin of life have come to occupy a central position. The surge of interest in geophysics in the past decade has stimulated a great deal of enterprising work. A better understanding of the relationships between the various chemical components of the present atmosphere has, for example, made speculation about the reducing atmosphere of the early Pre-Cambrian a much more certain process. The comparative study of the planets, now easier than it used to be, has worked in the same direction. Superficially unrelated processes such as continental drift have drawn attention to the need to know more about the processes of mountain building and to the feasibility of doing so. It should not be too long before people are

Tests for Pregnancy

THE Royal Postgraduate Medical School at Hammersmith Hospital was founded as a part of the University of London to provide a centre for specialist teaching and medical study. It receives approximately £340,000 a year in grants and benefactions as well as a grant from the university.

able to define stratigraphical horizons in the Pro-Cambrian with at least some of the certainty with which this can be done in the Pleistocene.

Molecular biology has been a great spur. It is true that the apparent simplicity and sureness of the genetic code raise awkward questions—how can such a simple and sure means of replication have come into being more or less in one fell swoop? And, if it did not, what else happened? But these are speculative questions. There is more profit in less ambitious directions. The understanding of photosynthesis has raised all kinds of questions about the origin of life. There is the possibility that photosynthesis may discriminate against the naturally occurring and stable isotope carbon-13 in such a sure way as to provide an indication that ancient samples of petroleum, for example. have been formed in ways like these. But photosynthesis has also emphasized the relative stability of the porphyrin structure. It is no wonder that people go about with excitement when they have found traces of porphyrins in ancient sediments, much as if they had found the fossil of some new kind of dinosaur.

In practice, recent developments of technique in biochemistry have given the hunt for traces of primeval chemicals a great stimulus, which brings obvious benefits but which creates problems as well. Some of the benefits are at once valuable in themselves and provoking of other questions—the ways in which amino-acids and other materials are being found in Pre-Cambrian materials (see Nature, 216, 561; 1967) are important illustrations of this principle. The nature of some of the problems is illustrated by the article by Professors Eugene McCarthy and Melvin Calvin on page 642, and proves how sensitively some of the problems must be dealt with. The argument is that quite complicated chemicals can be built up by inorganic processes—if matters were organized differently, the whole question of biogenesis would be enormously complicated. It follows, however, that there must be serious problems in distinguishing between fossil chemicals derived from living things and those which have been constructed by other means. At the same time, it is also clear that establishing criteria to decide issues like these will be an absorbing task. A great many of the other problems of biogenesis have this important quality. This is why these studies deserve more attention, more support and more respect than they have usually attracted.

Part of the research in the Department of Obstetrics and Gynaecology at the Medical School is a project concerned with foetal heart monitoring in early pregnancy by means of ultrasonics. Using the Doptone foetal pulse detector, the presence of the foetal heart can be detected before foetal movements are felt by

the patient and before the conventional foetal stethoscope can be of value. The guiding principle used is the doppler effect. A beam of low frequency ultrasound is directed into the uterus of the patient. Some of this is reflected at every density gradient which the beam traverses. Stationary structures reflect ultrasonic beams at a frequency different from that reflected by moving structures and this difference in frequency is used to demonstrate movement in vessels or in the foetal heart. The alteration in pitch is recorded on a tape and can be heard on a loudspeaker. Preliminary studies have shown that the foetal heart can be heard as early as the tenth week of pregnancy.

Women often forget the stage of pregnancy which they have reached; a simple technique has therefore been developed in the department to estimate accurately foetal maturity. This is a cytological test, based on the fact that, as the foetus matures, fat cells become more numerous and desquamate. A catheter is introduced into the uterus and 10 millilitres of amniotic fluid is removed. Samples of the liquor are placed on slides and stained with nile blue sulphate—cells containing lipid stain orange-brown. One can then count the number of cells containing lipid and compare the results with previously obtained values. For example, between 34 and 38 weeks of pregnancy, 1-10 per cent of cells contain lipid. After term, more than 50 per cent of cells stain orange and it is then safe to induce birth when necessary.

Bacterial Walls

BACTERIOLOGISTS have come a long way since Leeuwenhoek first observed and described bacteria under the microscope. During the last ten years, particular attention has been focused on the structure and function of the bacterial cell wall. This subject formed the basis of this year's Royal Society Leeuwenhoek Lecture, entitled "Teichoic Acids and the Molecular Structure of Bacterial Walls" and given by Professor J. Baddiley on November 9.

Professor Baddiley began by outlining the importance of the bacterial cell wall: it is important in conferring shape on the organism; it is interesting because of its strength and permeability to metabolites; it is the site of many serologically active substances and, furthermore, certain antibiotics such as penicillin and vacitracin effect their antibacterial activity by interfering with cell wall synthesis. Cell walls are also interesting in their own right, as they contribute up to 20 per cent of the dry weight of the micro-

Isolated walls can be prepared by several methods, including mechanical disruption with tiny glass beads, after which the material is subjected to filtration, washing, differential centrifugation and further washing. The chemical structure of the wall depends on the kind of micro-organism. Thus in Gram-negative bacteria, it is a complex of lipid, protein and carbohydrate; in Gram-positive bacteria, the structure is simpler, and it is on this latter group that Professor Baddiley concentrated. He pointed out that the cell walls of these Gram-positive bacteria contain two principal components—mucopeptides and teichoic acids. Mucopeptides give the cell wall its structural rigidity: they consist, essentially, of a polysaccharide chain containing acetylglucosamine and cetylmuramic

acid residues, to which are attached short peptide side chains. They constitute approximately 50 per cent of the cell wall.

Teichoic acids constitute 30 or 40 per cent of the dry They are phosphate polymers containing either ribitol or glycerol residues joined through phosphodiester linkages. In most cases, sugars are attached to the polyol units and D-alanine residues are in labile ester linkage with hydroxyl groups. The length of the teichoic acid chain is not accurately known but is probably in the region of 15 to 20 units and varies according to the size of the organism. Variations on this basic structure have been found in a number of bacteria, for example in Streptococcus griseus, Bacillus subtilis and Staphylococcus lactis, and an atypical teichoic acid has also been found in the Pneumococcus capsule. Professor Baddiley described how, using the enzyme lysozyme, a membrane associated teichoic acid has been demonstrated in addition to the wall teichoic acid. This suggests that teichoic acids could be concerned with regulating the transport of metabolites and ions, as the densely charged environment created in, or beneath, a cell wall by their presence must surely influence the passage of electrolytes.

The overall biosynthesis of teichoic acids from nucleotide precursors is largely understood, but the process of attachment of alanine and the exact sequence in building up the polymers are not yet clear. It has, moreover, been suggested that a lipid is involved as a carrier in the transfer of glycerol phosphate or ribitol phosphate from the nucleotide precursor to the polymer: this lipid is probably C55-polyisoprene.

Hummingbirds and Plant Speciation

from a Correspondent

In California many species of hummingbird are migratory, moving from lowland desert in winter and spring to the mountainous regions in summer. Grant and Grant observed (Aliso, 6, 51; 1966; and 103 and 107; 1967) that hummingbirds are the pollinators of many Californian plant species whose floral parts may be adapted to receive the bird's bill. The Grants now suggest (Evolution, 21, 457; 1967) there is a relationship between the migration of the hummingbirds and speciation of plants in areas occupied by the birds. One step in speciation may be a change in floral structure, another may be a change in the time of flowering; both changes facilitate contact with the hummingbirds to the advantage of both flower and bird.

The Grants have found that in the lowlands, the flowering period of plants pollinated by hummingbirds falls in the winter and spring, and this coincides with the occupation of these regions by the hummingbirds before migration. Here the hummingbirds are widely dispersed, the different species showing habitat preferences and territorial activity. The hummingbird flowers tend to be allopatric, that is, one species predominated in the areas studied. By contrast, in the mountainous regions the hummingbird flowers are summer flowering, and here they are sympatric-up to six species occurring together within the area studied. During the summer in the mountainous regions hummingbirds of different species are collected together. There seems to be a definite pattern of distribution of hummingbirds and the character of the species they pollinate. The suggestion is that the higher the density of hummingbirds the greater the selection pressure they exert to produce suitably adapted plants and hence new races and species. This is the situation in the mountainous regions which are sites of high selection pressure on each of the species present, and is revealed by the sympatric nature of the hummingbird flower population. The allopatric population in lowland areas indicates a less intense selection pressure. A similar correlation of hummingbird dispersion and plant speciation has been found in Arizona, and is expected to occur in other areas over which the hummingbirds migrate.

Underground Shrimps

AMPHIPOD crustaceans are among the largest and least studied groups of North American freshwater invertebrates. It is therefore useful that a complete reappraisal of the systematics, speciation and distribution of the subterranean amphipod genus *Stygonectes* has been reported by John R. Holsinger in Bulletin 259 of the United States National Museum.

With 29 species, Stygonectes is the largest of the nine genera of Gammaridae. Mature adults range from 4·50 mm-19·50 mm; they lack eyes and pigments and inhabit subterranean areas such as caves and solution channels in limestone regions, and interstitial habitats where there is underlying non-cavernous rock. The genus has been divided into six species groups which are geographically distributed in the eastern United States, the south-central United States and central Texas.

The origin of Stygonectes is difficult to work out because of the complete lack of a fossil record and because of the absence of marine forms which can be regarded as related to an ancestral stock. Nevertheless, Holsinger has postulated a theory based on a series of freshwater invasions by ancestral marine stygonectic stock beginning early in the Cenozoic or even in the Ancestral stock was probably Upper Cretaceous. already inhabiting shallow coastal waters at this time and later passed through a transitional stage in brackish waters during periods of marine embayment. As the sea waters fluctuated, ancestral forms slowly migrated into freshwater areas lying adjacent to the old coastline. Finally, as the sea water permanently receded, they became tolerant of changing salinity and, as they established themselves in fresh water, moved slowly inland to occupy a whole series of newly created niches. Subsequent inland dispersal occurred through interstitial habitats developed in flood plains along principal rivers.

Three lines of evolution are indicated within the genus on the basis of morphology, geographic distribution and, to some extent, ecology. Patterns of insular speciation in the emarginatus and spinatus groups of Stygonectes in the central Appalachians, and in the flagellatus and hadenoecus groups of the Edwards Plateau, can be explained in terms of geographic isolation. Thus in the Appalachians, species in limestone-floored valleys were isolated from each other by high ridges of shales, sandstones and conglomerates. Similarly, ranges in the Edwards Plateau were isolated by barriers in the form of extensive faults and stratigraphic changes. Species with the widest range are found in the tenuis group and disperse freely through ground waters which are situated close to the surface.

Some instances have been reported in Maryland of breakdown of ecological isolating mechanisms between two otherwise phenotypically and genotypically distinct species.

Dr Holsinger also considers recommendations for future study on the evolutionary biology of the genus. These include experiments to determine toleration to varying concentrations of salt water and improving methods to clearly delineate patterns of geographic variation.

Protein Protons

from our Molecular Biology Correspondent

THE past year has seen the burgeoning of proton magnetic resonance as a means of looking at the behaviour of particular functional groups in biological polymers. Some new examples in the current literature demonstrate again the scope and potential of the method.

Following on earlier work, Bradbury and Wilairat (Biochem. Biophys. Res. Commun., 29, 84; 1967) have examined the characteristic resonances of certain protons-specifically the C-2 ring protons-of the histidine residues in several proteins. It was shown previously that in ribonuclease, signals corresponding to different histidines could be distinguished. It is now shown that in hen's egg lysozyme the solitary histidine is clearly observable and that, in native insulin, cytochrome c and myoglobin peaks likewise show up in the expected region. On the other hand, in trypsin, chymotrypsin and their zymogens no histidine signals are detected until the protein is denatured. The simplest explanation, and the one offered by the authors, is that in the latter group of proteins the histidine rings are immobilized by interactions with their environment, whereas in the others they are presumably essentially free to rotate. Since protonation of the imidazole ring leads to a downfield shift, it is possible to follow its ionization curve, and Bradbury and Wilairat give the pK of the histidine residue of lysozyme as 6.3 in D_2O (corresponding to about 5.8 in H_2O).

The same experiment (with happily the same result) has been described by Jardetzky's group (Meadow et al., Proc. US Nat. Acad. Sci., 58, 1307; 1967). In a human lysozyme these workers find the quite different, and anomalous, pK of 7.6. The addition to hen's egg lysozyme of a specific inhibitor leads to no detectable shift in the histidine resonance. In pancreatic ribonuclease, however, interesting effects are observed. In the first place, the 100 Mc instrument used by Meadows et al. makes possible the resolution of signals from all four histidine residues. Their hydrogen ion titration curves have thus been individually measured, and pKvalues of 5.6, 5.9, 6.1 and 6.6 ensue. Further, it is known that two histidines, his-12 and his-119, flank the active site, and their chemical modification annihilates the activity of the enzyme. Progressive addition of the competitive inhibitor, cytidine monophosphate, leads to quite large shifts in the signals from first one and then another of the histidines, and ultimately the remainder also show appreciable effects. There is no doubt, however, that two of the residues are most strongly affected, and it seems highly probable that these are near the active centre.

A staphylococcal nuclease, which requires calcium for activity and has also four histidine residues, has been similarly studied. Here, however, addition of inhibitor leads to no significant shifts, and it therefore seems likely that in this enzyme histidines are not directly involved in the active site. The pK values of the four residues have been measured, and one—the least readily protonated—shows evidence of interaction with calcium ions.

A minute study of the NMR spectrum of lysozyme has been described by Sternlicht and Wilson (Biochemistry, 6, 2881; 1967). This represents a beginning to the formidable task of allocating all the observed resonances: from the crystallographic co-ordinates it has been possible to calculate interactions (for example, ring-current shifts of methyl protons close to aromatic rings), which has made possible a near-certain identification of several signals from side chains and backbone. The changes occurring on thermal unfolding have been described.

A review, including much new work, of results obtained with the celebrated new 220 Mc Varian instrument also requires mention (Ferguson and Phillips, Science, 157, 257; 1967). This shows yet again, and in spectacular fashion, the resolution of the four histidine residues of ribonuclease, and the changes observed when ribonuclease and lysozyme are denatured. It will be noted that all the other work which has been mentioned was performed with 60 or 100 Mc instruments. Wider applications in enzymology may be expected.

More about Chain Initiation

from our Cell Biology Correspondent

GIVEN the great variety of proteins in ribosomes, it has always been a puzzle why ribosomes consist of two subunits; there seems to be no a priori reason why a single particle could not fulfil all the ribosomal functions. To explain the existence of subunits it has been suggested that during protein synthesis there is a cycle involving their separation and also that there is some division of the ribosomal functions between them. Several recent experiments, most of which are reported in the last two numbers of *Proc. US Nat. Acad. Sci.*, confirm both these suggestions.

Nomura and his collaborators (Proc. US Nat. Acad. Sci., 58, 946 and 1487; 1967), Eisenstadt and Brawerman (ibid., 1560) and Hille et al. (ibid., 1652) have all reported on the specific role of the 30S ribosomal subunit in the initiation of polypeptide chain synthesis in E. coli. The following general scheme emerges from their data—which are in gratifying agreement—and from previous results. First, initiation factors, which stimulate formyl methionyl tRNAF binding to ribosomes programmed with the initiating codons AUG or GUG, bind to the 30S subunit. Then the 30S subunit binds mRNA and F-met tRNAF to form an initiation complex, an obligatory intermediate of initiation. It is now generally agreed (Ohta, Sarkar and Thach, ibid., 1638) that this binding of F-met tRNAr is GTP dependent although GTP may not be split at the binding stage but during the formation of the first peptide band. And according to Economou and Nakamoto (ibid., 1033) the methionyl residue is formulated before it binds to a ribosome, but this is still a disputed point. Once the initiation complex has formed a 50S subunit, it couples with it to produce a 70S ribosome attached to mRNA and with the chain initiator in place. Only when this has happened can other amino-acyl tRNA bind and the

first peptide bond be formed. The requirement of the 50S subunit for peptide bond formation is in agreement with Munro's results (J. Mol. Biol., 26, 147; 1967) which suggest that the enzyme necessary for this, peptidyl transferase, resides in the 50S subunit. Once polypeptide synthesis has been initiated Eisenstadt and Brawerman envisage the initiation factors leaving the 70S ribosome and binding to another free 30S subunit. This would explain why initiation factors must be added to get initiation in vitro with 70S ribosomes while the 30S subunit forms an initiation complex without added factors and can then couple with a 50S subunit. It also explains why initiation factors are not found free in the soluble extracts of E. coli.

At chain termination and release of the completed protein the 70S ribosome dissociates into 30S and 50S subunits and if the 30S subunit picks up more initiation factor the cycle can be repeated. Mangiarotti and Schlessinger's (1966) discovery that the ribosomal population of E. coli consists of free 30S and 50S subunits and polysomes without a pool of free and stable 70S ribosomes clearly provides evidence for this. Moreover, Schlessinger et al. (ibid., 1782) now report that free 30S and 50S ribosomal subunits from E. coli do not spontaneously associate to form 70S ribosomes but will associate in the presence of mRNA, tRNA, K+ and Mg++ ions. The 70S ribosomes seem to be stabilized by peptidyl tRNA, for when peptide chain release is effected with puromycin they dissociate into 30S and 50S subunits.

So far, of course, all the experimental evidence for this scheme comes from work with *E. coli*, but it is unlikely that the process of protein synthesis should differ fundamentally in eucells. Indeed, there have been several reports—the latest by Sells and Takahashi (*Biochim. Biophys. Acta*, 134, 69; 1967)—that in nucleated cells mRNA associates with the small ribosomal subunit in the nucleus and then migrates to the cytoplasm; it would be no surprise if these complexes prove to be initiation complexes involving newly synthesized mRNA and small ribosomal subunits.

The Genetic Code in vivo

by our Molecular Genetics Correspondent

THE detailed relationship between amino-acids and their trinucleotide codons has been established for Escherichia coli over the past six years. Of the sixtyfour codons, three are nonsense (UAG, UAA, and UGA) and the remainder code for the twenty natural aminoacids. The evidence for constructing this dictionary of codons and amino-acids has come essentially from two different types of biochemical experiments, both carried out in the test-tube. The first of these is the triplet binding technique in which a single trinucleotide is used to select and bind the correct transfer-RNA molecule carrying its amino-acid to the ribosome. This experimental approach, developed in Nirenberg's laboratory, is limited because of the rather small The second experimental set-up, effects observed. developed in Khorana's laboratory, utilized defined messenger RNAs with simple repeating sequences to direct the synthesis of polypeptides whose sequence was then determined. This, then, has provided us with the genetic code of E. coli in vitro.

It is far from easy to establish that this is exactly

the way in which the code is translated in vivo. There are two rather different tests which can be applied at present to see whether the in vitro code agrees with the in vivo code. First, base analogue-induced mutations are a single change of one base for another which should lead to the change of one amino-acid for another in a predictable fashion. Thus, a mutation which changes methionine (AUG) to valine (GUG) produces a change consistent with the in vitro code. Many such examples are known.

A more powerful approach utilizes a different type of mutation—the acridine mutation. In this case, as shown by Crick, Brenner and their colleagues in 1961, a whole segment of the gene is misread because an extra base has been inserted into the nucleotide sequence of the DNA, giving rise to a similar extra base in the messenger RNA. This extra base throws the translation of the messenger RNA out of its normal reading frame as the triplet codons are read, one at a time. However, if another base is deleted in a second mutation farther down the gene, the original phase of reading will be restored with a piece of phase-shifted sequence in between the two mutations. the original gene reads . . . ,AAA,BBB,CCC,DDD,EEE, ..., insertion of an extra base Y will give the following misreading: . . . ,AAY,ABB,BCC,CDD,DEE,E . . , . . . Finally, a further deletion of a C will give ..., AAY, ABB, BCC, DDD, EEE, ..., so that we finish up in the original phase with a bit of sequence altered in between. Two tests of this have now been made in E. coli; in the first case Streisinger and his colleagues studied such double acridine mutants of the phage T4 lysozyme (Proc. US Nat. Acad. Sci., 56, 500 and 1692; 1966), and recently Yanofsky and his group have determined the sequences of similar mutants in the tryptophane synthetase A protein of E. coli (Proc. US Nat. Acad. Sci., 58, 1499; 1967). Below are illustrated some of the mutants these groups obtained:

```
lysozyme (wild):
... thr. lys. ser. pro. ser. leu. asN. ala ...
... ACU.AAA.AGU.CCA.UCA.CUU.AAU.GCC ...
... ACU.AAA.GUC.CAU.CAC.UUA.AUG.GCC ...
lysozyme (mutant):
... thr.lys.val.his.his.leu.met.ala ...
tryp. synthetase (wild):
. thr. tyr. leu. leu. ser. arg. ala. gly ...
... ACC.UAU.UUG.CUG.UCA.CGA.GCG.GGU ...
... ACC.UUU.UGC.UGU.CAC.GAG.CAG.GGU ...
tryp. synthetase (mutant):
```

The collective results show that all the acridine mutants so far reported confirm the codons assigned to amino-acids by the *in vitro* system. This covers about one-third of all the triplets.

thr.phe.cys.cys.his.glu.glN.gly . . .

Furthermore, these data not only confirm codon assignments but show us which codons are in fact used in vivo. Since T4 phage has a higher AT base composition in its DNA than $E.\ coli$ (the (A+T)/(G+C) ratio is about 0.66 and 0.50 respectively), we might not expect T4 and $E.\ coli$ to use the same codons. Although the samples of codons used are still small, it is nevertheless striking that T4 appears to prefer to use triplets ending in A or U instead of equivalent (standing for the same amino-acid) triplets ending in a G or C. Just the reverse appears true of $E.\ coli$.

Phage RNA Replication

from our Cell Biology Correspondent

When I last wrote on the replication of single stranded RNA bacteriophage (see Nature, 214, 1287; 1967), Spiegelman's group had finally convinced themselves that this replication involves the synthesis of complementary minus strands and thus accepted the principle that in general replication of single stranded nucleic acid molecules requires synthesis of a complementary strand. Weissmann's group, however, had challenged the conventional view that replication of RNA phage genomes involves a replicative form (parental plus and minus strands hydrogen bonded in a duplex) which serves as a template for synthesis of progeny strands and so gives rise to a replicative intermediate (the duplex with nascent single stranded progeny molecules attached). They suggested that the minus strand (not the hydrogen bonded duplex) is the template for progeny plus strands and that the replicative form and intermediates are artefacts derived from the actual replicative complex during isolation.

The only way to test this idea is to isolate pure minus strands and determine their template function. Weissmann et al. (Proc. US Nat. Acad. Sci., 58, 766; 1967) and Inglewski and Frankin (ibid., 1019) have achieved the first step, the isolation of Q and R17 minus strands. Both groups used essentially the same method which involves annealing denatured replicative form with fragmented plus strands and then separating the minus strands from the hybrid of minus strand and plus strand fragments. The pure minus strands are, as expected, not infective and now the crucial experiments are doubtless being done.

Meanwhile Kelly and Sinsheimer (J. Mol. Biol., 29, 229 and 237; 1967) have reported a new technique for isolating replicative intermediate and have used it to test whether nascent progeny strands displace parental plus strands from the duplex (semi-conservative replication) or whether the original parental plus strand remains in a duplex with a minus strand while the progeny strand is displaced (conservative replication). Their experiment is based on the assumption that the replicative intermediate is a precursor of progeny strands and contains a double stranded core and this ignores any changes in structure that might occur during isolation which, of course, is just what worries Weissmann. Kelly and Sinsheimer find that, when the nascent strand is labelled with a short pulse of radioactivity, only about half of the label is displaced from the replicative intermediate. It is surprising that, when the parental plus strand of the duplex is labelled before replication, then again about half the label is displaced. As there is evidence that no duplexes are conserved throughout a complete infective cycle this result indicates that there is an about equal probability that any particular replication cycle will be either semiconservative or conservative and that all molecules are involved in both mechanisms at some time during the infective cycle.

The sugar phosphate chain of all nucleic acids has chemical polarity because of its 5' 3' phosphodiester links, and the usual direction of synthesis of nucleic acids is from 5' to 3'. Bishop, Pace and Spiegelman (*Proc. US Nat. Acad. Sci.*, 58, 1790; 1967), however, report evidence that although the synthesis of the minus strand during Q β RNA replication begins at the 5'

terminus, as usual, the polarity of synthesis of the progeny plus strands in the replicative intermediate is reversed and proceeds from 3' terminus to 5' terminus. This so far unique example of 3' to 5' synthesis predicts that the first part of progeny plus strands present in early replicative intermediate have a base composition similar to the 3' end of the completed plus strand and that progeny plus strands can be initiated before the

minus strand template has been completed. Apparently, both these predictions have been verified by experiment but, characteristically, the details are to be published separately. As translation proceeds in a 5' to 3' direction, the 3' to 5' synthesis of progeny plus strands must surely mean that translation of these strands cannot begin until their transcription has been completed.

Mathematics in 1984

Dr Brian Thwaites, the new president of the Institute of Mathematics, believes that Britain is failing to exploit the powers of the computer in mathematics. The following is an extract from his presidential address, given on November 16.

My subject is comprehensive in its own way because it embraces indiscriminately the whole of mathematics. It arises from a belief that the practice of mathematics is destined to be entirely changed by the computer. Our present pencil-and-paper method will, in the historical perspective, be likened to the industrial methods of the Stone Age.

I would therefore like to outline some of the reasons which have led me to this certainty. I have made no particular effort to pick out the most significant examples or to mention the most advanced techniques.

First, the ultimate objective of mathematical investigations into physical deterministic systems is the production of numbers. Soon, computers will be able to produce whatever numbers are required far more effectively than our conventional theoretical methods. Second, the analytical apparatus in our mathematical tool-kit is, in fact, extremely primitive; the computer offers us a power of technique which will transcend all our present capabilities.

The power of future machines therefore faces the present-day applied mathematician with the brutal question "What am I after and how do I achieve it?" I fear that, in this country, most of us would answer this in too cautious and conservative a fashion. In the other two major world mathematical communities, in the USA and Russia, there is evidence of a much more vigorous approach. Let me quote just two papers from Russia. These refer to the equations of three-dimensional supersonic and hypersonic flow, equations to which an enormous theoretical effort has been devoted, with Russian mathematicians having made particularly notable contributions. Now come two papers by Babenko et al. (Nat. Aero. Space Admin. Tech. Transl. 380) and by Belotserkovskii et al. (Basic Developments in Fluid Dynamics, edit. by Holt, M.; Academic Press, New York) which seem to me to mark a decisive turning point in the theoretical study of this particular area of science. They set out, from the start, to exploit the resources of the computer and, in doing so, tackle problems far outside the limits of analysis.

As an aside, one may wonder whether such papers are the first fruits at the research level of the great emphasis which has been placed since the War on computers in mathematical education in Russian schools, technical colleges and universities. The report of the Ditchley Mathematical Conference and a recent report soon to be published by the Schools Council (Thwaites, B., Mathematical Education in Russian Schools (to be published)) highlight this situation, whose significance our own Department of Education and Science seems to have quite failed to grasp if one may judge from the recent report on

computer education by an interdepartmental working group (HMSO, 1967).

In regard, therefore, to the famous canonical equations of mathematical physics, one can say that it is no longer fanciful to imagine the facility of calling up standard programmes for the solution of, say, the complete Navier-Stokes equations stored as a kind of sub-routine in a large computer, so that the only actual programming that needs to be done by the user is to input the boundary condition of his problem, the degree of accuracy he requires in the solution, and the type of output. Certainly the construction of such a sub-routine is an immense task, but once completed it is there for good. It is a little odd that the British scientific community seems deliberately to shun the undertaking of this type of task which would bring such tremendous benefits.

I am, of course, aware of the argument that research into fundamental physical mechanisms, the discovery of effects which are strongly coupled, and the interpretation of natural observations all require more than merely the production of numbers. But these activities of the research mathematician are akin in character to those of the pure mathematician and I hope to indicate later how the computer is already active as an indispensable laboratory assistant to the pure mathematician. Moreover, the applied mathematician needs to go further than the pure; his theories are not sufficient unto themselves: whatever their sophistication, they must still be tested against observation. And, since the essence of scientific observation is quantitative measurement, numbers are, in the end, inescapable. They are, in my submission, becoming inescapable throughout.

Input and Output

So far, I have been talking in pretty trad terms and I must now move on to my second line of argument, namely that our mathematical tool-kit is soon to be enlarged beyond recognition. Whatever you may think of the potentialities I have been describing, those in the non-numerical field are far more startling. Our pencil and paper, therefore, give way to the teleprinter and the cathode-ray tube.

The teleprinter is no longer primarily the means for the input of a programme or its output; it is the medium of conversation between the mathematician and his mathematics. Mathematics is written into the machine which will ask questions back if it does not quite follow the argument; manipulations are made and calculations requested; chunks of mathematics are stored away to be retrieved later or fitted into a larger scheme. All this requires a language of conversation. At present, there are almost as many different languages as there are specialists working in this field, though they show strong affinities to each other. Far too little effort is being put in this country into this crucially important field of conversational languages; indeed, as far as I have been able to discover, the only reasonably comprehensive and readily available programme in the United Kingdom is at the Department of Machine Intelligence at Edinburgh University.

[Dr Thwaites then went on to describe the application of the cathode-ray tube, allied to lightpens, and the use of computers in pattern recognition. He also discussed the role of computers in abstract mathematics, in the finding or proving of theorems. One day, he said, computers would be programmed to emulate the feats of the finest mathematicians. He then gave examples of the use of the computer in the manipulation of real functions of a single real variable. But what conclusions should be drawn from these demonstrations of the power of the

computer ?]

All that I have described should have the most profound influence on the content of mathematical courses, in schools and universities, and on teachers' attitudes to mathematics. As to the content, there should first be much greater emphasis on discrete mathematics than there has been in the past. In the lower stages of education, where in any case attention has always been focused on the integers and on various practical uses of the integers, the notion of finite, rather than infinite, sets, and the idea of closed binary operations defined on such sets, should now be made explicit—as indeed is typical of some of the projects for the reform of main-school mathematics. In the higher stages—in the sixth form and above -the stress which is laid on continuous and on differentiable functions should be relieved by much greater emphasis on the discrete analogues of the kind I exempli-In particular, the notorious "methods" fied earlier. courses should be drastically overhauled in an attempt to anticipate the kinds of methods which today's students may be using in ten years' time.

Equally, mathematics must no longer be presented to the student—at whatever level—as a system involving proofs, demonstrations and other chunks of argument which are prescribed by some immutable and probably divine law. It seems to me essential that our students should be consciously reared on the algorithmic approach, duly spiced with heuristics; essential, too, that they should be taught explicitly that the methods to be employed in any piece of mathematics, and the chances of success, must depend on the means available, on the acceptable cost in time for man and machine, and on the use to which the result is to be put. In particular, the idea of graphs—that is, of the possible states of a system, and of the operative connexions between the states—is one which should now permeate all the teaching of mathematics.

Next, I want to suggest that the character of an intellectual discipline in its higher reaches, and the attitude which its practitioners adopt towards it, depend strongly on the manner in which the subject is presented during the period of formal education. Or, to put it differently but quite starkly, if schoolchildren are using techniques with which university dons (for example) have yet to catch up, then there is trouble ahead.

This is, in fact, now the situation. Young children at the very beginning of their conscious lives are doing mathematics by computer—with their own keyboards, CRTs and lightpens; in constant dialogue with the computer which is responding to their own efforts in an individual personalized manner which far exceeds what they could experience from any teacher facing a class of thirty-five children; and, as a final twist, the computer is also talking to them through the earphones.

This is not a vision of the future; it is with us now, being developed with immense vigour by people such as Suppes at Stanford University and exploited with equal vigour by firms such as I.B.M. This is what the practice of mathematics means to a coming generation of children; and this is how they will expect to practise mathematics when they are older.

Having been a schoolmaster myself for a number of years, and with a deep nostalgia for the classroom atmosphere, I can well understand the misgivings that many teachers will have in connexion with such developments. Prophecy turns to certainty, however, when in the glass ball one sees at least one of the two major world communities actively evolving such teaching methods. It is another measure of England's coming technological decline that, as far as I know, no group or institution in this country is even planning such activity.

A Short View

Earlier this evening, I took a long view of the development of mathematics, in recognition that there are those who will deny the changes that I have tried to exemplify. Now I am bound to take a shorter view; indeed to confess that I myself have erred on the side of conservatism in choosing 1984 as characteristic of these changes.

For I have not been indulging in any flights of imagination; I have kept my feet most firmly on the ground by describing what is actually now being done. I am not really looking forward seventeen years at all—if I were, I should have much more startling and controversial things to say. After all, east your minds back seventeen years to 1950: the "state of the art" now in 1967 far transcends what would then have been realistically predicted for 1967. There are solid reasons for supposing that a shot now at a realistic prediction for 1984 would fall equally short of the target.

I am therefore concerned not so much with the potentialities for 1984, but with the absence of potentialities in 1967. The lethargy which seems to be inhibiting British mathematics from realizing the changes inherent in computers has many manifestations.

First there is the evidence of the literature. Even if one goes no further than to say that the use of computers is one of the strongest growing points in mathematics. the British effort is already dangerously small in comparison with that of Russia and the United States.

Then there are the consequences of the future experimental nature of mathematics. We now need a great variety of sophisticated laboratory equipment, each piece precisely designed for the type of problem to be tackled. Thus university departments should be clamouring insistently for specific and detailed requirements for software, programming languages, and multi-access facilities. As it is, I suspect that the Computer Board is showing more imagination than mathematics departments. refer, for an example, to my own university, London; the very generous provision recently announced includes no facilities of any substance which are, in a significant mathematical sense, forward looking. That this may be inevitable in the present economic climate is beside the point; it remains that in their submission last year the university's mathematicians—and I am one of them—did not show the imagination and the professionalism which should be expected of them.

Finally, in looking at the more general national need of education in computer usage, I refer again to the recent Government report on an interdepartmental working group on computer education. This is a derisory piece of work. The members of the working party may shelter behind their terms of reference; if so, the terms of reference are derisory. In either event, it must be a matter of deep national concern if this document in any way reflects Government thinking on the applications of computers either to mathematics in particular or to the technological society in general. It is of high importance that within the next two or three years, this problem of computer education be examined by another body with knowledge and foresight.

Organic Geochemical Studies. 1. Molecular Criteria for Hydrocarbon Genesis

by EUGENE D. McCARTHY MELVIN CALVIN

Laboratory of Chemical Biodynamics and Space Sciences Laboratory, University of California, Berkeley, California In organic geochemical studies, it is important to develop criteria for distinguishing between chemicals produced by inorganic means and those which are the product of living processes. Molecules which consist of isoprene units have been particularly important, and abiogenic construction now seems possible.

THE search for forms of life in the earliest periods of geological time has been carried on not only at the morphological level but also at the molecular level. This has been possible as a result of the increase in biochemical knowledge and with the advent of analytical techniques that make it possible to describe the intimate molecular architecture of individual molecules in fine detail. The fundamental premises upon which this organic geochemical approach rests are that certain molecules, possessing a characteristic structural skeleton, show a reasonable stability to degradation over long periods of geological time, that their structural specificity can be understood in terms of known biosynthetic sequences and that their formation by any non-biological means is of negligible probability. In this article it is proposed critically to re-examine these premises and to establish criteria whereby one can differentiate molecules derived from biological systems from those which originate from non-biological One important reason for establishing such criteria is that they can be used to determine whether life exists, or has existed, on other planets. It may be possible to provide an initial answer to this question in the very near future when the first lunar samples are returned to the Earth for analysis.

Almost all classes of organic compounds which are recognized as the constituents of living organismsnucleic acids, the proteins, the organic pigments, the carbohydrates and the lipids-have been sought in sedimentary deposits of the geological environment. All these fulfil, to a first approximation, the requirements of structural specificity, but only the lipids and, to a lesser extent, the class of organic pigments known as porphyrins, survive from the earliest periods of geological time to be related to their original form today. All organic compounds in the geological environment are unstable with respect to temperature. The rates of thermal degradation processes therefore depend on the nature of the organic compound and upon the geothermal temperatures to which it has been subjected. Because some geothermal temperatures are often quite low and may not have exceeded 30° C (ref. 1), organic compounds may survive for long periods of times in the geological environment. Conway and Libby² measured the rate of a very slow reaction—the dicarboxylation of the amino-acid alanine—under various conditions and with radioactive labelling and low-level counting techniques. At room temperature, the half-life for decarboxylation was found to be about 10° years; at higher temperatures (420-430° K) the half-life was only 100 years. This experiment suggests that at low temperatures, amino-acids derived from the hydrolysis of proteins could be stable over millions of years. In agreement with this prediction, Abelson³ has identified a series of aminoacids, including alanine, in a Trilobite fossil believed to be 450 million years old.

Other classes of organic compounds might be expected to survive these relatively mild thermal conditions of geological environment. The stable tetrapyrrole portion of the porphyrin molecule, for example, has been identified in sediments of Pre-Cambrian age⁴. On the other hand, the

carotenoid groups of organic pigments are easily destroyed at temperatures of about 200° C (ref. 1), which is in keeping with the almost total absence of these compounds in sediments greater than 20,000 years old.

Of all the classes of organic compounds, the lipids have been most widely sought in the geological environment and the occurrence of a series of hydrocarbons, with structures based on the C_5H_3 isoprene unit, has been invoked as evidence for life-forms in Pre-Cambrian times. In particular, two hydrocarbons, phytane, thought to be derived from the phytol side chain of the chlorophyll molecules, and pristane, derived from phytol and also present as such in some marine organisms, have been sought in Pre-Cambrian sediments.

Their architectural skeleton, which has a methyl branch every four carbons, is stable over long periods of time. Hydrocarbons which can be related to the steroids and triterpenoids have also been found in ancient sediments. Examples from this class are cholestane⁵ and gammacerane⁶ whose structures are shown:

The saturated hydrocarbons are more stable than the porphyrins¹ and would be expected to survive in the geological environment through periods of time greater than the presently accepted age of the Earth (4·5 × 10° years, approximately). It is therefore not surprising that isoprenoid hydrocarbons have been consistently found in Pre-Cambrian sediments¹-1¹. Simple calculations of the degradation of hydrocarbons by thermal cracking mechanisms¹² indicate that hydrocarbons should be stable for more than 10²² years, at room temperature, and for 10¹² years at 100° C. The presence of catalysts in the geological environment will presumably accelerate the rates of these processes.

The characteristic architecture of the isoprenoid hydrocarbon molecule has until recently been generally accepted as a marker of biological origin. This is why the idea has arisen that the isoprenoid hydrocarbons might be used to designate the period in geological history when the transition from chemical evolutionary development to biological evolutionary development was made.

The absence of isoprenoid hydrocarbons in the organic extract from a Pre-Cambrian sediment might serve as a criterion for the period of chemical evolutionary development. That such a transition should exist and that evolution should be continuous, not only in the domain of living organisms, was a concept

that Darwin himself had clearly recognized.

The expectation that such a transition might be recognized at the molecular level of evolutionary development has not been realized for several practical reasons. Most important among these is the scarcity of known Pre-Cambrian sediments that might form a chronological sequence from the earliest periods of the Earth's history to the advent of the Cambrian (600 million years). Moreover, only a few of these sediments have been studied from the organic geochemical standpoint. A more fundamental reason why this transition may not have been recognized, however, has now arisen with the development of a nonbiogenic stereospecific polymerization of the isoprene molecule to produce polymers which are identical with the isoprenoid polymers synthesized in the living system. The characteristic structure of the isoprenoid molecule may not be as specific as once was thought. In attempting to recognize this transition in evolutionary history, therefore, it is necessary to establish criteria which will distinguish between those organic compounds—particularly the hydrocarbons—that are derived from abiogenic sources and those that are derived from living systems. Only when this has been achieved can this transition point be recognized with any reliability.

Biosynthesis of the Isoprenoid Structure

The biosynthetic pathway of the isoprenoid hydrocarbons has been fully elucidated (for a general review, see refs. 13 and 14). In the biological system, isopentenyl pyrophosphate plays an important part. This five carbon fragment is the precursor of all the intermediates involved in the isoprenoid pathway.

$$\begin{array}{c} \mathrm{CH_2} \\ \parallel \\ \mathrm{CH_3--C--CH_2--CH_2O--(PP)} \end{array}$$

Polymerization of this five-carbon unit takes place by displacement of the pyrophosphate group to give a 10-carbon fragment, or monoterpenoid compound. The displacement, which involves a head to tail linkage, takes place in a stereospecific manner¹⁵. This biological mechanism is repeated in further polymerization reactions. The head to tail mechanism is, however, replaced by a tail to tail linkage at two places:

(1) The C₁₅ compound farnesyl pyrophosphate reacts with another molecule of farnesyl pyrophosphate to give the hydrocarbon squalene.

(2) In the biosynthetic pathway to the carotenoids, C_{40} terpenoid compounds, an analogous tail to tail linkage is formed between two C_{20} compounds.

The biosynthetic pathway has other stereospecific characteristics. Lindgren 18 has shown that homologous aliphatic $\rm C_{30}-\rm C_{45}$ terpenols occur in extracts of birch wood (Betula verrucosa Erh.). These alcohols have the general formula

$$H--(CH_2--C(CH_3)=CH--CH_2)_n--OH$$

where n is 6,7,8,9. About 60 per cent of their double bonds have the cis configuration. Thus C_{30} terpenoid and C_{40} terpenoid structures containing both the tail to tail linkage and the head to tail linkage have been isolated from living organisms. Higher terpenoid compounds have been isolated from pig liver, which is a rich source of dolichol.

Dolichol has fifteen or sixteen of its eighteen internal isoprene units in the *cis* configuration¹⁷.

$$\begin{array}{c|cccc} CH_3 & CH_3 & CH_3 \\ & | & | & | \\ CH_3-C=CH-CH_2-(CH_2-C-CH-CH_2)_{18} & -CH_2-CH & CH_2-(CH_2OH) \\ \end{array}$$

Isoprenyl alcohols, whose structures have been character ized as undecaisoprenol-1 and dodecaisoprenol-1, and solanesol have been isolated from silk worm facces 18 . Solanesol had been characterized earlier by other workers 19 , who had isolated it from flue-cured tobacco. Its structure was confirmed by Erickson et al. 20 and by Kofler et al. 21 as a 12 -isoprenoid compound. Mevalonic acid, a precursor of isopentenyl pyrophosphate in the biosynthesis of choles terol, is also an intermediate in the biosynthesis of ubiquinone, vitamin K and the tocopherols, all of which contain the isoprenoid side chain 22 .

The biosynthetic pathway to rubber and gutta, which is also a polyisoprenoid and is produced by a small number of tropical species, has been discussed by Bonner²³. Both rubber and gutta are polymeric substances containing iso prene units linked together through 1,4 linkages. Rubber contains from 500 to 5,000 isoprene units, while gutta contains about 100 units. It is of interest that the double bonds of the individual isoprene molecules are in the cis configuration for rubber and in the trans configuration for gutta. No high polymers have been found which contain both the cis and trans geometry.

Non-biogenic Synthesis of the Polyisoprenoid Structure

The biological polymerization mechanism involves a stereospecific 1,4 linkage of the individual isoprene molecules and it is this feature that has been hitherto assumed a unique aspect of the biosynthetic pathway. But recent studies on the non-biological polymerization of small molecules, such as propylene and butadiene, have indicated that these reactions proceed with considerable stereospecificity.

Natta was the first to show that butadiene, isoprene and 1,3 pentadiene are converted to polymers containing 99 per cent of linear 1,4 trans structures by means of a highly stereospecific catalyst Al(Et)₃-VCl₃ (molar ratio 2:1) in heptane²⁴. The presence of even small amounts of impurities impairs the stereo-specificity of the reaction. The infrared spectrum of the polymer resembles that of natural rubber. X-ray diffraction studies show the linearity of the polymers and a periodicity of 4.82 Å along the main axis. The use of TiCl3 produces a stereospecific 1,4 cis linked polymer. The exact mimicking of the stereospecific features of the biosynthetic pathway in the terpenoid series dispels the notion that the head to tail linkage is unique to biological systems. Natta has also shown that not only the 1,4 trans configuration of the biopolymer, gutta, but also the 1,4 cis configuration of the natural rubber can be reproduced by non-biological methods. These findings seriously question the validity of the isoprenoid compounds as "biological markers". Certainly the structure of the isoprenoid hydrocarbons taken in isolation can no longer be considered to be unambiguously derived from a biological precursor. Instead their occurrence in crude oils and sediments must be viewed against the background of the other components present25.

Non-biogenic Polymerization Processes and Chemical Evolution

An empirical approach to the evolutionary development of the first living organisms is to simulate in the laboratory the types of chemical reactions that could have taken place in the primitive atmosphere and given rise to the simple organic molecules that constitute living organisms today.

Such experiments have met with considerable success. By analogy with the atmospheres of other planets, the primitive Earth probably had an essentially reducing atmosphere consisting of hydrogen, methane, ammonia and water. Most of the fundamental building units of living systems, including the amino acids, the sugars, the purines and the pyrimidines, have been synthesized in varying amounts in "primordial atmosphere" experiments where energy, such as ionizing radiation in the form of particulate or gamma radiation, acts on this mixture of gases.

Any chemical evolutionary theory which accounts for the synthesis of the polyisoprenoid compounds in the primitive atmosphere by invoking polymerization reactions of the Natta type, utilizing reduced metal catalysts, must, at the outset, provide a feasible experimental route to the isoprene molecule. No such pathway has been proposed which has been justified on an experimental basis.

Consideration of a well-established commercial process which has been used in industry for many years suggests a possible synthetic route to isoprene in the primordial atmosphere. Isoprene is produced in significant quantities as a side-product in the thermal cracking of a mixture of propane and ethane²⁶. These two hydrocarbons are fed into a pyrolysis furnace in various amounts and undergo thermal cracking at temperatures approaching 1,100° K. In the primitive atmosphere, the starting materials for this pyrolysis reaction, ethane and propane, could be generated from methane in the presence of an external energy source. At 1,000° K these reactions proceed endergonically with a change in free energy amounting to about 17 and 32 kcals/mole, respectively. In a typical feed in addition to ethylene and propylene, sizable quantities of isoprene and butadiene are produced by thermal cracking at 1,100° K (see Table 1).

Table 1
Starting materials: 50 per cent ethane, 50 per cent propane.

	Rel. amount (per cent of product)*					
Ethylene	30					
Propylene	6-8					
Butadiene	2					
Isoprene	0.2					
Renzene	2					

^{*} The remaining 60 per cent is H_2 , CH_4 , and unreacted C_2H_5 .

In a natural environment the shock wave of a sizable meteoritic infall²⁷ would produce the high temperatures and high pressures necessary for this reaction in a pulse of only microseconds to milliseconds duration.

There is no reason to suppose that subsequent polymerization processes in the primitive atmosphere should be confined exclusively to isoprene. Indeed, one would expect that polymerization processes involving isoprene would proceed more slowly than polymerization processes involving the other three molecules. Unlike the molecule of ethylene, the propylene molecule is structurally asymmetric and so it can polymerize in various steric configurations. Under carefully specified conditions, stereospecific polymers may result—the so-called "iso-tactic" and "syndiotactic" polymers. Stereospecific Stereospecific polymerizations of butadiene may also give rise to ordered configurations²⁸. Natta has also shown that a *cis* polybutadiene can be synthesized with a steric purity exceeding the steric purity of the 97 to 98 per cent cis units found in natural rubber.

One may reasonably question the relevance of such polymerization reactions to the kinds of reactions that took place in the primitive atmosphere. All these polymerizations require specific catalysts to produce polymers of a given steric configuration. The likelihood of these particular catalysts being available on the primitive earth is somewhat remote. On the other hand, the overall simplicity of the polymerization reaction, taking place in the presence of metals in their reduced oxidation states

and using only isoprene as a starting material, gives credence to the hypothesis that stereospecific polymerization reactions similar to those developed by Natta might have taken place in the primitive atmosphere, and that the characteristic 1,4 trans head to tail linkage of the polyisoprenoid compounds may not be confined solely to biosynthetic mechanisms.

Even if this hypothesis is accepted, one has to explain why only the polymerization mechanisms giving rise to polyisoprenoid compounds survived to become an integral part of the biological system. There is no obvious reason why the polyisoprenoid compounds should be retained in evolutionary development in preference to the analogous polymers based on propylene or butadiene. Many of the polyisoprenoid compounds belong to the general class of biological components known as the lipids; the lipids themselves form an important part of the membranes of The role of lipids in the transition from living cells. chemical evolution to biological evolution may have been especially significant. Oparin²⁹ has suggested that an essential step in the transition between these two evolutionary stages might be the formation of membranes around droplets of organic substances produced in the primitive atmosphere. It may be that there is some important structural feature, as yet unrecognized and confined only to the polyisoprenoid compounds, that makes these polymers the best structural constituents of cell membranes. As a result, only the polyisoprenoid compounds would have survived the transition into biological evolution. At present this is a purely speculative hypothesis without experimental foundation.

The Fischer-Tropsch reaction has been considered by protagonists of the abiogenic theory for hydrocarbon formation to be a significant process in the formation of petroleum. (The Fischer-Tropsch reaction is a catalytic reaction involving carbon monoxide and hydrogen at temperatures between 200° C and 300° C and at atmospheric or higher pressures.) Friedel and Sharkey30 were the first to demonstrate that a similarity exists between low molecular weight alkane isomers (up to C₈) in crude oil and those characterized in the Fischer-Tropsch reaction product. This hypothesis has recently been extended by Hayatsu and Anders to account for the formation of hydrocarbons in the early solar system³¹. Using a mixture of carbon monoxide and deuterium to eliminate the contamination danger, they provided mass spectrometric evidence for the presence of a series of deuterated isoprenoid hydrocarbons, ranging in carbon number from C₉ to C₁₄. One member of this series, C₁₁D₂₄, had a structure identical to the C11H24 hydrocarbon, 2,6 dimethylnonane, which these workers had identified in the Murray meteorite. Ambiguities can arise in the structural characterization of isoprenoid hydrocarbons where the evidence is based on mass spectrometry alone³². However, if the identification of a homologous series of isoprenoid hydrocarbons is confirmed in the Fischer-Tropsch reaction product this series would be identical with that reported by Göhring, Schenck and Engelhardt in an Italian cretaceous shale³³. In effect this would provide an abiogenic route to the lower molecular weight isoprenoid hydrocarbons that can be vindicated on an experimental basis.

One of the weaknesses of the Fischer–Tropsch theory as a possible inorganic origin of petroleum is that it has not been able to account for the higher molecular weight isoprenoid hydrocarbons (C₁₅–C₂₁), including pristane and phytane, which are found ubiquitously in crude oils and shale extracts as the major components of the "branch-cyclic" hydrocarbon fractions. Furthermore, steranes, triterpanes and hydrocarbons derived from carotenoid precursors have not been demonstrated in the Fischer–Tropsch product, whereas these classes of organic compounds have been identified in both Pre-Cambrian and more recent sediments^{4,5} and in crude oils. The Fischer–Tropsch reaction product therefore only partially resembles

the hydrocarbon constituents of crude oils; it does, however, show a closer resemblance to hydrocarbon extracts from meteorites. As a possible route to the polyisoprenoid hydrocarbons in chemical evolutionary development, this process seems to be subject to the same limitations as in accounting for the isoprenoid distribution in crude oils.

Criteria for Biogenic Origin

The experimental evidence points overwhelmingly to a biogenic origin for almost all the organic extracts of crude oil and shales. However, the origin of the organic material extracted from meteorites^{34–36}, thucholite samples³⁷ and hydrothermal deposits³⁸ is much less clear cut. As the recent polymerization studies of Natta have brought into question the validity of isoprenoid hydrocarbons as "biological markers", it is important to search for criteria which will unambiguously resolve these uncertainties. The same uncertainties about the origin of the organic extracts may very well arise when lunar samples are returned to Earth for analysis.

One of the many approaches adopted in endeavouring to find criteria which will determine whether organic material has an abiogenic or biogenic origin involves the determination of the precise stereochemistry, and particularly the absolute configuration of the optical centres, of the individual constituents of organic extracts.

Optical activity measurements in the past have, in general, been confined to a complex mixture of extracted organic material. Nagy et al. 39 observed optical activity in the organic extract from the Orgueil meteorite, and on the basis of this and other evidence invoked a biological origin for the organic material. Subsequent work on carbonaceous chondrites by Urey⁴⁰ and Nagy⁴¹ provided additional evidence for the presence of optically active material in such sources. Hayatsu42 has consistently criticized the experimental foundation on which this evidence rests. Even if the experimental foundation were verified, however, it is doubtful what the significance of optical activity in a complex mixture would be unless it were possible to relate the origin of the optical activity to a specific component within the mixture, and to correlate this optical rotation, in magnitude and direction, with a known biological compound. Furthermore, the spontaneous formation of optically active substances from an inactive material, without interference from a directing, asymmetric agency, has been demonstrated by experiments. Havinga43 showed that methyl-ethyl-allylanilinium iodide may crystallize spontaneously into either enantiomer under certain experimental conditions. In general, if a nucleus of one type of enantiomeric crystals should begin to form by spontaneous, statistical fluctuations, an autocatalytic crystallization process may set in, because such a nucleus tends to grow by the addition of enantiomeric molecules of similar configuration. observation suggests a mechanism for the formation of the first optically active substance and indicates that optical activity may not be a unique property of the biological system.

In any experimental approach it is necessary to carry out as many measurements as possible of an individual compound to designate an unambiguous structure. We have, however, made no attempt to determine the stereochemistry of the isoprenoid hydrocarbons isolated from crude oils and shales nor, in general, have such determinations been made by other research workers in organic geochemistry. There are very good reasons for this. Until very recently the very specific structural architecture of the "biological markers" was in itself considered adequate evidence for a biological origin. With the isoprenoid hydrocarbons, for example, the characteristic methyl branch at every fourth carbon atom is so specific that it has generally been accepted to be indicative of a biogenic origin. Further, it is very difficult to isolate the individual components in sufficient quantity and purity from the

very complex organic mixtures to obtain a reliable optical measurement. Finally, with the isoprenoid hydrocarbons in particular, the optical rotations are so small that much larger quantities of the compound than normal (20–50 mg). as opposed to the optical measurements carried out by Hills and Whitehead⁴⁴ on milligram amounts, are required. It is impossible to obtain such quantities of individual compounds from most interesting organic geochemical samples and so few optical measurements have been made^{44,45}.

Despite this experimental difficulty it is important to correlate the stereochemistry of the geological samples with that of the biological precursor from which they are thought to be derived. It might be possible to separate diastereoisomeric forms of a particular isoprenoid hydrocarbon using capillary gas chromatography; preliminary attempts to bring about this separation have so far not been successful in our laboratory. Pristane can exist in two forms, and pristane isolated from marine sources is thought to have the mesoconfiguration.

(1) Meso-form

D on C(6), D on C(10)

(2) Mixture of d,l isomers

The separation of very small amounts of pristane into the diastereoisomers by capillary gas-liquid chromatography would provide a major breakthrough in experimental techniques in organic geochemistry. More significantly, it would establish a criterion to distinguish organic compounds derived from abiological sources from those derived from biological sources for, a priori, one would not expect a C₁₉ saturated isoprenoid hydrocarbon, having exclusively the mesoconfiguration, to be produced in an abiogenic synthesis.

Another criterion for biogenic origin stems from the biosynthetic pathway to the polyisoprenoid compounds in the living system. This pathway is dominated by the occurrence of the head to tail linkage in the polymerization of isopentenyl pyrophosphate, but a characteristic tail to tail linkage seems to take place exclusively at C15 to give C₃₀ compounds such as squalene, the precursor of the steranes and triterpanes, and at C20 to give C40 compounds such as lycopene. It may be that the non-branched fourcarbon unit in this tail to tail linkage is the criterion we are searching for to assign a biological origin to compounds isolated from crude oils and sediments. We have made several as yet unsuccessful attempts to identify such a 4-carbon unit in organic geochemical material32. The occurrence of a C21 saturated isoprenoid hydrocarbon 2,6,10,14-tetramethylheptadecane, in a series of sediments suggests that a C40 isoprenoid hydrocarbon such as lycopene, containing a tail to tail linkage, might be a pre-cursor⁴⁶. But it does not demand such a precursor and higher head to tail polyisoprenoids, such as solanesol and dolichol, can be postulated as precursors. The experimental evidence also seems to indicate that the C30 isoprenoid hydrocarbon, squalene, does not play a significant part as precursor to the isoprenoid hydrocarbons.

When isoprene is polymerized abiogenically, one might predict, a priori, that the reaction should not proceed stereospecifically, and that three compounds should be formed—the head to tail linkage (h-t), the tail to tail linkage (t-t) and the head to head linkage (h-h). This is illustrated below:

Isoprene
$$(h-t)$$
 $(h-t)$

When we consider the addition of another molecule of isoprene to produce a C₁₅ compound, and a further molecule to give a C₂₀ compound, then several products should result whose structural skeletons are shown in Fig. 1. These compounds might be expected to be present in hydrocarbon mixtures if they were derived by an abiogenic process which did not proceed stereospecifically. Thus, the presence of such structures in organic extracts might be used as a criterion for abiogenic origin.

there is a tendency to terminate with propylene, thus producing linear odd and isohydrocarbon homologies⁴⁸. Pyrophoric metal catalysts can also bring about copolymerization reaction⁴⁹. One such catalyst, pyrophoric iron, might have been present in the reducing environment on the primitive Earth. These catalysts have the effect of reducing the reactivity of ethylene relative to propylene by an order of magnitude; on reduced cobalt catalysts they appear to react at the same rates⁴⁹.

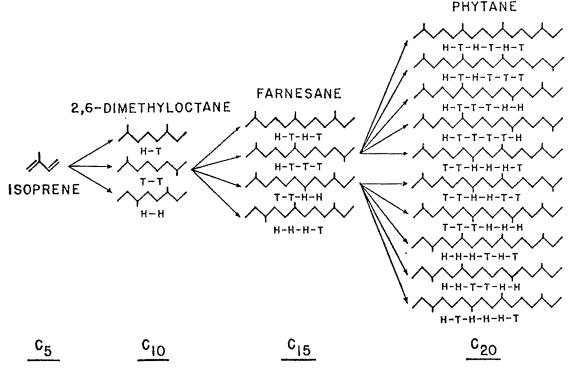


Fig. 1. Polymerization of isoprene.

The copolymerization of the simple olefines and diolefines ethylene, propylene, butadiene and isoprene⁴⁷—which are produced in the thermal cracking of ethane and propane can lead to polymeric products which have methyl groups located at irregular intervals along the linear polymer chain. If the non-biogenic polymerization of isoprene with reduced metal catalysts is invoked to explain the occurrence of polyisoprenoid compounds it is to be expected that copolymerization reactions would have occurred simultaneously. The polymeric products of these copolymerization reactions might serve as a criterion, therefore, of abiogenic origin. The structure of these products might be predicted from what is already known about copolymerization reactions. Ethylene is by far the most reactive of the simple olefines and will be the major constituent of the resulting product. The nature of the transition metal compound has been shown to have major control over the monomer composition of ethylene-propulene copolymers prepared with Ziegler-Natta propylene copolymers prepared with catalysts⁴⁸. Two examples are given below:

 $\begin{array}{c} & \text{Ethylene}(r_1)-\text{Propylene}(r_2) \\ \text{With } Al(\text{Et})_2 \\ \text{TiCl}_4 \\ \text{VCl}_2 \\ \end{array} \begin{array}{c} 38.7 \\ 1,040 \\ \end{array}$

Such polymerization products would be virtually straight chain in character with the occasional methyl branch and

Other criteria for biogenic origin such as the odd to even distribution of normal alkanes are only reliable for most recent sediments. The identification of "biological markers", other than saturated isoprenoid hydrocarbons, within a given organic extract is a reliable criterion in complementing the evidence for biological origin. Except for the porphyrins, however, and the steranes and triterpanes, other classes of "biological markers" do not survive into the most critical region of interest, the Pre-Cambrian, which imposes a severe limitation on this approach. The finding of homologous series in crude oils and sediments, while not in themselves necessarily indicative of biological origin, does augment the evidence for the isoprenoid hydrocarbons, when identified in the same extracts. A further point, which is often not sufficiently emphasized in establishing criteria for biogenic origin, is the relative amounts in which these isoprenoid hydrocarbons are found. In almost all cases they constitute the major components of the "branch-cyclic" hydrocarbon fraction; an observation which argues strongly for a biogenic origin.

Yet another criterion for biogenic origin is the carbon isotope ratios of the organic extract. This approach is based on the fact that photosynthetic organisms discriminate against carbon-13 in preference for carbon-12 (refs. 50, 51). Although not always definitive such measurements on organic extracts have already cast some doubt on certain proposals for an abiogenic origin of the hydrocarbons from hydrothermal deposits. Further insights into this problem might also be obtained from a development of the carbon

isotope approach. The study of the carbon isotope ratios of individual molecules is still rudimentary52 and the experimental problems involved can be overcome. This is a new field of study which could provide valuable information in ascertaining the origin of hydrocarbons.

The problem of the origin of the isoprenoid hydrocarbons remains inconclusive. The evidence still indicates that isoprenoid hydrocarbons are, in most cases, derived from biological sources, even in the Pre-Cambrian samples. It is in this geological time period, however, that we are looking with renewed interest for the transition between chemical evolution and the advent of biological systems. Natta has already demonstrated an experimental, nonbiogenic route to the isoprenoid compounds. The possibility of non-biogenic isoprenoid hydrocarbons is a very real one, and criteria must be established which will distinguish between those derived from an abiogenic origin and those derived from biological systems.

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Catenated Circular DNA Molecules in HeLa Cell Mitochondria .

bу

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Closed circular mitochondrial DNA molecules which are catenated, or connected like the links in a chain, have been identified in extracts of HeLa cells.

MITOCHONDRIAL DNA from a variety of organisms has been shown to occur in the form of closed circular duplex molecules with a uniform length of about 5μ and a molecular weight of 10 million daltons¹⁻³. Closed circular duplexes display a variety of special properties which arise from the inability of the two polynucleotide strands to unwind. One of these properties, the restricted uptake of the intercalating dye ethidium bromide (EB)4, forms the basis of a convenient method for isolating and detecting closed circular DNA molecules. When this method was first applied in a study of extracts of HeLa cell mitochondria, a paucidisperse system of closed circular molecules was seen in the electron microscope⁵. micron dimers accounted for 10 per cent of the molecules; higher oligomers were present in smaller amounts. The structure of these oligomers, however, was not determined, and it was possible that they consisted of monomers that were joined to each other after extraction of the DNA from the mitochondria. We now report experiments which show that mitochondrial DNA dimers and higher oligomers are stable, isolable DNA molecules that consist of independent, double stranded, closed circles that are topologically interlocked or catenated like the links in a chain. Because of the nature and stability of this topological bond, these oligomers must exist in the HeLa cell. A plausible mechanism for the formation of the catenated molecules is the physical recombination or crossing over of circular mitochondrial DNA molecules.

When ethidium bromide binds to DNA, the strands in the Watson-Crick duplex partially unwind. The unwinding results in a rotation of the molecule about the duplex axis if the DNA is linear or if it is circular and has a single-strand scission or nick. We will refer to both of these types of molecules as open molecules and to closed circular molecules as closed. A closed DNA with no site for rotation resists the uptake of dye in high concentrations and thus binds a smaller amount of dye than an open DNA. The binding of ethidium bromide, which has a low density, lowers the buoyant density of DNA in caesium chloride density gradients. The differential binding causes the closed DNA to band at a higher density than the open DNA. A mixture of closed and open DNAs forms two well separated bands after centrifugation of a mixture of ethidium bromide, caesium chloride, DNA and water. A compound molecule consisting of one closed and one nicked circular DNA would be expected to form a band approximately midway between the closed and open bands.

A fluorescence photograph of an ultracentrifuge tube containing mitochondrial DNA from HeLa cells and nuclear DNA from a subcellular fraction enriched with mitochondria is shown in Fig. 1a. The DNA-dye bands stand out over the background of dye because of the enhanced efficiency of fluorescence when the dye binds to DNA⁶. The positions of the upper and lower bands correspond to the previously reported⁶ positions for open (upper) and closed (lower) DNAs in the conditions used. The middle band, as shown in the microdensitometer record of this photograph (Fig. 1b), is about equally distant from the centres of the upper and lower bands. When examined with the electron microscope, the bottom band was seen to contain about 10 per cent dimers and 90 per



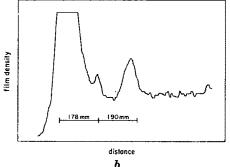


Fig. 1. A caesium ohloride—ethidium bromide density gradient showing the resolved species of HeLa DNA. a, Proparative tube photographed in ultraviolet light through an ultraviolet filter with a Polaroid camera as described* but with type 46 film. The darkest band contains the open DNA. The next two bands in descending order contain the open-closed dimers and the fully closed species. The lowest band is a carbohydrate as judged by the density, turbidity and low dye binding. b, Microdensitometer tracing of this photograph showing the band positions. The left-hand side of the tracing corresponds to the upper part of tube. HeLa S3 cells were grown in suspension culture in Eagle's medium containing 10 per cent calf serum, washed in TD buffer (0·005 M tris, 0·15 M NaCl, 0·005 M KCl, 0·001 M NaH, PO₂), suspended in 0·01 M tris, 0·01 M KCl, 0·005 M KCl, 0·001 M NaH, PO₂), suspended in 0·01 M tris, 0·01 M KCl, 0·005 M KCl, 0·001 M NaH, PO₂), suspended in 0·01 M tris, 0·01 M KCl, 0·005 M KCl, 0·001 M NaH, PO₂), suspended in 0·01 M tris, 0·01 M KCl, 0·005 M KCl, 0·001 M NaH, PO₂), suspended in 0·01 M tris, 0·01 M KCl, 0·005 M KCl, 0·001 M NaH, PO₂), suspended in 0·06 per cent sodium dodecy sulphate—0·01 M EDTA. After 2 h at room temperature enough 7 M CsCl was added to make the solution 1 M in CsCl; it was cooled to 0° C, and centrifuged at 10 K r.p.m. for 30 min to remove most of the caesium dodecy! sulphate—0·10 M EDTA. After 2 h at room temperature enough 7 M CsCl was added to make the solution 1 M in CsCl; it was cooled to 0° C, and centrifuged at 10 K r.p.m. for 30 min to remove most of the caesium dodecy! sulphate. The supernatant was adjusted to 1·56 g/ml, with solid CsCl, and ethidium bromide was added to 200 μg/ml. A sample (3-5 ml.) was centrifuged in a Spinco SW 50 rotor at 43 K r.p.m. at 20° C for 36 h. The bottom and middle hands from four preparations, 3 ml. of packed cells each, were pooled, stored at ~70° C for 1-3 weeks and rebanded to obtain the tube shown. A significant fraction of top band

cent monomers, and the middle band about 60 per cent dimers and 40 per cent monomers. Higher multiples were not included in this count. The dimers contained crossover points at or near the middle of the molecule. Representative electron micrographs of DNA molecules from the middle band are shown in Fig. 2. Dimers were observed on sparsely populated grids. Some dimers were made up of one extended molecule joined to one twisted molecule as shown (Fig. 2c).

We conclude from these observations that the middle band contained joined molecules in which one molecule is a closed circular duplex and the other a nicked circular duplex. The presence of monomers in the middle band is accounted for by the overlap inherent in the breadth of the bands (Fig. 1b) and the size of the fraction taken for electron microscopy. When middle and lower bands from several experiments were pooled and rebanded after some nicking had occurred, the fractional amount of material in the middle band increased substantially while the material in the lower band decreased. Repetition of this procedure resulted in a loss of middle band material. These experiments allow us to dismiss the possibility that the middle band consists of dimers banded at the intermediate position because of a different content of guanine and cytosine.

Measurements of the DNA molecules in electron micrographs made from the middle band (Fig. 3) show that the dimers consist of two molecules both the length of the unjoined monomers. The approximate 5μ length of these molecules from subcellular fractions enriched with mitochondria indicates their mitochondrial origin. The hypotheses that dimers consist of pairs of unequal length or of two populations containing long pairs and short pairs were tested with the χ^2 test and were found not to be statistically significant. The dimers, therefore, consist of two monomeric mitochondrial molecules joined together so that one monomer may be nicked while the other remains closed. The dimers found in the lower band contain joined monomers which are both closed.

The joint between the two molecules is stable in 4.5 molar caesium chloride and survives the hydrodynamic and surface forces which develop when specimens are being prepared. We now consider two possible kinds of joints between the monomeric constituents. kind involves ordinary chemical bonds, either covalent or secondary, between the two constituents, and possibly including joining agents such as proteins. The alternative is that the circular monomers are joined by a topological bond formed by interlocking the two circular monomers. The electron micrographs of dimers from the middle band (Fig. 4) show the interlocked nature of the joint. The molecules were successively shadowed while the specimen was rotated and then while stationary. This procedure reveals the three dimensional details of the two overlaps that are involved in the topological bond. We conclude from photographs of this type that the dimeric mitochondrial molecules are topologically linked circular monomers.

Many rotary shadowed dimeric molecules were examined and they gave the following further evidence that dimers are not joined by ordinary chemical bonds. two intersections between the monomers appeared, under the electron microscope, to be overlapping fibres when the focus was varied about the setting for best focus. In most cases the relations among the levels of the fibres at the two intersections showed that the monomers were interlocked. (b) Photographs of fourteen molecules like those in Fig. 2 were carefully examined by us and by six other people in the laboratory; everyone agreed that there were seven molecules which were catenanes. The rest of the molecules were ambiguously identified (the two possible orientations at each intersection were chosen about equally often). In no case was there agreement that a test dimer represented overlapped molecules. The agreement in the assignment at each crossover was based on

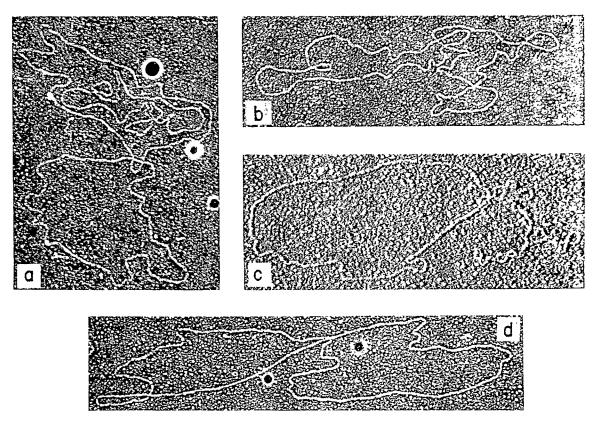


Fig. 2. Representative dimeric molecules from the middle band shown in Fig. 1. The density gradient shown in Fig. 1 was fractionated into approximately 50 μ L fractions. In these conditions the tenth fraction below the centre of the open band contains the peak fraction of the closed band⁴. In this experiment the resultant fractions were diluted with 20 μ L of 10 mg/ml, of cytochrome c and 80 μ L of 0·01 M EDTA, pH 7, and specimen grids were prepared by the procedure of Kleinschmidt and Zalni' with 'Pariodion' covered 200 mesh grids. The hypophase was 0·15 M ammonium acetate, pH 7·4. The DNA-cytochrome films were aged for 10–30 min. The grids were shadowed while rotating with platinum-palladium (except c which was also shadowed from a single direction) and examined in a Philips EM 200 electron microscope. The molecules shown were found in the sixth fraction from the open band. The fourth fraction showed a predominance of the molecules shown in Fig. 5. The eleventh and twelfth fractions contained mostly 5μ monomers with only 10 per cent dimers. (× c, 20,600.)

the continuity or lack of continuity of a light halo (absence of grains) along the edges of the fibres. This halo may be a result of the gathering of the cytochrome from the bulk of the film on to the DNA. (c) Both overlaps otherwise had the same appearance, and the fibres at the joint were not thickened as might be expected if they were held by large protein joiners. (d) Except when the molecules appeared to be pulled from each other and contained only one overlap, there were never sharp corners at the critical overlaps as would be expected if the fibres were joined by mechanically restrictive chemical bonds. From the foregoing we have concluded that at least most of the dimers contained in the middle band (Fig. 1) are catenanes. Our use of the term catenane to describe these compounds follows the usage of Wasserman⁸. The term concatenate has been used to describe oligomers of DNA of unknown structure⁹.

Higher oligomers, n > 2, were also reported previously⁵ and have been found in this study in the lower band and at a higher frequency in the middle band. Trimers, tetramers, pentamers and a septamer have been found, and some examples are shown in Fig. 5. The interlocking nature of these molecules was confirmed by examining the photographs and in some cases by focusing the electron microscope.

We have considered the possibility that accidental overlapping of molecules could result in apparent catenation. Such accidental overlaps occur only infrequently for circular molecules of this length. We have examined photographs of polyoma circular DNA (1.5μ) , and λ phage circular DNA (15μ) as well as mitochondrial DNA, and have found that even at high surface concentrations the DNA molecules seem to avoid one another (Fig. 2a) rather than overlap. A plausible explanation for this

effect is that the complex of DNA and cytochrome is able to move at the surface of the hypophase. Because the DNA-cytochrome films are allowed to age for from

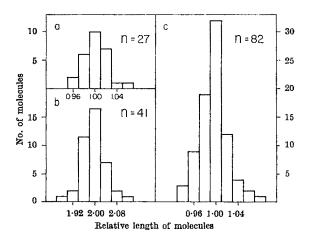


Fig. 3. Length measurements of mitochondrial DNA molecules from the middle band shown in Fig. 1a. Single length (monomeric) molecules. The approximately 5μ lengths were normalized by the mean length for the population. The standard deviation was 2·1 per cent. b, Double length (dimeric) molecules. The lengths were normalized by the factor used in a. The normalized mean length was 1·994 with a standard deviation 2·2 per cent about 2·000. c, Monomeric units contained in dimers. The lengths were again normalized by the mean length of monomers in a. The mean length was 0·997 with a standard deviation of 2·5 per cent about 1·000. The normalization procedure reduces the effect of drift in the magnification factor and stretching of the 'Parlodion' specimen supports. This latter factor is an important cause of scatter. In effect we are using the approximately 5·3\(\mu\) monomers as internal relative standards in photographs of one grid hole. All these data were obtained with one specimen grid. Results from another grid were quantitatively similar. Length measurements were made on low gloss prints with a map measure.

10 to 30 min, the complexes are able to separate from each other and expose to the surface the most possible protein side groups. Careful examination of the dimers which had been shadowed from one direction showed that only very few apparent dimers were accidental overlaps. There is a phenomenon known as flower pattern formation which causes the aggregation of DNA into large complex structures. Flower patterns were observed infrequently on one or two of the grids and were easily distinguishable from the higher oligomers by their very compact nature.

These catenated molecules represent a new class of naturally occurring compounds. Molecules with topological bonds¹⁰ were first identified by Wasserman⁸, who formed catenated dimers of thirty carbon rings. Wang¹¹ prepared non-covalent catenanes by cyclizing viral λ DNA in solutions containing high concentrations of hydrogen bonded phage 186 circular molecules. Topo-

logically bonded DNA molecules have been known for some time. Monomeric mitochondrial DNA and all other closed circular duplex DNAs contain single stranded DNA molecules which are topologically bonded to their respective complements. The pairs of single rings in closed duplex molecules are interlocked by one topological bond which is characterized by a topological winding number, α , of about 450 in polyoma^{12,13} and SV 40⁴ DNA and about 1,500 in closed circular mitochondrial DNA. The quantity α represents the number of times one strand winds about the other when the molecule is constrained to lie in a plane. The corresponding quantity, A, for interlocked duplexes is unity in all the catenanes described here.

In the discussion which follows the circular duplex will be considered as a single circular system (the individual polynucleotide strands will not be considered). Catenated

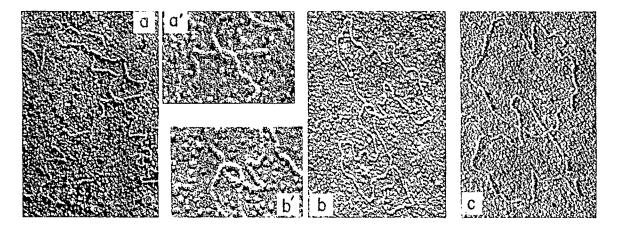


Fig. 4. The interlocking nature of catenated dimers. The specimen grid prepared as described in Fig. 2 was subsequently shadowed from one direction to reveal the three dimensional nature of the intersections. ($\times c$. 18,775 except a' and b' which are $\times c$. 34,600.)

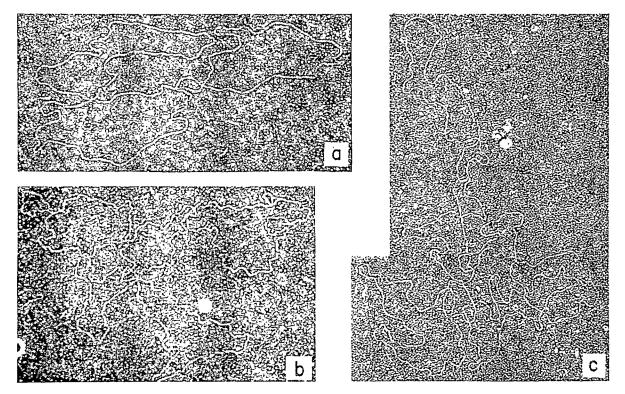


Fig. 5. Higher oligomers of mitochondrial DNA. The specimens were prepared as described in Fig. 2. a, A trimer b_2c . b, A tetramer b_2c_2 . c, A pentamer b_2c_2e . ($\times c$. 20,600.)

trimers differ from dimers in that at least one ring is joined to two others. In a linear trimer two of the molecules have a topological bonding number (TBN) of one, and the third a bonding number of two. All three molecules in the cyclic trimer have a TBN of 2. Isomerism based on the TBN results in a large number of species in the case of higher oligomers. Figure 6 presents the isomers for $n \le 4$ together with a notation system based on the The criterion for determining the TBN is the number of circles that must be cleaved in order to free an intact circle. In this system a stands for TBN=0, b for TBN = 1, and so on. Subscripts indicate the number of monomers of a given topological bonding number in the oligomer. The pentamer shown in Fig. 5c is characterized by the formula b_2c_2e . This system does not specify the topological winding number in the bonds, nor does it distinguish structural isomerism based on the three dimensional arrangement of the topological bond. Figure 7b shows the optical isomers for the cyclic trimer, and

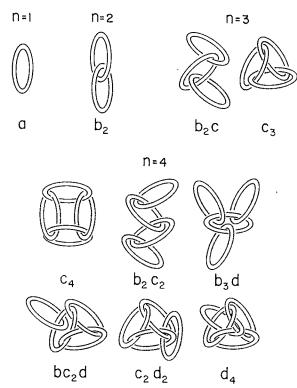


Fig. 6. Topological bonding number (TBN) isomers. The simple connexion of two circles by a single winding (A=1) is regarded as the only type of bond. The formula representations are based on α for TBN=0, b for TBN=1, and so on.

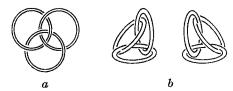


Fig. 7. Examples of topological isomers other than topological bonding number isomers 10. a, A Borromean ring, a trimeric structure with topological bonds based on a linkage type other than simple catenation. b, The pair of enantiomers for one of the c₂ species. There is another pair of enantiomers for a second c₂ species. The latter pair differ from the one shown in the order of the overlaps of the crossings.

Fig. 7a represents a trimer with topological bonds based on a higher type of topological linkage than simple catenation. There is a further type of isomerism, based on the polarity of the strands in the Watson-Crick duplex. which gives rise to two polarity isomers for the catenated dimer and to many further isomers for the higher oligomers.

We now consider the origin of the oligomers in terms of known processes in which the backbone bonds of DNA are formed or re-arranged. We postulate that these molecules arise either in the course of replication or recombination. The mode of replication of closed circular molecules is not yet understood adequately to permit predictions of the possible structures that might be formed as replication intermediates or as a result of errors of replication. The breakage and reunion model for genetic recombination, on the other hand, enables us to formulate a plausible scheme for the formation of the catenated oligomers described. It also enables us to correlate the results presented here with those obtained with mitochondrial DNA from human leucocytes as described in the following article. The model also suggests the existence of structures that have not as yet been The catenated molecules, according to the recombination model, result from a double recombination between two circular mitochondrial DNA molecules. This process could occur as two consecutive single recombination events or as a concerted process in which both recombination events occur within the same pairing region (Fig. 8). Higher oligomers would be formed by double recombinations between a monomer and catenated dimers. Doubly interlocked dimers (A=2) should result from four recombination events. The recombination model for the origin of catenated circles predicts that circular dimers without crossing points should also occur. Such species have been observed at a very low frequency in the bottom (closed) band. It is possible that the low frequency is in part a consequence of the loss of cyclic dimers from the closed band because of statistical nicking. Also, twisted circular dimers are difficult to distinguish from catenated dimers. These circular oligomers of mitochondrial DNA

Fig. 8. The circular mitochondrial molecules first pair (a) and are then "broken" either once (b) or twice (c) as shown. If broken once, reunion results in an open dimer (d) which can pair again (e) and recombine. Half of the products of the second recombination will be catenanes (f) while the other half will be separate circles. If broken twice (c) half of the recombinations will result in separate circles (i) while half will be catenanes (h). Double recombination events between dimeric catenanes and monomers result in trimeric catenanes and this can be continued to higher species.

are more frequent in other systems and are discussed in more detail in the following article. Species involving 10μ circular dimers catenated to 5μ monomers should also be seen but have not yet been observed in mitochondrial DNA of HeLa cells. Double interlocked dimers (A=2) have not as yet been conclusively identified.

The recombination model places the various types of mitochondrial DNA species in a sequence running from circular monomers to circular dimers, to catenated dimers, to higher oligomers. In particular, it predicts that catenated dimers will occur before higher oligomers and that, as catenated dimers increase, circular dimers will decrease. The population of mitochondrial DNA is viewed as an equilibrium population, and the various possible distributions of mitochondrial species as different positions in a multiple equilibrium. The equilibrium position for HeLa cells seems to be well to the right of catenated dimers. Other equilibrium positions are known for human leukaemic leucocytes (see following article).

The sequence of events depicted in Fig. 8 depends somewhat on the particular mechanism of the recombination process. An alternative mechanism which has been proposed, does not involve the initial formation of a four-stranded pairing region between the duplex molecules. Instead the molecules are specifically broken, matched with another specifically broken molecule and reunited. Reciprocal recombinants are not formed in the same recombination event but are statistically equally likely. The ends of the molecule are free to move in solution. The most reasonable proposal for the specific recognition system for the matching of the broken molecules is that the initial breakage gives rise to single stranded cohesive ends on the two fragments. This model of the recombination event differs from the homologous pairing model (Fig. 8) in that catenated dimers can arise from monomers without the intermediate formation of a cyclic dimer. If linear molecules with cohesive ends are formed in low concentration and then recyclize in the presence of closed circles some catenanes will form. According to this model catenanes are only a by-product of recombination; the two members of a dimer have not undergone a recombination event with each other. In conditions of high concentrations of linear molecules with cohesive ends, cyclic dimers would form. Another mechanism for catenane and cyclic dimer formation involves the opening and reclosure of mitochondrial DNA molecules at one specific point in the molecule. This process would be similar to the closure and reopening of λ phage DNA and is presumably not related to recombination.

Genetic recombination between cytoplasmic genomes in Chlamydomonas has been demonstrated in the experiments of Sager¹⁴. Possibly the observed fusion of mitochondria in the cytoplasm¹⁵ is a manifestation of a recombination event. Interaction between the mitochondria of different strains of maize has been reported to result in a new type of mitochondria in the hybrid strain¹⁶. On the other hand, because the number of DNA molecules in specific mitochondria is not known, it is possible that the recombination process described here could occur between molecules in each mitochondrion. Our results show that in some mitochondria, possibly those which are structurally or functionally abnormal, there are at least two to seven mitochondrial-length molecules. The monomeric units of the oligomers described here are all the same length and have approximately the same base composition and are therefore, probably identical genomes. Polymerization of mitochondrial DNA molecules may result in gene duplication which would otherwise not occur.

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Circular Dimer and Catenate Forms of Mitochondrial DNA in Human Leukaemic Leucocytes

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Oligomer forms of circular mitochondrial DNA have been identified in mitochondrial extracts of human leukaemic leucocytes. The oligomers occur as circular dimers with a contour length of $10 \, \mu$ and catenanes made up of interlocked 5μ monomers.

MITOCHONDRIAL DNA from a variety of organisms¹⁻³ has been reported to occur in the form of covalently closed circular duplex molecules approximately 5μ in contour length. These cytoplasmic DNAs resemble polyoma viral DNA4 in several physical chemical properties that are shared by all closed circular DNAs. One such property, the restricted uptake of the intercalating dye ethidium bromide (EB), forms the basis for a convenient centrifuge method for isolating substantially pure mitochondrial DNA7. The finding in this laboratory that HeLa cell mitochondrial DNA preparations obtained by ethidium bromide method contain 10 per cent multiple length molecules, or oligomers prompted us to examine the structure and size distribution of mitochondrial DNA in preparations from animal cells in a variety of physiological states. This report presents the result that mitochondrial DNA from human leukaemic leucocytes contains circular dimers, closed circular duplex DNA molecules



Fig. 1. A caesium chloride—ethidium bromide density gradient showing the two bands obtained with mitochondrial DNA preparations from leucocytes of the chronic granulocytic leukaemic patient, M.C., from March 8 to May 20, 1967. The 3 ml. solution of DNA in caesium chloride at a density of 1-55 g/ml., and 100 \(\textit{\mu}g/ml\) of ethidium bromide, \(\textit{\mu}H = 8-0\), was centrifuged 36 h at 43 K r.p.m. and 20° C in an SW 50 rotorin a Beckman \(L^2 = 65B\) ultracentrifuge. The preparative tube was photographed in near ultraviolet light. In the usual procedure a 500 ml. unit of blood was withdrawn into ACD. The inverted bottle was stored for 3 h in the refrigerator and the red cells were re-infused. The leucocytes and the remaining plasma were then chilled to 4° C. The leucocytes were spun down and resuspended in the homogenizing medium, 0.01 M KCl, 0.01 M tris chloride, 0.005 M Na EDTA, pH 7-5. After homogenization with a tight fitting "Teflon" pestle, sucrose was added to 0.25 M, and the nucle and cell debris removed by centrifugation at 3 K r.p.m. in an SS34 Sorvall rotor for 5 min. Resuspension and centrifugation were repeated. A greenish material, presumed to contain myeloperoxidase, was then pelleted at 5 K r.p.m. for 5 min. The mitochondrial DNA and linear nuclear DNA. The lower band contains closed circular mitochondrial DNA, the threshold level of detection in visible light of a DNA—EB band, from 5 × 10° leucocytes. The recovered material corresponds to 600 mitochondrial DNA molecules in a mitochondrion vary between two and six in different organisms. On the basis of these results we calculate that the lower bands contain 15–50 per cent of the mitochondrial DNA in the cell population.

approximately 10 μ contour length. The preparations also contain catenated dimers, interlocked pairs of 5 μ closed circular DNAs, such as have been shown to occur in HeLa cell mitochondrial preparations (preceding article).

Leucocyte samples were obtained at approximately weekly intervals from the chronic leukaemic (M. C.). Samples from the lower band (Fig. 1) obtained at the beginning of this study were pooled and recentrifuged. The lower band contains the intact mitochondrial DNA molecules which exhibit restricted dye uptake. Examination of the DNA molecules from the lower band in the electron microscope revealed that 26 per cent of the molecules were circular dimers (Table 1). Fig. 2 presents electron micrographs of the types of molecules found. Only 3 per cent catenated dimers and 2 per cent trimers were observed. In addition to the molecules which were scored (Table 1) from prints of fields with high contrast and showing molecules with few overlaps, several thousand molecules were viewed under the electron microscope and scored for the fraction of total dimers This percentage of circular dimers was confirmed by the sedimentation velocity analysis described The histograms (Fig. 3) demonstrate that the dimers have twice the contour length of the monomers.

We note that 49 per cent by weight of the mitochondrial DNA in the lower band obtained from this patient (M. C.)

is in the form of oligomers, and for the most part in the form of circular dimers. It is not yet possible to relate this number—probably a minimum—firmly to the distribution in the leucocytes of the donor. The frequency of oligomers, f, will be affected by any process of selection for or against closed oligomers over closed monomers. The following processes lead to selection if they occur to a significant degree. (a) An incomplete recovery of mitochondria from the different cell types biases the results is the cell types contain different amounts of the variouf oligomer species. Inspection in the light microscope of homogenates stained with azure C revealed that all cell types were effectively disrupted. This effect is therefore not likely to be significant. (b) Hirt11 has shown that very high molecular weight nuclear DNA is spun down with the sodium dodecylsulphate (SDS) precipitate in the isolation procedure. It is conceivable that some very high oligomers may have been lost, and that f was reduced. (c) In the approximately 4 h period between withdrawal of whole blood and the disruption of the mitochondria, nuclease may have been active within the mitochondria. We have no way of assessing this effect, except to note that we have recovered approximately 15-50 per cent of the mitochondrial DNA as closed DNA. (d) After the SDS treatment all the mitochondrial molecules are in a common environment and subject to nicking by reducing agents and active endonucleases that may be present. Because the oligomers are the larger targets, f may have been reduced. Barring an enhanced stability of the oligomers over the monomers in the mitochondria, after withdrawal of blood, the estimates of the frequency of oligomers f (Table 1) are considered to be minimal.

The physical chemical properties of this new form of mitochondrial DNA, the circular dimer, were studied by means of ultracentrifugation. The material from the lower band diluted with an equal volume of twice distilled water and freed of dye by chromatography through a 50 µl. bed volume of 'Dowex-50' cation exchange resin was examined by the band sedimentation velocity procedure12 in 2.85 molar caesium chloride (Fig. 4). The standard sedimentation coefficients calculated for sodium DNA for the three components are $51 \pm 1.4S$, $36.5 \pm 0.8S$, and 26.9 ± 1.7S. Similar experiments in alkaline 2.85 molar caesium chloride showed two discrete components with incorrect sedimentation coefficients of 112S and 80S (Fig. 5). Figure 6 presents a double logarithmic plot of sedimentation coefficient against molecular weight for closed circular DNA with additional data obtained from the literature. The 80S and 112S alkaline components fall on the line for closed circular DNA in alkali. The slow 27S species at neutral pH falls on the line II for nicked circular DNA. The fast 51S component falls above the line I for in vivo closed circular viral DNA at neutral pH, as do all other mitochondrial DNAs so far reported. We conclude that the neutral 51S component represents the compact superhelical form of the circular dimer. 80S and 112S components in alkali represent the fully titrated forms of the closed monomer and dimer respectively. The intermediate 37S component is apparently a mixture of nicked dimers and intact monomers. Figure 6 indicates that the closed monomer should sediment slightly faster than the nicked dimer.

We have calculated from the fractions of intact dimer and nicked monomer observed in Fig. 4 that the original

Table 1. DISTRIBUTION OF MITOCHONDRIAL DNA FORMS ISOLATED AS INTACT CLOSED CIRCULAR MOLECULES FROM NORMAL AND LEUKAEMIC LEUCOCYTES

	Patient M. C.*			Leukaemic Patient S. T. Patient			atient S.	S. B. Normal				
	No.	%	Wt. %	No.	%	Wt. %	No.	%	Wt. %	No.	%	Wt. %
Monomers	205	68	51	639	89	78	1.146	95	91	1,926	98-6	97
Oligomers	95	32	49	80	11	22	57	5	9	27	1.4	3
Circular dimers	79	26	39	27	4	7	22	2	3.5			
Catenated dimers	10	3	5	39	5	10	32	3	5	27	1.4	3
Trimers and higher oligomers	6	2	4.5	14	2	6	3	0.2	0.7	-	_	
Total molecules scored		300			719			1,203			1,953	
Catenane index †		0.1	1		0.5	9		0.59)			

Pooled lower bands from leucocytes obtained from March 8 to June 7, 1967.
 †Catenane index, catenated dimers/total dimers.

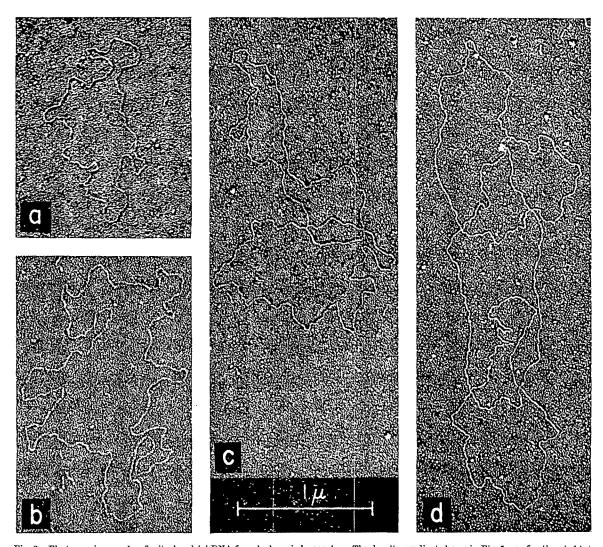


Fig. 2. Electron micrographs of mitochondrial DNA from leukaemic leucocytes. The density gradient shown in Fig. 1 was fractionated into approximately fractions of 50 μl. and specimen grids were prepared with material found ten to twelve fractions below the centre of of the upper band. A sample 125 μl. of the fraction was diluted with 30 μl. of 1 mg/ml. of cytochrome c and 40 μl. of 0.01 M EDTA, pH 8-0. The specimen grids were prepared by the procedure of Kleinschmidt and Zahn¹o with 0.15 M NH,Ac, pH 7-4, as the hypophase. The grids were shadowed while rotating with platinum-palladium and examined in a Philips EM 200 electron microscope. a, Single length mitochondrial DNA; b, circular dimer form; c, catenated timer; d, catenated timer. The catenation was established by focusing at the microscope and by careful examination of the negatives as described by Hudson and Vinograd (preceding article).

lower buoyant band contained 33 per cent dimers. The calculation was carried out with the assumption that statistical nicking occurred after the band was isolated. The agreement between this result and that obtained by electron microscopy (29 per cent, Table I) suggests that there was no significant selection for or against dimers in the specimen grid preparation or in the electron microscope examination.

Further evidence that the 51S component represents a closed circular dimer form was obtained by analytical ultracentrifugation. Mitochondrial DNA recovered from the experiment described in Fig. 4 was centrifuged to equilibrium in buoyant caesium chloride containing ethidium bromide. Conditions were selected so that the closed DNA was separated from the open DNA in the caesium chloride-ethidium bromide gradient. The ratio of amounts of closed to open DNA in the buoyant gradients was 0.5, compared with 0.75 expected for this material in the absence of further nicking. If, on the other hand, the leading component had consisted of nicked dimer and closed monomer, the middle component of nicked monomer, and the slow component of linear DNA, the ratio would have been 0.15.

The buoyant densities of the dye-free mitochondrial and nuclear DNA differed by 0.010 g/ml. (Table 2) in separate experiments with a crab dAT marker. The increment was

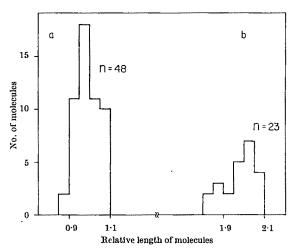


Fig. 3. Normalized contour lengths of mitochondrial DNA from the lower band in a caesium chloride-ethidium bromide density gradient. The DNA was obtained from leucocytes from the chronic granulocytic leukaemic patient, M. C. a, Single length molecules. The approximately 5μ lengths were normalized by the mean length. The standard deviation was ± 0.05 dimensionless units. b, Double length molecules which were predominantly circular dimers. The approximate 10μ lengths were normalized by the mean monomer length. The standard deviation was ± 0.06 dimensionless units.

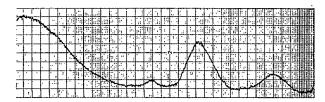


Fig. 4. Band sedimentation velocity pattern of originally closed mitochondrial DNA from M. C. in 2.85 M caesium chloride, pH 8-0, 20° C. Photoelectric scan of the liquid column 40 min after reaching 30 K r.p.m. The field is directed to the right. The leading band contains the 51S closed circular dimer. The middle band contains the 37S unresolved mixture of nicked circular dimer and closed circular monomer. The slowest band contains the 27S nicked circular monomer. The absorbing material between the slowest band and the meniscus is EDTA. No material sediments from this region after longer times at higher speed.

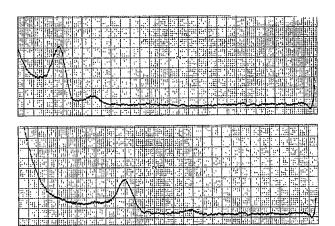


Fig. 5. Band sedimentation velocity pattern of originally closed mitochondrial DNA from M. C. in 2.85 M caesium chloride, pH 12.3, 20° C. Photoelectric scans of the liquid column at 8 min intervals at 28 K r.p.m. The fast band contains the 1125 fully titrated closed circular dimer. The slow band contains the 805 monomer. At higher speeds a broad 265 band sediments out of the region obscured by the absorption of light by EDTA.

also observed in a synthetic mixture of mitochondrial and nuclear DNA (Fig. 7). The buoyant densities of nuclear DNA from leukaemic and normal leucocytes were indistinguishable. The mitochondrial DNA, containing 39 per cent by weight circular dimers, formed a symmetrical buoyant band in caesium chloride in the analytical ultracentrifuge. This result proves that the circular dimer has about the same base composition as the circular monomer and suggests that circular dimers consist of two connected monomer genomes. The clinical and diagnostic data for M.C. and the other two patients in this study are shown in Table 3.

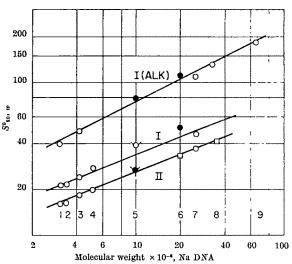
The mitochondrial DNA from leucocytes from the sub-acute granulocytic leukaemic (S.T.) formed three fluorescent bands (Fig. 8). The lower band contained 9 per cent dimers, of which approximately 60 per cent were catenated dimers (Table 1). A histogram of 277 molecules is presented in Fig. 9. We do not regard the difference in oligomer content between M. C. and S. T. as significant

Table 2. Buoyant densities of mitochondrial and nuclear dna from normal and leuraemic human leucocytes on caesium chloride at $25^{\circ}\,\text{c}.$

	'	•	No. of
Source	Nuclear	Mitochondrial	experiments
М. С.	$1.689, \pm 0.0002$	$1.700_0 \pm 0.0003$	3
S. T.	1.689	1.700	i
S. B.	1.689	1.700	ï
Normal donors	1.6895		ĩ

Buoyant densities were calculated from the distances between the above DNAs and a crab dAT marker. In these calculations the buoyant density of the marker was taken to be $\theta=1.669_0$, a value determined by the absolute method. The buoyant density gradient, Vinograd and Hearsi's, was used in the calculations. If these buoyant densities are calculated with an assumed value of 1.710 g/ml. for an $E.\cos DNA$ marker and with a gradient uncorrected for the effects of pressure, the buoyant densities of the nuclear and mitochondrial DNAs are 1.6954 and 1.7055, respectively. These values correspond to a G-C content of 36 mole per cent for nuclear DNA and 46 mole per cent for mitochondrial DNAs.

because the time between the withdrawal of blood and homogenization was 10 h for S. T. compared with 4 h for M. C., and extensive nicking may have occurred. The middle band was midway between the closed and open DNA bands. This position corresponds to the expected



Molecular weight × 10-4, Na DNA

Fig. 6. Sedimentation coefficients of three forms of circular DNA as a function of molecular weight. The results for the fully titrated mitochondrial circular monomer and dimer and for the neutral nicked monomer fall on the best least squares lines for data previously published or in the press. The alkaline sedimentation coefficients represent partially corrected values obtained from experiments at 20° C in approximately 2·85 M caesium chloride, 1·35 g/ml. The data were corrected only for the small effects of solution density when the concentration of caesium chloride departed from the above value. The least squares slope of this line is 0·49. The neutral standard sedimentation coefficients were measured at 20° in caesium chloride or sodium chloride and when necessary have been corrected by the method of Bruner and Vinograd. The slopes for the closed circular DNA, I, and the nicked circular DNA, II, as determined by a least squares method are 0·38 and 0·39. 1, Polyoma⁴; 2, RF φX174 (ref. 14); 3, RF M13 (ref. 15); 4, human papilloma¹⁵; 5, mitochondrial monomer (ref. 2 and unpublished work of Vinograd. Pikó and Blair); 6, mitochondrial dimer; 7, in vivo closed λε₁λε₂ (Lale; I, II, unpublished results of Kyer, Young and Sinsheimer); 8,9, λcl₁s₂ monomer and dimer¹⁷. ♣. This work; □, 6, value calculated from S versus M relation given by Studier of circularization; □, 8,24g DNA¹⁸. The value was raised by the factor 1·14.

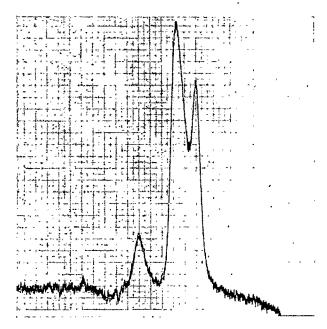


Fig. 7. Photoelectric scan of a synthetic mixture of nuclear and mitochondrial leucocyte DNA from the patient M. C. in neutral buoyant caesium chloride; 25°C, 44 K.r.p.m., Moseley X-Y parallel recorder. The field is directed to the right. The light band contains crab dAT, the middle and dense bands contain nuclear and mitochondrial DNA, respectively.

position for a catenane containing one open and one closed molecule as described for HeLa cell mitochondrial DNA.

The catenane index, the ratio of catenated dimers to total dimers, varies by a factor of six between the preparations from M. C. and S. T. This index for the DNA in the lower band is representative of the index at the time of dissolution of the mitochondria by SDS, and is insensitive to the extent of statistical nicking that may have occurred.

In extreme cases it was easy to discriminate between circular dimers and catenated dimers, but molecules that contained several crossovers were often not scored. Completely open molecules or molecules in which the crossovers occurred only near the ends of the molecules were scored as circular dimers. Dimers consisting of an open monomer and a twisted monomer, dimers with only one crossover which divide it into statistically valid halves, and dimers in which the three dimensional character of the catenation could be seen in the electron microscope or the prints8 were scored as catenanes. We have made the assumption that highly twisted catenanes and circular dimers were rejected with the same probability. catenane index is the best available basis for comparison of the different DNA preparations that we have investigated.

The mitochondrial DNA from the case of chronic lymphocytic leukaemia (S. B.) also formed three fluorescent bands in the caesium chloride ethidium bromide gradient. The DNA in the lower band contained 5 per cent oligomers, of which approximately half were catenated molecules (Table 1, Fig. 9). There was 24 h between the withdrawal of blood and homogenization in this patient, and so the oligomer content may be artificially low.

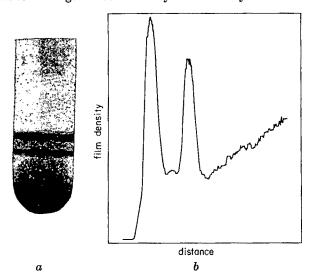


Fig. 8. a, Fluorescence photograph of a caesium chloride-ethidium bromide density gradient as in Fig. 1. b, A microdensitometer tracing showing band positions. The left hand side of the tracing corresponds to the upper part of the tube. The middle band indicates the presence of catenated dimers as shown by Hudson and Vinograd in the preceding article.

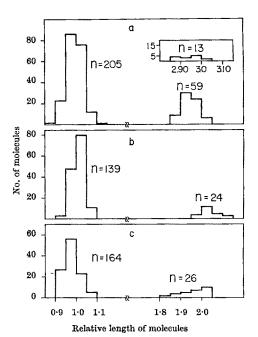


Fig. 9. Histogram of normalized contour lengths of mitochondrial DNA from the lower band in a caesium chloride-ethidium bromide density gradient as in Fig. 3. a, DNA from S. T.; b, DNA from S. B.; c, DNA from normal donors.

The closed mitochondrial DNA from leucocytes of normal donors was examined with material prepared from five samples of fresh blood. Only two fluorescent bands were found. Analyses of the lower band showed that 1.5 per cent of the mitochondrial DNA molecules were dimers (Table 1). A catenane index was not obtained because of the small number of dimers present in the electron micrographs. The period between blood withdrawal and dissolution of the mitochondria by SDS varied between 5 and 12 h.

The results so far can be summarized as follows. (a) Oligomer forms of mitochondrial DNA occur in leucocytes from normal and leukaemic human donors. gomers are principally circular and catenated dimers. Catenated trimers and a very small percentage of catenated higher oligomers also occur. (b) There are differences in the distribution of types of oligomers in the DNA preparations from the three leukaemic patients studied. The preparations from the two patients with clinically more advanced leukaemia (S. T. and S. B.) had a higher catenane index. (c) The circular dimers and catenated dimers represent new forms of mitochondrial DNA. The catenated forms have also been found in HeLa cells (preceding article).

Mitochondrial DNA isolated from M. C. during April to May, 1967, showed only two bands (Fig. 1) while pooled DNA preparations obtained during June to July, 1967, yielded three fluorescent DNA bands. This indicates that

Table 3. CLINICAL DATA FOR THE THREE DONORS OF LEUKAEMIC LEUCOCYTES*

	22-3-67	12-6-67	10-7-67	Patient S. T. 6-7, 22-7-67‡	Patient S. B. 24-7, 4-8-67‡	Normal †
Myeloblasts		3.3%	13.7%	6.0%		_
Promyelocytes	*****	8.3	10.2	23.0		_
Myelocytes	4.0%	17.3	22.0	28.0		
Metamyelocytes	10.0	22.0	27.3	14.0		3.0-5.0%
Band and segmented cells	68-0	44.8	16.0	26.0		54.0-62.0
Eosinophiles			0-3	0.2		1.0-3.0
Lymphocytes	18.0	1.5	4.5	0.5	99%	25.0-33.0
Monocytes		1.0		0.5		3.0-7.0
Nucleated red cells		1.0	6.0	2.0	_	

Patient M. C. had chronic granulocytic leckaemia confirmed by bone marrow aspiration on December 1, 1966. Patient felt well. Patient S. T. had subacute granulocytic leukaemia confirmed by bone marrow aspiration on June 23, 1967. Patient was 70 per cent functional. Patient S. B. had chronic lymphocytic leukaemia confirmed by bone marrow aspiration on May 11, 1967. Patients M. C. and S. T. had received no chemotherapy before the leucocytes were sampled. Patient S. B. had received 'Prednisone' and 'Cytoxan'.

^{*} Wright's stain smears of buffy coats.
† Range of leucocyte counts in normal adults. Normal blood also contains a low percentage of myelocytes²¹.
† Values listed are means.

$$\bigcirc\bigcirc\bigcirc \xrightarrow{a}\bigcirc \xrightarrow{p}\bigcirc \xrightarrow{c}\bigcirc$$

Fig. 10. Formation of oligomers by a postulated breakage and reunion mechanism. a, The circular dimer is formed in a single recombination event between two monomer molecules. b, The catenated dimer could result from a double recombination event between two monomers or a single recombination event within a circular dimer. c, A double recombination event between a catenated dimer and monomer molecule results in the formation of a catenated dimer. This scheme can be continued to form higher oligomers.

there was an increase in the fraction of catenated dimers in the preparations. Electron microscope analysis showed that the fraction of oligomers had remained essentially constant and that the catenane index approximately doubled. The change paralleled a significant shift in the leucocyte population: myeloblasts and promyelocytes increased and there was a corresponding decrease in mature forms.

A genetic recombination pathway based on the breakage and reunion model has been proposed as a possible scheme for the formation of the oligomers described here (Fig. 10) and in HeLa cell mitochondrial DNA (preceding Changes in the equilibrium distribution of oligomers in the leukaemics studied may have resulted either from changes in the proportion of the various cell types or from changes within a given cell type. In either case it seems that there are various equilibrium positions in the monomer-oligomer equilibria.

Other investigations in this laboratory have shown that there are interlocked mitochondrial molecules in a variety of other systems. Mitochondrial DNA of unfertilized sea urchin eggs, for example, contains catenated dimers and a small proportion of higher catenated oligomers (unpublished results of Blair, Pikó and Vinograd). A middle band containing catenated oligomers has been obtained from the mitochondrial DNA of 3T3 mouse cells transformed by SV 40 virus. These results suggest the possibility that oligomers were present but not reported in the mitochondrial DNA preparations earlier described¹⁻³. The elucidation of the relationship between the occurrence of the oligomers and the physiological state of a cell will require further quantitative investigation of a variety of cell systems. The dye-buoyant density method used in this work provides highly purified closed mitochondrial DNA, but as discussed the method selects against open DNA that may be present. In our continuing study of the composition and structure of mitochondrial DNA in normal individuals and in leukaemic patients, nonselective methods are being used to isolate the total complement of DNA in the mitochondria.

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Thermal Poly- α -amino-acids containing Low Proportions of Aspartic Acid

by

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Synthetic poly- α -amino-acids (proteinoids) are prepared by heating together suitable proportions of eighteen common amino-acids. Proteinoids which more closely resemble proteins in amino-acid composition can now be prepared from reactants augmented with lysine and neutral amino-acids.

THE feasibility of preparing poly-α-amino-acids by heating suitable proportions of dry amino-acids has been demonstrated by Fox and associates 1-6. The products of the thermal condensation reactions can range from homopolymers of aspartic acid2 or lysine3 to heteropolymers, termed proteinoids, which contain some proportion of each of the eighteen common amino-acids⁴⁻⁶. Proteinoids exhibit many properties in common with proteins1, and, because they are prepared under geologically plausible

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conditions, they have been regarded as models of abiotic

The amino-acid compositions of proteinoids can in part be regulated in a controllable and reproducible manner⁵⁻⁸. The content of amino-acids in the products reflects, however, the large proportion of dicarboxylic amino-acids or of lysine which is commonly used in the reactants. For example, proteinoids prepared from reactants containing a large proportion of aspartic and glutamic acids typically contain 40-70 per cent aspartic acide; lysine-rich proteinoids, prepared from reactants enriched with lysine free base (as opposed to the hydrochloride salt), contain about

50 per cent lysine⁵. These high proportions of non-neutral amino-acids are not typical of proteins. The proportion of aspartic acid in acid proteinoids has to some extent been reduced by lowering the proportion of aspartic (or aspartic and glutamic) acid in the reactants4,6, but low yields of quite dark products have limited this approach4. method is described here of preparing proteinoids which contain a very low proportion of aspartic acid, using conditions which do not result in diminished yields or dark products. These polymers were obtained from reactants which contained lower proportions of aspartic acid than normally used, but which were augmented with lysine free base. Less satisfactory results were obtained when lysine was omitted or used as the hydrochloride salt. Furthermore, the content of neutral amino-acids in proteinoids containing low amounts of aspartic acid has been considerably increased.

Proteinoids were prepared and processed by a modification of the method of Fox and Harada4, using L-aminoacids. The compositions of the various reactants are given in Table 1. The reactants were heated in an oil bath at 175° C under prepurified nitrogen purge for 3.5 h. After cooling under nitrogen, the crude products were dissolved in normal sodium hydroxide and the resulting solutions were dialysed against running distilled water for 2-3 days and were filtered. The filtrates were acidified to pH 2 and were dialysed for another day. The solutions were again filtered and were then lyophilized. The amounts of insoluble material were quite small; only the soluble, nondiffusible portions were used in later experiments. technique used ensures that the product is purified by true dialysis, rather than by dialytic washing of a suspension; the yields are accordingly lower than those previously reported4.

Samples of proteinoids were hydrolysed, before amino-acid analysis, in constant boiling hydrochloric acid in evacuated, sealed tubes, at 106°-110° C, for 48 h. The analyses were performed on either a Phoenix or Beckman automatic amino-acid analyser. The recoveries of amino-acids, uncorrected for ash or moisture content, were usually about 70 per cent, although a progressive drop in recovery was found in Series A as the proportion of aspartic acid in the polymer decreased. Clear trends in the contents of histidine, arginine and individual neutral amino-acids were not evident, and these have not been separately reported; tryptophan, destroyed by acid hydrolysis. but shown previously to be incorporated into proteinoids, was not determined. The absorption spectra of proteinoids in 0·1 normal acetate buffer, pH 5·0, were determined on a Turner model 210 spectrofluorimeter,

used as a spectrophotometer. The absorption increased rapidly as the wavelength decreased in the range 400–300 mµ; a slight shoulder occurred near 350 mµ, and this wavelength was used to evaluate relative intensities of colour. Optical rotations at the sodium D line, using about 5·0 mg polymer/ml. in 0·067 molar phosphate buffer, pH 6·8, were determined using a Rudolph model 80 photoelectric polarimeter equipped with an oscillating polarizer. Estimates of relative molecular size were made using a 'Bio-Gel P-4' column. The values reported are the percentages of the total sample (determined by the optical density at 220 mµ) which eluted from the column in the same volume required for the elution of bovine albumin. The solvent was 0·1 normal in acetic acid and sodium chloride.

The polymers of Series A (Table 1) were prepared from reactants in which the proportion of aspartic acid was progressively lowered. Lysine was not present, except for the $0\cdot16$ g contained in the equimolar mixture of basic and neutral amino-acids. As the amount of aspartic acid in the reactants decreases, the proportion in the product decreases to 40 per cent. This decrease is accompanied, however, by an increase in the proportion of glutamic acid, such that the content of both dicarboxylic amino-acids is reduced by only 11 per cent. Furthermore, the yield of the soluble, non-diffusible products markedly decreases, and the products become very dark; these results amplify earlier observations⁴.

In Series B, increasing amounts of lysine hydrochloride were added to reactants in which the amount of aspartic acid was progressively reduced. (For each quantity of aspartic acid deleted, a molar equivalent of lysine was added.) In the products of this series, the proportion of aspartic acid drops to 23 per cent; the percentage of glutamic acid again increases, but the sum of the two is decreased by 27 per cent. This drop is accompanied by an increase in the proportion of lysine. The intensity of the colour of the products in Series B decreases somewhat; however, as in Series A, the yield of the product greatly decreases, thus off-setting the advantages gained by using lysine hydrochloride in the reactants.

The reactants in Series C were similar to those in Series B except that lysine was used in the form of the free base instead of the hydrochloride. The yield of the product, instead of decreasing as in the previous series, increases somewhat as the proportion of aspartic acid in the reactants is lowered, and the intensity of the colour of the product substantially drops. The proportion of aspartic acid in the products decreases to less than 10 per cent; although the percentage of glutamic acid again increases,

Table 1. VARIOUS PROPERTIES OF FOUR SERIES OF PROTEINOIDS

		Comn	osition of r	eactant	s (a)			Compo (g/100	sition of pro g amino-a	oducts cids) Sum of		Yield (weight	Optical density at 350 mµ/g	$[a]_{D}^{as}$
Polymer	Asp	Glu	Band N*		Neutrals†	Asp	Glu	Lys	Acidic	Basic	Neutral	per cent)	polymer/1.	(degrees)
A-1 A-2 A-3 A-4	2·0 1·5 1·0 0·5	2·0 2·0 2·0 2·0	2·0 2·0 2·0 2·0	0·0 0·0 0·0	0·0 0·0 0·0	65 58 53 40	14 17 20 28	4 6 6 6	79 76 73 68	$\begin{array}{c} 7 \\ 8 \\ 9 \\ 12 \end{array}$	13 16 18 22	2·2 2·0 1·6 0·9	1·1 2·1 3·2 — ‡	
$\begin{array}{c} B-1 \\ B-2 \\ B-3 \\ B-4 \end{array}$	2·0 1·5 1·0 0·5	2·0 2·0 2·0 2·0	2·0 2·0 2·0 2·0	0·0 0·7§ 1·4§ 2·1§	0·0 0·0 0·0	75 51 38 23	11 19 23 36	4 18 29 28	86 70 61 59	6 20 30 30	8 9 8 12	2·8 2·7 1·6 0·6	1·7 1·3 1·3 1·2	=
C-1 $C-2$ $C-3$ $C-3a$ $C-4$ $C-5$	2·0 1·5 1·0 0·75 0·5 0·25	2·0 2·0 2·0 2·0 2·0 2·0	2·0 2·0 2·0 2·0 2·0 2·0	0.0 0.55¶ 1.1¶ 1.4¶ 1.65¶ 1.9¶	0∙0 0∙0	68 49 25 16 9 5	15 19 32 34 35 42	4 17 25 34 32 36	82 68 58 50 43 47	6 19 30 37 37 39	11 12 13 13 21 14	3·6 3·8 3·8 4·0 4·2 2·0	1·4 1·0 0·6 0·6 0·5 0·5	-6.6±0.3 -6.6±0.4 -7.2±0.2 -6.9±0.3
D-1 D-2 D-3 D-4	0·5 0·5 0·5 0·5	1.5 1.5 1.5 1.5	2·0 2·0 2·0 2·0	1.65¶ 1.65¶ 1.65¶ 1.65¶	2·2 2·95	9 8 8 7	31 32 29 29	35 32 31 32	40 40 37 37	37 35 34 34	28 25 29 29	1·4 1·3 1·0 1·3	0·8 0·9 1·0 0·9	$\begin{array}{c} -6.1 \pm 0.9 \\ -5.2 \pm 0.9 \\ -5.8 \pm 1.0 \\ -4.7 \pm 0.8 \end{array}$

^{*} Equimolar mixture of 16 basic and neutral amino-acids (cf. ref. 4); 2.0 g contained 0.16 g lysine hydrochloride.

[†] Equimolar mixture of 13 neutral amino-acids, in addition to those present in basic and neutral mixture.

[‡] Very dark and partially insoluble.

 $[\]S$ Lysine hydrochloride, additional to that present in basic and neutral mixture.

I Lysine free base, in addition to the lysine hydrochloride present in the basic and neutral mixture.

the sum of the two dicarboxylic amino-acids is 50 per cent or less in three cases. The content of lysine again increases. The relatively high value for neutral amino-acids noted for polymer C-4 is probably atypical; the value obtained with a similar polymer of another (untabulated) series was 15 per cent. Results with other series of polymers similar to A, B and C, however, have given comparable results in terms of yield, intensity of colour and amino-acid com-

Although the amount of glutamic acid in the reactants of the first three series was not varied, the proportion of this amino acid in the products increased regularly as the amount of aspartic acid present decreased. These results provide further evidence (compare ref. 1) that the thermal polymerization of amino-acids is internally directed in a non-random manner.

The reactants in Series D contained relatively large proportions of neutral amino-acids, in addition to appropriate quantities of dicarboxylic amino-acids and lysine free base. The proportions of neutral amino-acids in the products are about twice as large as those found in Series Band C. Within the series, the content of neutral aminoacids in the product increases slightly as their proportion in the reactants is increased; concomitantly, the percentage of dicarboxylic amino-acids decreases slightly. intensities are low and uniform, and yields, although somewhat lower than in Series C, are uniform. Comparable series of polymers, in which increasing proportions of basic and neutral (instead of only neutral) amino-acids were used, gave similar results, except that the yields decreased progressively. In these series, as in Series D, the products obtained contain about a third each of neutral, acidic and basic amino-acids. (Independent of this work, Waehneldt and Fox have obtained proteinoids that contain about 60 mole per cent neutral amino-acids¹².)

The polymers tested were optically active, although the magnitude of the optical rotation was small compared with that of proteins¹³. These specific rotations are similar to those observed for acid-type proteinoids7; thermal polymers of lysine have shown little or no optical activity14. Some of the proteinoids were tested for catalytic action for decarboxylating oxalo-acetic acid. Unlike thermal polymers consisting predominantly or solely of residues of lysine 14,15, the present polymers exhibited little or no catalytic activity.

The percentages of each polymer eluting from a 'Bio-Gel P-4' column in the same volume required for bovine albumin were 12, 16, 22, 32, 36 and 34, respectively, for the polymers C-1 to C-5. This increase parallels the increase in lysine content, an observation that is consistent with the earlier finding⁵ that proteinoids rich in lysine are of greater molecular weight than are acid proteinoids.

This investigation has shown that the addition of lysine free base to reactants comprised predominantly of dicarboxylic amino-acids leads to thermal condensation products of which the contents of aspartic acid can be easily controlled without adversely affecting either the yield or appearance of the product. In the presence of a sufficient proportion of neutral amino-acids and lysine free base, products are obtained which contain about a third each of basic, dicarboxylic and neutral amino-acids. These proportions of amino-acids, which are intermediate between those of acid and lysine-type proteinoids. resemble more closely the composition of proteins than earlier described for thermal polyamino-acids. Such polymers thus may be more suitable for use as models of biogenic and of abiogenic proteins.

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The Thymus and the Precursors of Antigen Reactive Cells

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The thymus is important in providing an adequate pool of immunologically competent cells. Thymectomy does not reduce the number of precursor cells, but removes the influence necessary for their differentiation into antigen reactive cells.

THE bone marrow is a source of primitive stem cells which are characterized by their capacity for extensive proliferation, self renewal and differentiation into a variety of more mature cells. The colony forming unit is a stem cell which gives rise, in irradiated hosts, to large clones of erythroid and myeloid cells1. In both irradiated and non-irradiated animals there is a steady stream of cells

from the bone marrow to the thymus and to secondary lymphoid tissues, such as the lymph nodes and spleen. There are thus, in the bone marrow, cell types capable of repopulating the lymphoid compartment of the thymus and the pool of immunologically competent cells²⁻⁴. It is not clear whether there are separate stem cells for the myeloid tissues, the thymus and the immuno-competent, cell pool, although recent work suggests that the colony forming unit may also function as a lymphoid stem cell⁵.

The thymus plays an important part in building up an adequate pool of immunologically competent cells. When thymectomy is performed before this pool has been built up, for example, at birth, the number of cells in it is markedly less than normal and an impairment of those immunological reactions known to be mediated by these cells is immediately evident^{6,7}. When thymectomy is performed in adult life, or after an adequate pool has been built up, no immunological defects become evident until months later⁸⁻¹⁰, presumably after the pool has been depleted as a consequence of the limited life span of some of its cells and the immunological commitment of others. If, however, mice which have been thymectomized in adult life are subjected to total body irradiation, the anatomical and functional regeneration of the immune system is impaired¹¹⁻¹³. The exact relationship between the thymus, the pool of immuno-competent cells and precursors of them is unknown. Very few lymphocytes within the thymus can be detected as antigen-reactive cells¹⁴ and very few cells can be shown to have emigrated from the intact thymus¹⁵. It is unlikely that the thymus exerts an effect on the population of immuno-competent cells once they have been produced, for antigen-reactive cells respond to antigen by differentiation and proliferation to antibody-producing cells as efficiently in the normal as in the thymectomized animal⁶⁻⁷. Neonatal thymectomy thus does not inhibit the response of antigen-reactive cells to antigen and must therefore impair the production of antigen-reactive cells from some more immature, antigenindependent, precursor cells.

We report here the results of a time course study on the appearance of antigen-reactive cells in normal and thymectomized irradiated mice injected with bone marrow cells, thymus cells or both. Antigen-reactive cells are detected by their ability, when injected into heavily irradiated mice together with sheep erythrocytes¹⁴, to produce discrete clusters of haemolysin-producing cells in the spleen¹⁴. In this system, antigen-reactive cells are assumed to be the immediate precursors of antibody-producing cells and to belong to the same cell lineage. They are defined as cells which react to antigen, not by the production of detectable amounts of antibody, but by undergoing proliferation and differentiation to give rise to a line of antibody-producing cells.

Precursors of antigen-reactive cells are detected by incubating, in irradiated hosts and for varying periods of time, cell populations which lack antigen-reactive cells (such as bone marrow or thymus) in the absence of the relevant antigen. At the end of the incubation period, the spleen and thoracic duct lymphocyte pool of the irradiated recipients are assayed for their content of antigen-reactive cells. Mice of the highly inbred CBA strain were used in all experiments. Thymectomy of neonatal or adult mice was performed as described earlier16. Bone marrow cells were obtained from the femurs and tibiae of 2 month old normal mice and 2 month old clinically healthy mice thymectomized at birth, and were injected intravenously into 2-3 month old recipient mice of the same strain. Neither thymus cells nor sheep erythrocytes were injected into the irradiated hosts in the first experiment reported here. Some of the recipient mice had been thymectomized when 6 weeks old and all had been subjected to 900 rads of total body irradiation 4 h before marrow injection. They were killed at weekly intervals and the number of cells in their spleens capable of reacting to sheep erythrocytes was determined by the haemolytic focus assay technique of Kennedy et al.14.

As Fig. 1 shows, bone marrow from either normal or neonatally thymectomized donors contains precursors which, after a period of incubation of between 1 and 2 weeks in irradiated hosts, have developed into sheep erythrocyte-reactive cells. After between 3 and 4 weeks, the numbers of such cells produced in the spleens of the

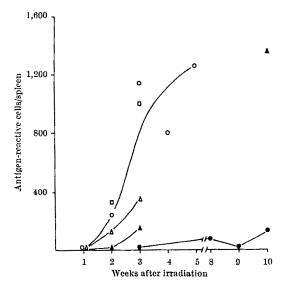


Fig. 1. Number of antigen-reactive cells detected at weekly intervals in the spleens of heavily irradiated CBA mice injected with bone marrow cells: \bigcirc , mice injected with 10° to 10° bone marrow cells from normal donors; \triangle , mice injected with 10° bone marrow cells from normal donors; \bigcirc , mice injected with 10° bone marrow cells from normal donors; \bigcirc , mice injected with 10° to 10° bone marrow cells from normal donors; \bigcirc , mice injected with 10° to 10° bone marrow cells from normal donors; \bigcirc to 10° bone marrow cells from normal donors. Each point represents the average value of five to forty determinations.

non-thymectomized irradiated mice inoculated with 106 or 107 bone marrow cells have reached the levels found in normal mice, that is, 1,000-2,000 (refs. 6 and 7). In contrast, the number of antigen-reactive cells detected in the spleens of adult thymectomized irradiated hosts never exceeded 200, even as late as 10 weeks after irradiation. The irradiated mice injected with marrow had their thoracic duct cannulated 2-9 weeks after irradiation using a method modified from that of Boak and Woodruff¹⁷, and the number of circulating lymphocytes and antigenreactive cells was determined as described previously4. Figure 2 shows that the number of cells recoverable in 48 h in sham-thymectomized irradiated mice increased from less than 10 million 2 weeks after irradiation to 40 million 7 weeks later. By contrast, no significant increase in the size of the 48 h pool was recorded from 2 to 9 weeks after irradiation and marrow protection in adult thymectomized mice. The total number of antigenreactive cells in the 48 h mobilizable pool increased between 4 and 9 weeks after irradiation from 120 to 1,000 in the sham-thymectomized irradiated controls but only from 15 to 66 in the thymectomized irradiated mice The number of antigen-reactive cells per million thoracic duct lymphocytes was virtually the same in mice of both groups 4 weeks after irradiation and then increased significantly only in the controls. By 9 weeks after irradiation the thymectomized irradiated mice, in comparison with controls, showed not only an absolute deficiency of antigen-reactive cells but also a reduced proportion of such cells per million lymphocytes. similar deficiency has been reported before in neonatally thymectomized mice4.

All these data clearly indicate that the precursor cells, which are antigen-independent and derived from marrow, differentiate and proliferate into antigen-reactive cells

Table 1. Number of sheep-erythrocyte-antigen-reactive cells in the 48 h mobile lymphocyte pool of sham-thymectomized and thymectomized, marrow protected, irradiated mice

Group	Weeks after irradiation	reactive cells/	no. of antigen- reactive cells/ 48 h mobilizable pool
Sham-thymectomized	4	5	120
irradiated	9	25	1,000
Thymectomized	4	3	15
irradiated	9	6	66

largely under the influence of the thymus. Precursor cells of marrow origin cannot, on their own and in the absence of the host thymus, differentiate into antigenreactive cells. Furthermore, it is evident that the neonatally thymectomized mouse does not lack precursor cells but simply lacks the influence necessary to drive these cells along the path leading to the production of antigen-reactive cells. Two questions are immediately evident: what is the nature of this thymus influence and why is there a lag period of about one week before antigenreactive cells become detectable?

The simplest relationship between the thymus and the pool of antigen-reactive cells would be one in which lymphoid precursor cells of marrow origin are transformed in the thymus to lymphocytes, some of which migrate out and mature further to become antigen-reactive cells. If this is true, the one week lag period already noted could be explained by the failure of bone marrow cells to repopulate the thymus of irradiated hosts during the first week after irradiation¹⁸. Furthermore, antigen-reactive cells ought to be detected in populations of thymus lymphocytes incubated for prolonged periods of time in irradiated hosts. Accordingly, experiments were set up to check these possibilities. One hundred million marrow cells or 100 million thymus cells were given by slow intravenous injection to two sets of irradiated mice, and spleen cells from these were transferred after one week into two further sets of irradiated mice. Cell suspensions from the spleens were passaged serially at weekly intervals into further irradiated recipients, one spleen equivalent being passaged each time. The controls received no initial inoculum and only irradiated spleen was serially transferred. At each passage, aliquots of the cells pooled from the spleens of one set of mice were assayed for their content of antigen-reactive cells by the haemolytic focus assay method14. There was no histological evidence of thymus lymphocyte repopulation in irradiated mice killed at weekly intervals in this experiment. It can be seen from Fig. 3 that incubation of the original thymus cell suspension in successive generations of irradiated hosts for up to 3 weeks yielded no more than fifty antigenreactive cells. Similarly, incubation of the original bone marrow cell suspension for a total period of 3 weeks gave values not significantly different from those obtained when only irradiated spleen was passaged. This last result is in contrast to that obtained when the bone

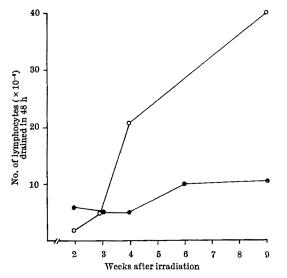


Fig. 2. Total number of lymphocytes drained in 48 h from the thoracic duct of adult *CBA* mice cannulated at intervals from 2 to 9 weeks after sham-thymectomy (\bigcirc) or thymectomy (\bigcirc) at 2 months of age, 900 rads and injection of 10° syngenet marrow cells. Each point represents the average of determinations made on three to four mice.

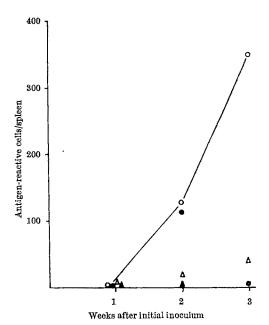


Fig. 3. Number of antigen-reactive cells detected in the spleens of heavily irradiated mice after the injection of bone marrow or thymus cells. ○, Number detected in mice receiving one initial inoculum of 10³ bone marrow cells from normal adult donors (from Fig. 1); ♠, in mice incubating for only 1 week spleen cells transferred at weekly intervals from successive sets of irradiated mice initially inoculated with 10³ bone marrow cells; △, in mice incubating for only 1 week spleen cells transferred at weekly intervals from successive sets of irradiated mice initially inoculated with 10³ thymus cells; ♠, in mice incubating for only 1 week spleen cells transferred at weekly intervals from successive sets of irradiated mice initially inoculated with 10³ thymus cells; ♠, in mice incubating for only 1 week spleen cells transferred at weekly intervals from successive sets of irradiated mice not initially inoculated. Each point represents the average of five to ten determinations.

marrow inoculum was incubated for 3 weeks in a single irradiated host. It can be argued that, because the original suspension is diluted at each transfer, the number of marrow cells incubated for the entire period of time must be less than 10⁸. But, even if a ten-fold dilution at each passage is assumed, so that only the equivalent of 10⁵ cells was incubated for 3 weeks, at least 350 antigenreactive cells would be expected in each spleen in the final host (Fig. 1).

These results, taken at their face value, suggest that the thymus lymphocyte population lacks cells capable, on their own, of giving rise to cells producing haemolysin, even after 3 weeks in successive generations of irradiated hosts. This does not support the hypothesis that bone marrow precursors become antigen-reactive cells only after differentiating within the thymus. The failure of bone marrow precursors to become antigen-reactive cells in the absence of thymus repopulation (Fig. 3) does, however, suggest the possibility that thymus lymphocytes might be essential for the rapid differentiation of precursors derived from marrow into haemolysin-producing cells. Experiments were therefore set up in which either 108 thymus cells or 108 bone marrow cells were incubated with or without sheep erythrocytes for one week in heavily irradiated hosts; cells from the spleens of these mice were transferred to a second group of irradiated mice together with either sheep erythrocytes only or with 107 bone marrow cells and sheep erythrocytes. The capacity of the second host to produce haemolysin-plaque-forming cells was assayed 4, 6 and 8 days after transfer according to the technique of Jerne¹⁸. Figure 4 shows the results obtained when spleens were transferred from thymusincubating donors and Fig. 5 shows results obtained when spleens from bone marrow incubators or uninoculated donors were transferred. It is evident that a significant plaque forming cell response occurred within 6 days in mice which received bone marrow cells, sheep erythrocytes and spleen transferred from those irradiated donors inoculated with thymus cells and sheep erythrocytes one week

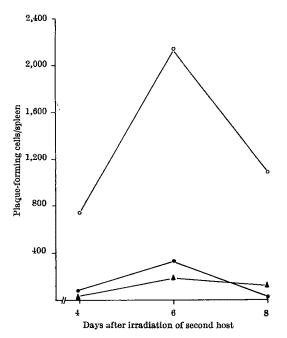


Fig. 4. Production of haemolysin-plaque-forming cells in the spleens of heavily irradiated mice (second hosts) injected with cells (sheep erythrocytes (SRBC) and/or bone marrow cells (BM)) and spleen transferred from a first irradiated host injected with thymus cells (T) and/or SRBC.

Group	Cells injected into first host	Cells in into seco	Symbol denot- ing number of plaque-forming cells in second host	
1	10°T + 10°SRBC	Spleen from first host	+10° BM +10° SRBC	0
2	$10^8\mathrm{T}+10^8\mathrm{SRBC}$	Spleen from first host	+10° SRBC	•
3	108 T	Spleen from first host	+107 BM +108 SRBC	A

Each point represents the average of three to twelve determinations.

before. There was no significant response if thymus cells were incubated without sheep erythrocytes in the first irradiated host, and a similar result was obtained if bone marrow cells were not given to the second irradiated host. We do not think that the response in the latter host can be ascribed to the transfer of lymphocytes of the type found in the circulating pool contaminating the thymus cell population and stimulated by the antigen given to the first host. If this had been so, one could have expected, in the second host, a response which was not dependent on the simultaneous presence of bone marrow cells.

Our results corroborate those obtained by Claman et al.20, who showed that suspensions containing a mixture of adult marrow and thymus cells were far more active in producing haemolysins against sheep erythrocytes when transferred to irradiated syngeneic recipients than could be accounted for by summating the activities of each cell population alone. They also show that the thymus lymphocyte population has had to react with antigen in some way before interaction with bone marrow cells could be expected to result in significant haemolysin production. The nature of the reaction between thymus cells and antigen is obscure, but it is presumably the same as that which has been observed when cells derived from a thymus graft responded vigorously to antigenic stimulation by mitosis^{21,22}, a response which did not, by itself, lead to antibody production23.

All the experimental evidence thus indicates that there is some sort of interaction between thymus cells, bone marrow cells and antigen. Two alternative interpretations of the results will be offered here. One possibility is that antigen-reactive cells are derived from the thymus but fail to react to sheep erythrocytes by producing plaque

forming cells in heavily irradiated hosts, because of the destruction or disruption of an essential mechanism for trapping antigens. It has been shown that the splenic follicles and marginal zone cells play a part in trapping antigens²⁴, and that they are destroyed by irradiation²⁵ and rapidly reconstituted by cells derived from bone marrow¹⁸. In these experiments, cells reactive to sheep erythrocytes could be derived directly from some of the thymus lymphocytes, the bone marrow population providing cells essential for the repair of the antigen trapping apparatus.

Our failure to detect precursors of antigen-reactive cells in populations of thymus lymphocytes would suggest an alternative interpretation; that the precursors of the haemolysin-producing cells are derived from marrow. The thymus might then provide cells necessary for trapping antigen or, on the other hand, thymus cells may be essential for the rapid differentiation of marrow precursor cells into antigen-reactive cells. It is difficult, however, to envisage the mechanism of action of these thymus cells.

In summary, sheep erythrocyte-antigen-reactive cells are the progeny of antigen-independent precursor cells, the differentiation of which is dependent on the thymus. The thymectomized animal does not lack precursor cells but lacks the influence necessary for their differentiation into antigen-reactive cells. Such reactive cells have not been detected after the incubation of thymus lymphocytes for up to 3 weeks in irradiated hosts. When, however, marrow cells were introduced, haemolytic, plaque forming cells appeared within a week in response to a challenge of sheep erythrocytes. One interpretation of these results is that the thymus does indeed provide antigen-reactive cells but that these cannot be detected in irradiated hosts

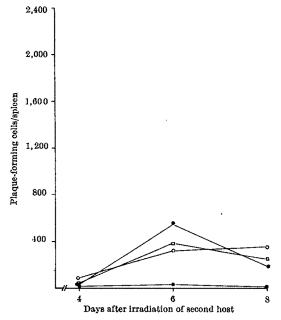


Fig. 5. Production of haemolysin-plaque-forming cells in the spleens of heavily irradiated mice (second hosts) injected with cells (sheep erythrocytes (SRBC) and/or bone marrow cells (BM)) and spleen transferred from a first irradiated host injected with BM and/or SRBC.

Group	Cells injected into first host	Cells into seco	njected ond host	Symbol denot- ing number of plaque-forming cells in second host
4	10° BM + 10° SRBC	Spleen from first host	+10°BM +10°SRBC	0
5	$10^8 \mathrm{BM} + 10^8 \mathrm{SRBC}$		+10° SRBC	•
6	108 SRBC	Spleen from	+10° BM	α.
7	108 SRBC	first host Spleen from	+10° SRBC +10° SRBC	

Each point represents the average value of four determinations. The graph is on the same scale as that in Fig. 4.

unless marrow cells are made available presumably to repair an essential antigen-trapping apparatus disrupted by the irradiation. Another interpretation is that thymus lymphocytes lack antigen-reactive cells or their precursors but act to provide a stimulus to ensure the rapid differentiation of precursor cells derived from marrow.

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Note added in proof (November 6, 1967). Since this paper was submitted, we have demonstrated that the precursors of the haemolysin forming cells are derived not from either the thymus or thoracic duct lymphocytes but from bone marrow²⁶.

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Haemoglobin Hammersmith (β 42 (CDI) Phe \rightarrow Ser)

by

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The forty-second amino-acid of the β-chain of human haemoglobin and its equivalent in all known globins (CDI) is phenylalanine; it stabilizes the haem by direct contact. In Haemoglobin Hammersmith, replacement by serine causes instability of the molecule and thereby severe haemolytic Heinz body anaemia.

A HEAT precipitable haemoglobin fraction has been reported in a female child suffering from a congenital anaemia who was studied for several years at the Royal Postgraduate Medical School, Hammersmith^{1,2}. recently, an unrelated infant suffering from an apparently identical disorder was observed in Coventry. Both patients have a severe haemolytic anaemia unrelieved by splenectomy and red cells containing large inclusion bodies This article summarizes studies on (Heinz bodies). the haemoglobin of these patients. In both cases, it was established first that the precipitable haemoglobin did not separate from haemoglobin A on electrophoresis at alkaline pH, and that there were no peptide chains carrying charges different from those of normal α and β chains of haemoglobin A $(\alpha_2 \beta_2)$ when the haemoglobin of either patient was submitted to starch gel electro-phoresis in 6 molar urea³. The haemoglobin was precipitated by adding one part each of 0.1 molar phosphate buffer, pH 7.4, and 0.01 molar zinc chloride to one part of 10 per cent haemoglobin solution. The washed precipitate was dissolved in 0.1 normal hydrochloric acid and precipitated with acetone4, and fingerprints and peptide analysis were carried out as described before. This procedure showed that the position of the tryptic peptide (βTpV), which comprises residues 41-59 of the 146 aminoacid residues of the \beta-chain, had changed. Some fingerprints showed only one peptide with the electrophoretic mobility of βTpV but with a much lower chromatographic (vertical) mobility (Fig. 1). Other fingerprints (Fig. 2)

showed three peptides with the electrophoretic mobility of βTpV and βTpV oxidized (ox). "Ox" indicates that the methionine in position β55 has been oxidized to methionine sulphoxide during the handling of the haemoglobin The three peptides were in the position of specimen. β TpV, β TpV ox and the third was below β TpV ox at about the same distance below the peptide at which βTpV ox is found below βTpV . A positive staining reaction for methionine was given by the uppermost peptide in the position of βTpV , and also by that in the position of βTpV ox, whereas in the case of haemoglobin A the peptide in this position does not stain for methionine. The lowest peptide did not give this reaction.

These findings were interpreted as indicating an aminoacid abnormality of \$TpV which involves a decrease in the hydrophobic properties of this peptide. The upper peptide which gave a faint reaction only for methionine and also for ninhydrin would represent the normal BTpV and arise from some coprecipitation of normal haemoglobin; the principal peptide in the middle would be the new \$TpV, and the lowest peptide would be its oxidized

The principal abnormal peptide was purified by successive paper electrophoresis and chromatography. To prevent contamination by normal \$TpV ox, which would be found in the same area of the fingerprint, the isolated vertical BTpV strip obtained after electrophoresis was exposed to hydrogen peroxide vapour for 6 h. This treatment converts methionine into methionine sulphoxide6



Fig. 1. Fingerprint (peptide chromatogram) of a nearly pure haemoglobin Hammersmith obtained by zinc precipitation. The upper arrow indicates the area where the β TpV is found in haemoglobin Å. The lower arrow indicates where β TpV is found in haemoglobin Hammersmith.

and confers on the principal abnormal peptide the chromatographic mobility of the lowest of the three peptides discussed here, thus freeing it from any possible contamination with normal \$TpV ox.

One-third of the purified peptide obtained from paper was hydrolysed with 6 normal hydrochloric acid for 42 h in a sealed evacuated tube. A control sample of the The prolonged normal peptide was treated similarly. hydrolysis time was used throughout this work, in view of the resistance to acid hydrolysis of the β41-42 Phe-Phe bond. The results of amino-acid analysis are given in Table 1, where it will be seen that the abnormal peptide differs from $\beta ATpV$ by an extra serine residue and the loss of one residue of phenylalanine. Such a replacement of a hydrophobic residue by a polar residue explains the decreased chromatographic mobility of the abnormal peptide.

Table 1. Amino-acid composition of normal and abnormal $\beta {\rm TpV}$ and chymotryptic peptides β 41–45

Chymotryptic peptides 41-45 βTpV Normal Abnormal No. of residues of: Normal Abnormal Amino-acid Aspartic acid Threonine Serine Glutamic acid 1.0 3.0 1.2 1.9 2·1 1·0 Proline Glycine Alanine Valine Methionine 0.8 1.1 2.0 Leucine Phenylalanine Lysine Yield/residue 3.0 2.0 0.9 0.020 0·9 0·043 0.030 0.023

As Table 2 shows, βTpV contains three residues of phenylalanine7. To determine which phenylalanine had been replaced, the remaining two-thirds of the purified abnormal peptide were digested with chymotrypsin for 4 h. The double rules in Table 2 indicate where chymotryptic cleavage would be expected, and the arrows indicate where most important splitting occurred. Figure 3 shows a fingerprint of the chymotryptic digest—a similar digest of the normal \beta TpV was prepared at the same time and a

fingerprint was also prepared. The dotted line in Fig. 3 shows the area where a peptide was found in the fingerprint of the normal digest but not in that of the digest of the abnormal \$TpV, and below a peptide is found which is absent in the digest of the normal peptide. This is the only difference between the two digests. Table 1 shows the analysis of these two chymotryptic peptides. In the case of the control, the amino-acid composition corresponds to βA 41-45, that is, Phe, Phe, Glu, Ser, Phe, but the abnormal peptide has the composition Phe, Ser, Glu, Ser, The substitution is provisionally placed in the second position, as the ninhydrin colour in the abnormal peptide was blue whereas an N terminal serine would cause the ninhydrin stain to be brown, and the presence of the C terminal phenylalanine was indicated by a chymotryptic cleavage at β 45.

This deduced sequence was confirmed by dansylation of the chymotryptic peptide according to Gray and Hartley and Smellie and Hartleys, By this means it could be demonstrated that the N terminal sequence of the abnormal tryptic peptide BV and of the abnormal chymotryptic peptide β 41-45 was Phe-Ser. The formula of the haemoglobin Hammersmith can thus be written α₂β₂ 42 Phe-→Ser or in the helical notation β CD1 Phe-→ Ser.

To determine the proportion of the abnormal haemoglobin in the whole haemolysate a fingerprint was prepared after oxidation of the globin with hydrogen peroxide vapour. The oxidized normal and abnormal BV peptides were eluted, their amino-acids were analysed and the yields of each peptide were compared. In addition, the total BTpV from the whole haemolysate was analysed after electrophoresis followed by gel filtration ('Sephadex G-25') to remove any contaminants from paper. proportions from the integrated values of serine were compared with other residues in the combined peptide. The result obtained from these two experiments showed that the abnormal haemoglobin amounted to 30 per cent of the total. This proportion of haemoglobin Hammersmith in the haemolysate underestimates the original amount of the abnormal fraction, because part of it would have been made unavailable in the form of Heinz bodies.

				Table !	2. AMI	NO-ACI	SEQU	ENCE (OF B T	DV OF	HUMAN	HAEM	OGLOB	N A					
Sequential No.	41	42 CD	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57 Te	58	59
Helical No.	17	1	2			5					2								
	Phe	Phe	Glu 	Ser	Phe	Gly	Asp	Leu !	Ser	Thr	Pro	Asp	Ala	Val	Met	Gly	Asn	Pro	Lys
			n.			•										•			

The double rules (||) indicate expected points of chymotryptic cleavage, and arrows indicate the position of principal breaks.

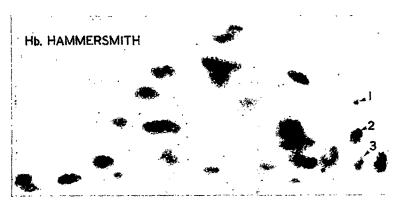


Fig. 2. Fingerprint (peptide chromatogram) of partially purified haemoglobin Hammersmith. (1) Normal β TpV staining positive for methionine. (2) Abnormal β TpV staining positive for methionine. This peptide is found in the position of normal β TpV oxidized, which would not yield a positive methionine reaction. (3) A small amount of abnormal β TpV oxidized.

When Grimes et al.2 labelled reticulocytes with radioactive leucine, almost 50 per cent of the newly formed haemoglobin was heat precipitable.

The abnormality in haemoglobin Hammersmith is of the same type as that observed in haemoglobin Köln β FG4 Val \rightarrow Met¹⁰, Genova β B10 Leu \rightarrow Pro⁴ and Sydney β Ell Val→Ala¹¹. When haemoglobin Sydney was described the repeated findings in unstable haemoglobins of amino-acid substitutions not involving a change of charge were suggested to be in a definite pattern. An internal hydrophobic residue the hydrophobic bonding of which contributes to the stability of the molecule is replaced by another of different dimensions and thereby the stability of the molecule is decreased. A replacement at such a site by a charged residue would presumably not be compatible with the survival of the tertiary structure¹². In the case of haemoglobin Genova when the leucine of a helix is replaced by a proline which cannot enter the helical sequence, and in another unstable haemoglobin, haemoglobin Freiburg¹³ where a residue is deleted in the B helix (β B5 Val), the distortion of the molecule which occurs must be sufficient to explain the instability of the molecule. In haemoglobins Köln and Sydney the residues which are replaced form hydrophobic (van der Waals) bonds with the haem. The phenylalanine β CD1 which is replaced in haemoglobin Hammersmith is an invariant residue which is present in all haemoglobins and myo-

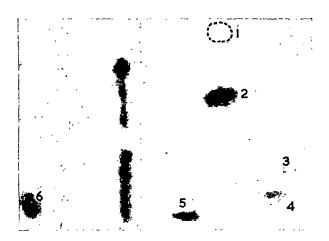


Fig. 3. Fingerprint (peptide chromatogram) of the chymotryptic digest of the abnormal \$T{\rm pV}. (1) Area where \$\beta\$ 41-45 is found in the fingerprint of the chymotryptic digest of normal \$T{\rm pV}. Amino-acid analysis indicated the composition of the other peptide as (2) \$\beta\$ 41-45 of haemoglobin Hammersmith. (3) 41-55; (4) 48-55; (5) 41-59; (6) 56-59.

globins. In a model it can be seen to lie closely to the porphyrin of the haem and in planar relation to it. It can therefore form strong van der Waals bonding with the haem and must be an important stabilizing force within the molecule. Its replacement by the non-charged but polar serine residue should cause a more severe instability than the Val-Met and Val-Ala replacements of haemoglobins Köln and Sydney respectively. This would agree with the greater clinical severity of haemoglobin Hammersmith haemoglobinopathy.

It is interesting that in both instances of this condition we have been unable to demonstrate the abnormal hacmoglobin in either parent—whereas haemoglobins Köln, Genova and Sydney are patently familial. We conclude that the chance of a child affected with haemoglobin Hammersmith surviving and producing children has been (and still is) slight, and its observation is therefore likely to be limited to patients in whom it has arisen as a new mutation.

The amino-acid substitution described here represents the first observation in human haemoglobin involving phenylalanine. The messenger RNA codons for phenylalanine are uracil-uracil-cytosine (UUC) and UUU, and two of the codons for serine are UCC and UCU14. single mutation in the second base from U to C in either of the two phenylalanine codons can therefore be responsible for the Phe→Ser substitution in haemoglobin Hammersmith. This is in agreement with the general pattern according to which human haemoglobin variants can be considered to have arisen from single point mutations¹⁵.

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Attachment of Rapidly Labelled RNA to Polysomes in the Absence of Ribosomal RNA Synthesis during Normal Cell Differentiation

bу

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Department of Experimental Biology, National Institute for Medical Research, Mill Hill, London Anucleotate mutant tadpoles of Xenopus laevis synthesize rapidly-labelled RNA but no detectable ribosomal RNA or ribosomes. Experiments demonstrate that high molecular weight RNA synthesized by these mutants attaches to polysomes active in protein synthesis.

In animal cells the synthesis of rapidly labelled RNA in the nucleus and the subsequent appearance of such RNA in cytoplasmic polysomes usually take place at the same time as the synthesis of ribosomal RNA and cytoplasmic ribosomes. This and other relationships¹⁻³ have suggested that the synthesis of messenger RNA, its transport to the cytoplasm and its incorporation into polysomes may be dependent on, or in some way linked to, the synthesis of ribosomal RNA and ribosomes. This possibility can be tested most directly in conditions in which rapidly labelled RNA is synthesized but ribosomal RNA is not. There seem to be only two natural conditions in which these two kinds of synthesis are clearly separated. The first is during cleavage in frogs4, sea urchins5 and other animals. Only very small amounts of non-transfer RNA are synthesized during the early cleavage stages of embryos, however, and at this stage of development most ribosomes seem to exist as monomers; it is therefore difficult to demonstrate the attachment of rapidly labelled RNA to polysomes during cleavage in embryos. Nevertheless RNA synthesized by a sample of sea urchin embryos, mostly in early cleavage stages, becomes associated with polysomes which may not, however, be active in protein synthesis. The second condition in which the synthesis of rapidly labelled RNA and ribosomal RNA are clearly separated is during the life of Xenopus embryos and tadpoles, homozygous for the anucleolate mutation7 which prevents the synthesis of any ribosomal RNA⁸. Purified ribosomes from the anucleolate mutant contain very small amounts of labelled non-transfer RNA9, and it has not yet been demonstrated that any of the high molecular weight RNA synthesized by these mutants becomes associated with a population of polysomes active in protein synthesis. The experiments described here provide such a demonstration, and therefore show that the attachment of rapidly labelled RNA to polysomes is not necessarily dependent on or linked to ribosomal RNA synthesis during cell differentiation.

Polysomes in Xenopus Embryos

We have used the following criteria for the identification of polysomes: sedimentation properties before and after release from cytoplasmic membranes; behaviour after ribonuclease treatment; and activity in protein synthesis. These features have been demonstrated on the same sample of tadpoles derived from one mating. After hatching (stage 38 of Nieuwkoop and Faber¹o), sixty tadpoles were microinjected with a mixture of ¹⁴C-aminoacids (Radiochemical Centre, Amersham). This was prepared by adding ¹⁴C-leucine at 305 mc./mmole to mixed ¹⁴C-amino-acids lacking methionine and histidine; the mixture was freeze dried and taken up in modified

Barth's solution¹¹. After 15-25 min, these embryos and an excess of unlabelled embryos of the same age were washed several times and homogenized together in 3 ml. of 0.25 molar sucrose, made up in TKM buffer (35 mmolar tris hydrochloric acid at pH 7.4, 50 mmolar potassium chloride and 1 mmolar magnesium chloride), using ten strokes of a loose fitting glass ball homogenizer. homogenate was layered over 1 ml. of 1 molar sucrose-TKM and centrifuged at $13,000g_{\text{max}}$ for 15 min at 0° C (MSE 3×5 ml., swing-out rotor at 12,000 r.p.m.) to remove nuclei, pigment, etc. The supernatant was carefully withdrawn as far as the interphase and divided into three equal parts. One part was treated with 0.5 per cent 'Triton X-100' (Lennig Chemicals) for 0.5 h at 0° C, another was treated with ribonuclease at 1 $\mu g/ml$. and 'Triton' at 0.5 per cent for 0.5 h at 0° C, and the last remained untreated for 0.5 h at 0° C. Each part was layered on top of an 18 ml. 10-30 per cent linear sucrose-TKM gradient lying over a 1 ml. cushion of 60 per cent sucrose-TKM. After centrifugation at 0° C for 2.5 h at $90,000g_{\text{max}}$ (MSE 3×20 ml., swing-out rotor at 25,000 r.p.m.), fractions were collected, and their optical density read at 240, 260, 280 and 320 m μ . Each fraction was then precipitated onto Whatman GF 83 filters by addition of 50 per cent TCA and counted in a liquid scintillation counter. The results are shown graphically in Fig. 1a-c. The optical density profiles have been corrected for scattering by reference to absorption at 320 mu. From tube 5 onwards the optical density at 260 mu is largely the result of nucleic acid as judged by the 280: 260: 240 my. ratio. In all cases (Fig. 1a-c), monosomes have sedimented as far as tube 7. In the untreated sample (Fig. 1a), components containing RNA have sedimented faster than the monosomes and some have accumulated on the dense cushion at tube 23. These fast sedimenting components show substantial incorporation of ¹⁴C-amino-acids into a product which is insoluble in acid; they are therefore active in protein synthesis. 'Triton', like deoxycholate, releases polysomes from fragments of cytoplasmic membrane, and treatment with it has caused the RNA-containing particles which previously reached the 60 per cent sucrose cushion, to sediment more slowly, reaching tubes 10-15 where there is an increase in $E_{260\,\mathrm{m}\mu}$ (Fig. 1b). This would be expected of polysomes released from cytoplasmic membranes. Finally, Fig. 1c shows that treatment with low temperature ribonuclease causes these fast sedimenting components to sediment with monosomes, the optical density and ¹⁴C-radioactivity of which is proportionately increased (Table 1)—a result expected of polysomes but not of RNA attached to non-ribosomal components¹². All criteria that we have used indicate that the RNA-containing components, which sediment faster than single ribosomes, behave as polysomes active in protein synthesis.

Attachment of Rapidly Labelled RNA to Polysomes

Because tadpoles which are homozygous for the anucleolate mutation (0-nu) die before feeding, they must be obtained by crossing two 1-nu frogs, heterozygous for the mutation. Among the progeny of such a cross 88 0-nu tadpoles were identified individually by phase-contrast microscopy of excised tail tips¹¹. At the hatching stage (stage 37/38, ref. 10), each 0-nu tadpole was microinjected with about 30 mul. of 3H-uridine solution, that is 3H-uridine labelled primarily in the 5C position at 30 c./mmole (Radiochemical Centre, Amersham), and made up to 8 mc./ml. of modified Barth's solution. 3-4 h later, the injected 0-nu tadpoles were washed and homogenized together with the unlabelled and 14C-amino-acid labelled tadpoles described. The labelled and unlabelled tadpoles together numbered 450 and were all obtained from the The tadpoles labelled with same $1-nu \times 1-nu$ mating. The tadpoles labelled with ${}^{3}\text{H-uridine}$ were part of the sample analysed on the 10-30 per cent sucrose gradients described. 3H-radioactivity was counted at the same time as 14C-radioactivity in each sample using a three channel liquid scintillation counter. The channels ratio method was used to calculate the efficiency of counting (15 per cent for tritium and 50 per cent for carbon-14) and the proportion of counts present in the 3H-channel resulting from carbon-14 radioactivity. In tadpoles labelled for no more than 3-4 h, only 2-3 per cent of the radioactivity introduced with ³H-uridine enters DNA and the rest enters RNA¹³. ³Hradioactivity is plotted in Fig. 1a and b, and it is seen that labelled RNA is present in the polysome region of these gradients, whether treated with 'Triton' or not. Light ribonuclease treatment removes virtually all ³H-radioactivity from the polysome region (Fig. 1c. Table 1). In another experiment in which 0-nu embryos were labelled with 3H-uridine for 18 instead of 3.5 h, we also found heterogeneous RNA associated with poly-

We conclude from these experiments that non-ribosomal RNA synthesized by 0-nu tadpoles becomes associated with cytoplasmic components which behave like polysomes. A small amount of transfer RNA, which is synthesized as usual by 0-nu tadpoles^{8,9}, would be expected to become attached to polysomes, and the

Table 1. Distribution of 3 H-labelled rna and 14 C-labelled protein, and of total unlabelled rna (E_{280} m μ), in different regions of the gradients shown in Fig. 1a–c

		Region of gradient (and tube number)								
Gradient		Monomer (5-		(10-24)	Total (5-24)					
14	$E_{290~\mathrm{m}\mu}$ $^{14}\mathrm{C\text{-}cpm}$ $^{8}\mathrm{H\text{-}cpm}$	4·54 53% 1,675 27% 1,576 38%	4,628	73%	8-56 6,303 4,196					
1 <i>b</i>	E_{260} m μ ¹⁴ C-opm ³ H-cpm	4·84 56% 1,984 35% 1,578 39%	3,661	65%	8·58 5,645 4,056					
10	$E_{260 \text{ m}\mu}$ ¹⁴ C-cpm ³ H-cpm	6·73 79% 4,139 75% 968 59%	1,352	25%	8·56 5,491 1,638					

The regions of each gradient are defined by tube number. The recovery in each region is expressed as a percentage of total recovery (last column).

following experiment was therefore carried out to determine whether this was the only kind of RNA to become attached to polysomes in our experiments. From among the progeny of a second $1-nu \times 1-nu$ mating, fifty 0-nutadpoles were identified individually as described earlier. At stage 37/38 they were microinjected with the 3H-uridine preparation used before. After 3.5 h they were washed and combined with 130 unlabelled 1-nu and 2-nu tadpoles of a similar age. After homogenization in 0.25 molar sucrose-TKM, and centrifugation at 13,000g for 15 min as described before, the supernatant (3.0 ml.) was placed on top of a stepped gradient of 0.8 ml. of 1 molar sucrose-TKM and 0.8 ml. of 2 molar sucrose-TKM¹². fugation at 0° C for 2.5 h at 176,000 $g_{\rm max}$ (MSE 3×5 ml. swing-out rotor at 40,000 r.p.m.) yielded a pellet which contained 20-30 per cent of the total 260 mµ optical density added to the tube, and which must therefore have contained, as was intended, most of the components which previously sedimented faster than the monosomes. pellet was dissolved in 1 ml. of 0.01 molar sodium acetate at pH 5.0, 1 mmolar versene, 50 mmolar sodium chloride, 4 μg/ml. polyvinyl sulphate, and 0.5 per cent sodium dodecyl sulphate, a solution intended to dissociate protein from RNA without causing degradation of RNA. Purified ribosomes from 150 unlabelled tadpoles were added at this point to provide an appropriate amount of optical density at 260 mu in the ribosomal RNA region of the next sedimentation gradient. The RNA solution, from which sodium dodecyl sulphate had been removed, was layered

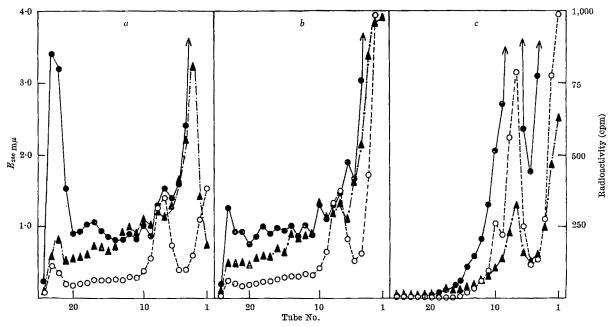


Fig. 1. Distribution of optical density at 260 mμ (O --- O), ¹⁴C-protein (•--- Φ), and ²H-RNA (▲-···· ▲) among the fractions of sucrose gradients. The labelled protein and RNA were attached to, or part of, ribosomes or polysomes. The embryos were labelled and the supernatant samples prepared for each gradient as described in the text. The three samples of embryos supernatant were incubated in sucrose-TKM medium supplemented in the following ways before being layered over the sucrose gradients: (a) no addition; (b) 0.5 per cent "Triton X-100"; (c) 0.5 per cent "Triton X-100" and I μg/ml. ribonuclease.

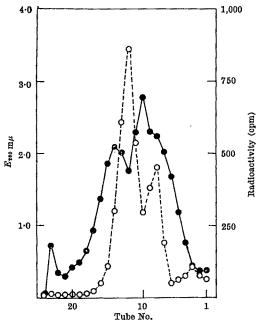


Fig. 2. Distribution of optical density at 260 m μ (\bigcirc - - - \bigcirc) and *H-RNA (\bigcirc — \bigcirc) among the fractions of a sucrose gradient. Embryos were labelled and the BNA extracted as described in the text.

on top of a linear gradient of 5-20 per cent sucrose containing sodium acetate, versene, polyvinyl sulphate and sodium chloride, as described earlier. After centrifugation at 5° C for 14 h at 75,000 g_{max} (MSE 3×20 ml., swing-out rotor at 23,000 r.p.m.), fractions were collected from the gradient and assayed for optical density and radioactivity as before. The result (Fig. 2) shows that the labelled RNA extracted from polysomes sedimented throughout the gradient with a peak of radioactivity lying between the typical optical density peaks of 28S and 18S ribosomal RNA. A similar sedimentation profile has been described previously for the total DNA-like RNA extracted from 0-nu embryos labelled for 3 h⁹. We conclude from this experiment that very little of the rapidly labelled RNA which is synthesized by 0-nu tadpoles and which becomes attached to polysomes is transfer RNA.

Interpretation of Results

We consider our results show that rapidly labelled RNA can become associated with polysomes in the total absence of ribosomal RNA synthesis. We discuss here some of the more obvious kinds of error which if not excluded would seriously affect the interpretation of our results. It is essential that our preparation of 0-nu tadpoles should not have been contaminated by any wild-type or heterozygous tadpoles, and that they should have been in a normal lifelike condition during the experiment. In this connexion, we wish to stress that each 0-nu tadpole was identified individually by a procedure which has previously been shown to be very reliable when tested for ribosomal RNA synthesis by sucrose gradient analysis. Furthermore, any contamination of our sample of 0-nu tadpoles by ones capable of synthesizing ribosomal RNA would have been clearly revealed as peaks of 28S and 18S radioactivity in Fig. 2. The 0-nu tadpoles used for these experiments were between stages 37 and 39 and were not morphologically distinguishable from wild-type tadpoles. They seemed entirely normal when homogenized at stage 39. Our principal conclusion is based on results obtained with 0-nu tadpoles from two different matings.

The possibility that we were dealing with aggregates of ribosomes or with RNA-containing cell components other than polysomes seems very unlikely, for ribo-nuclease converted the fast-sedimenting components into monosomes. Contamination of our polysome preparation by nuclear components containing RNA seems unlikely, not only because of the ribonuclease effect just mentioned but also because nuclei and other components were discarded after the initial centrifugation into a pellet at 13,000g for 15 min. Evidence that this effectively separated nuclear from cytoplasmic components is provided by the finding that the supernatant of this first centrifugation contained 95 per cent of the 14C-protein radioactivity, but less than 50 per cent of the ³H-RNA radioactivity originally present in the homogenate. We used a loose fitting ball-type homogenizer known to provide a high yield of unbroken tadpole nuclei14.

Bacterial contamination was excluded by removal of all unhatched embryos from jelly and membranes, the use of streptomycin sulphate and benzylpenicillin at 0.01 µg/ml. in all incubation media, and by extensive washing before homogenization. These precautions have been found to eliminate bacterial contaminations, and the presence of any labelled bacterial RNA in our samples would have been seen as 23S and 17S peaks of 3H-radio-

activity in Fig. 2.

In summary, our results permit the following conclu-First, they show that rapidly labelled RNA of anucleolate amphibian embryos becomes associated in a few hours with cytoplasmic components which have all the biochemical properties expected of polysomes. Because this takes place in anucleolate tadpoles, that is 3-4 days after ribosomal RNA was last synthesized, the synthesis, transport and attachment of rapidly labelled RNA to polysomes cannot be linked in any way to ribosomal RNA synthesis. A similar conclusion has been drawn from short-term experiments with HeLa cells treated with puromycin15 or infected with vaccinia virus16, but does not seem to have been reached previously for normal differentiating cells in natural conditions, or in circumstances in which ribosomal RNA synthesis is so clearly dissociated from the synthesis of other kinds of RNA. While our results demonstrate that the synthesis and translation of rapidly labelled RNA (presumably messenger RNA) are quite independent of ribosomal RNA synthesis, they do not exclude the possibility that mRNA attachment to polysomes is linked to the transport and assembly of ribosome subunits released from degraded ribosomes. As a second conclusion, our results provide direct evidence that newly synthesized non-transfer and non-ribosomal RNA can become attached to a population of polysomes, some of which are active in protein synthesis and the component ribosomes of which were synthesized 3-4 days before, during oogenesis. While this might have been expected in view of the substantial amount of DNA-like RNA synthesized by anucleolate embryos, it does not seem to have been demonstrated before.

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LETTERS TO THE EDITOR

ASTRONOMY

Arguments for the Presence of Undiscovered Satellites

In this communication we summarize the results of recent work leading to the prediction of the presence of three new satellites, one each for Jupiter, Saturn and Uranus, and specify those properties of the satellites and their orbits which can be deduced.

In the course of a rediscussion of the characteristics of the satellite systems of the principal planets we showed the following. (a) With the exception of the Galilean satellites of Jupiter, commensurability relations of the type

$$\sum N_i n_i = 0$$
 (1)

(where n_i is the mean orbital angular rate of the *i*th satellite of a planet, N_j is an integer or zero, and the summation is over all satellites) probably arise by chance and have no physical significance. (b) Simple resonance perturbation of particles in Saturn's ring does not explain the observed positions of gaps or relative minima; this conclusion has also been reached by Alfvén¹. (c) The eight inner satellites of Saturn, including the recently discovered Janus², obey the following rule

$$n_i + n_{i+2} = (1 + m_i k) (n_{i+1} + n_{i+3})$$
 (2)

where n_i is defined as in equation (1), m_i is a small positive integer, k a constant = 0·1505439, and that such a rule will certainly not arise by chance. (d) Similar equations can be formed for the satellite system of Uranus if an extra satellite is postulated in an orbit between the present inner pair, Miranda and Ariel. (e) Two solutions pertain to the Jovian system, both of which postulate an extra satellite between Amalthea and Io, with values of k=0.365514, n=432.189 deg/day or k=0.262087, n=417.802 deg/day.

We note that, to quite a high degree of exactness, the constant k_q (subscript q denoting the satellite system) is proportional to the mean density, ρ_q , of the corresponding primary. The second of these postulated Jovian satellites fits accurately into this scheme. The relation between k_q and ρ_q (in egs units) may be expressed as

$$k_q = (0.180 \pm 0.008) \rho_q + (0.026 \pm 0.022)$$
 (3)

To investigate the possibility that an extension of equation (2) might serve to portray the detailed structure of Saturn's ring, we calculated $n_{0,i}$ given by

$$n_{0,i} + n_2 = (n_1 + n_3) (1 + 0.1505439m_i)$$
 (4)

where n_1 , n_2 , n_3 are the angular rates of Janus, Mimas and Enceladus, respectively, and m_i a positive integer. It is inferred that these $n_{0,i}$ represent stable orbits, and hence maxima in the particle density in Saturn's ring; and for $m_i \leq 10$ this is found to be the case. We find that $n_{0,i}$ must be rejected because its value of 473.288 deg/day is less than that of n_i which is 480.667 deg/day, and that $n_{0,2}$ lies outside the ring with the value 585.23 deg/day. We note, however, that this latter value might represent an undiscovered satellite outside the Roche limit, provided its mean density exceeds 1.3 g cm⁻³. hypothesis receives support from Alfvén's proposal that the Cassini division and the relative minimum in the ring at 876.2 deg/day are the cosmogonic shadows of Mimas and Janus, respectively, caused by these satellites sweeping up part of the plasma which later condenses into ring grains, the semi-major axes of whose orbits are 0.65 those of the plasma particles. Alfvén defines the "fall-down ratio" as the ratio between the present semi-major axis of the sweeping satellite's orbit and the radial distance from the centre of the primary to the corresponding ring gap.

The ratios for the pairs (Mimas-Cassini division) and (Janus-876·2 deg/day gap) are $1\cdot541$ and $1\cdot492$, respectively. We find that the other major gap, the Lyot gap separating rings B and C and centred on $1,117\cdot8$ deg/day, would be the cosmogonic shadow of our proposed satellite, with a fall-down ratio of $1\cdot539$ agreeing very well with the ratio for Mimas. The photometric profile of the Lyot gap is at present uncertain, but it seems likely that the proposed satellite has a mass intermediate between those of Mimas and Janus (hence probably a magnitude at mean opposition of about 13), and orbital eccentricity similar to that of Mimas (0·02).

Some elements for the conjectured satellites are summarized in Table 1.

Table 1. PARAMETERS OF THE CONJECTURED SATELLITES OF JUPITER, SATURN AND URANUS

Primary	Conjectured satellite orbital angular rate (deg/day)	Period	Mean distance from primary (equatorial planetary radii)	kq	64
Jupiter	417·802 432·189	0d 20h 40m 47s 0d 19h 59m 24s		0·262087 0·365514	1.334 1.334
Saturn Uranus	585·23 214·558	0d 14h 45m 03s 1d 16h 16m 08s	2.31	0·1505439 0·316532	0.684 1.60

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PLANETARY SCIENCE

Theoretical Model for the Chandler Wobble

EULER is generally credited with having been the first to show that an axially symmetric rigid body, with a fractional difference between the equatorial and polar moments of inertia equal to that of the Earth, could undergo a free nutation with a period of about 300 days. That is, in a body-fixed co-ordinate system, the instantaneous axis of rotation would describe a cone about the polar axis with a 300 day period. It could have been expected that such a motion, even if present primordially, would have been damped almost completely by natural dissipative processes within the Earth. Such a motion would show itself in a periodic variation in astronomic latitude of a given site on the Earth's surface, because the rotation axis moves only slightly with respect to an inertial frame1. Despite the expectance of almost complete damping, repeated attempts were made in the nineteenth century to uncover indications of a variation in latitude with a 10 month period. None was definitely established, but in 1891 Chandler² announced a variation with a period of 428 days, about 40 per cent larger than predicted. Newcomb soon realized that the period of free nutation for the Earth would be greater than the rigid-body value, because of the fluid nature of the oceans and elastic yielding of the solid earth, and he proposed that Chandler's observations were indeed of the free nutation3. Systematic observations of latitude variations have been made since the turn of the century and clearly indicate the presence of an oscillation with this 14 month period (see Fig. 1). The amplitude of this oscillation has a maximum of about 0.3 sec of arc, that is the inclination of the instantaneous axis of rotation to the figure axis does not seem ever to exceed about 0.3 sec of arc.

What prevented this free nutation from being completely damped? Many suggestions of mechanisms to excite the Chandler wobble have been put forward during the past 75 yr, but none has proved satisfactory. question is still considered by most geophysicists to be largely unanswered. The purpose of this communication is to describe a new, and admittedly speculative, model that we conceived independently. The idea is based on Fig. 1 which, although extending over too short a time interval to be definitive, seems to have an envelope characteristic of "beat" phenomena. We therefore propose that these latitude variations do indeed exhibit beats, attributable to an interaction between core and We speculatively assume that the core has sufficient rigidity to have a free nutation frequency nearly equal to the corresponding frequency of the mantle alone. These separate frequencies, modified by coupling, can be expected to be present in the Chandler motion. The data (Fig. 1) suggest a beat period of about 40 yr. Results of theoretical calculations, although quite sensitive to the assumed rigidity and difference in equatorial and polar moments of inertia of the core, indicate that such a period is reasonable for our proposed model. (Of course, if the model proves adequate, the observed beat period can be used to infer these properties of the core.)

We suppose that this resonant oscillation is excited asynchronously, through a non-linear interaction, by the differential luni-solar torques exerted on the core and mantle. Using a suitably parameterized model, we are investigating numerically the possible motions of this complicated, three degrees of freedom, dynamical system. Apart from the well known expressions for the luni-solar gravitational torques, the model includes a restoring torque (inertial coupling term), which is proportional to the separation of the figure axes of core and mantle, and a viscous torque which is a non-linear function of the difference in angular velocity of the coupled parts. In first approximation both core and mantle are considered rigid. The transfer of the required energy between core and mantle seems possible on the basis of preliminary calculations. Quantitative results, to be reported in a subsequent communication, will include the relations between the model parameters and the amplitudes and frequencies of the Chandler motion.

How may this model be checked experimentally? The supposition that a beat frequency is present in the Chandler motion can, of course, be tested conclusively by awaiting future measurements. In fact, data have

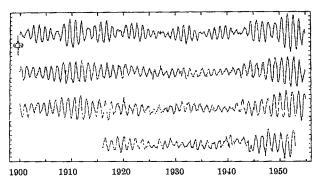


Fig. 1. Variations of the two orthogonal components of the position of the Earth's rotation axis with respect to the axis of figure (from ref. 1). The top two curves show the unsmoothed observations for one component, before (first curve) and after (second) removal of the seasonal variation. The last two curves, in reversed order, show similar variations for the second component. The first three curves were obtained by the International Latitude Service, the last with the Photographic Zenith Tube at Washington.

accumulated for 12 yr since Fig. 1 was compiled and would be expected to exhibit a decreasing amplitude in the oscillation. Although the nineteenth century observations may not be very reliable, we intend to re-examine the best of these and, after separating the annual term¹, seek evidence bearing on the predicted amplitude modulation of the Chandler term.

The presence of two important frequency components in the Chandler motion is, of course, also consistent with the apparent breadth of the power spectrum of the polar motion in the vicinity of 0.8 cycles/yr¹. In the past, the corresponding decrease in the amplitude of the autocorrelation of the Chandler data—approximately 10 per cent for a 14 month lag—was interpreted as indicating a damping time of approximately a decade and led to suggestions that the free nutation was being excited by random impulses¹. In our model, the amplitude of the auto-correlation will also decrease, as observed, but after longer intervals will rise again, the period being equal to that of the beat frequency. Furthermore, the problem of the anomalously small Q value for the Earth, inferred from the "damping" of the Chandler motion, is automatically disposed of.

If this model of the Chandler webble proves fruitful, it might be interesting to consider the more complicated case of a non-axially symmetric mantle which may be appropriate for Venus or the Moon.

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Sinking Rates of Radioactive Fallout Particles in the North East Pacific Ocean, 1961-62

THE resumption of nuclear weapon testing in the atmosphere in September 1961 provided an opportunity to investigate the sinking rate of radioactive fallout particles in the North East Pacific Ocean. The stopping of weapon testing in the atmosphere between November 1958 and September 1, 1961, gave sufficient time for radionuclides with relatively short half-lives which were released by tests before 1958 to decay. Weapon testing began in the Soviet Union on September 1, 1961, and by November 4, 1961, at least fifty nuclear devices had been detonated in the atmosphere, including fifteen megaton or multimegaton devices1. In the North East Pacific Ocean, the beginning of the 1961 test series coincided closely with the beginning of the winter storms and their heavy rainfall. This provided a mechanism for quickly depositing the fallout nuclides, rainfall being the principal mechanism for removing such particles from the atmosphere². Radioactive fallout material from these tests was detected in marine samples in mid-September 1961.

The gamma-ray activity was analysed in samples of sediment collected in July and August 1961 and in the summer of 1962. No activity caused by the presence of radionuclides from fallout was detected in thirty-nine samples collected in 1961 (general area sampled shown in Fig. 1), while twelve samples collected in June 1962 from

this area contained detectable amounts of several radionuclides from fallout.

Special sediment samples were collected in August and September 1962 for analysis of their gamma-ray activity. These samples were collected by siphoning off the muddy water above the interface between water and sediment in open gravity cores. I assume that the sediment particles suspended in the water were resuspended during the coring and retrieval operations and that this sediment was the most recently deposited material.

The muddy water recovered was transferred to a plastic container in which it was dried under infrared lamps aboard ship without washing or further handling, to minimize contamination. When the samples were dry the containers were sealed and wrapped in plastic which was changed just before analysing the gamma radiation. Depending on the amount of sediment recovered, the gamma radiation was analysed for 100-1,000 min using a 3×3 in. $(7.6 \times 7.6$ cm) sodium iodide (thallium) crystal connected to a photomultiplier and a multichannel analyser. The gamma-ray spectra obtained were analysed using a computer programme which estimated the abundance of eight radionuclides (potassium-40, cobalt-60, zinc-65, zirconium-95-niobium-95, ruthenium-103-106, bismuth-214, chromium-51, cerium-141) and estimated the 95 per cent confidence interval based on counting statistics3. The sample was considered to have no significant activity if the calculated activity was equal to or less than the estimated 95 per cent confidence interval.

Of the various radionuclides from fallout detected, the pair zirconium-95-niobium-95 was selected as the best for this study because of its relative short half-life (65 days), its abundance in fallout² and its known association with particles in sea water⁴. The apparent absence of zirconium-95-niobium-95 in sediments collected in July and August 1961, just before the resumption of testing.

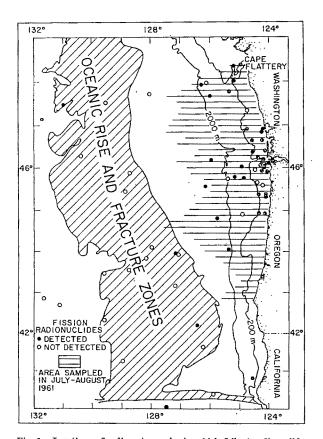


Fig. 1. Locations of sediment samples in which fallout radionuclides were detected (●) and not detected (○) in 1962. Sediment samples collected in July and August 1961 (area shown by horizontal rules) near the continent had no detectable quantities of radionuclides from fallout.

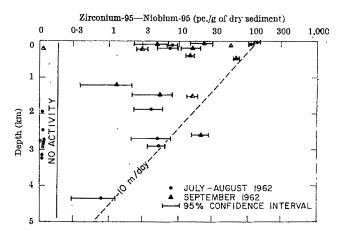


Fig. 2. Observed abundance of zirconium-95—niobium-95 in specially collected sediment samples taken in August-September 1962 from various depths in the region shown in Fig. 1. The dashed line indicates the expected decrease in zirconium-95—niobium-95 activity with depth assuming a constant supply of radioactive particles sinking at a rate of 10 m/day with no dilution by non-radioactive sediment after deposition or during sampling operations.

and its presence in shallow water deposits nearly everywhere in 1962 provides convincing evidence that the radioactivity detected was caused not by fallout derived from earlier weapons tested nor by the misidentification of the gamma-ray spectra of radionuclides occurring naturally in these sediments, but by fallout particles deposited after September 1961.

In the locations nearest the continent, the radioactive particles could have been transported and deposited either by processes near the ocean floor, or by sinking through the overlying sea water or by both. Thus the data for sample locations near the continent provide little information about sinking rates of the radioactive particles.

The deepest localities are apparently shielded from the accumulation of sediment by transport along the bottom of the ocean by the irregular bottom topography associated with the oceanic rise and fracture zones (Fig. 1). Thus the presence and abundance of fallout radionuclides in the sediment from these areas provide information about the sinking rates of the radioactive particles.

If we assume that the first radioactive fallout arrived in the area immediately after the testing began (September 1, 1961), the radioactive particles must have settled about 13 m/day to have reached a depth of 4.4 km (Fig. 2) by the time the sample was collected on August 8, 1962. Even if we discard the data for the deepest station because of possible contamination (for which there is no evidence) we must still assume sinking rates of at least 9 m/day to account for the radioactivity from fallout detected in three samples from depths between 2.6 and 2.9 km (Fig. 2). In short, it seems that the radioactive particles sank at rates near 10 m/day to account for the presence of zirconium-95—niobium-95 (half-life 65 days) in the sediment at these depths.

It is also interesting to note that the observed decrease in activity of zirconium-95-niobium-95 (Fig. 2) with depth corresponds rather closely to the expected decrease resulting from a steady state injection of fallout with the particles sinking at a rate of 10 m/day. This apparent relationship may be entirely fortuitous because it would demand not only a quasi steady state input of fallout particles, which seems scarcely likely, but also an absence of appreciable dilution of the recently deposited fallout particles by non-radioactive sediment during deposition and during the coring operations.

I have no data on the grain size, composition or density of these radioactive particles although fallout from other weapon tests has been studied^{1,2,5,6}. It is possible to calculate the probable grain size of the radioactive particles, having sinking rates of 10–13 m/day, by assum-

ing that the particles are spherical, have a density of 2.5 g/cm³ (typical of fused silicate glass), and obey Stokes law during gravitational settling through sea water $(S=35~{\rm p.p.t.},~T=5^{\circ}~{\rm C})$. Such a calculation shows that a sinking rate of 10 m/day corresponds to grains 16μ in diameter, and 13 m/day corresponds to grains 18µ in diameter.

These calculated grain sizes are consistent with the known behaviour of fallout particles where grains less than about 20μ in diameter tend to be transported primarily by atmospheric motions^{1,2}. Such grains could easily have been transported long distances from the test sites in the USSR and widely dispersed in the surface layers of the North East Pacific Ocean.

No doubt smaller particles also fell into the ocean surface layers, but my data do not provide any information about possible size distribution in the fallout. Not only do the larger particles sink more rapidly but they also have greater activities than the smaller particles because the activity of fallout particles is proportional to the volume of the particle. Thus the larger particles probably contributed the bulk of the radionuclides from fallout in these sediments when collected in 1962.

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Lake Levels and Quaternary Climates in the Eastern Rift Valley of Kenya

For more than 20 yr, the accepted sequence of pluvial and interpluvial climatic fluctuations during the late Pleistocene and Holocene in East Africa has been based on the evidence of high level shorelines and lake sediments in the Nakuru-Elmenteita basin in the Eastern Rift Valley, Kenya. The nomenclature was formally established in 19471 and 19482 but it had been published several years earlier. The sequence of lake level variations was originally studied by Leakey^{3,4}. The pluvial (high lake level) phases shown in Table 1 were suggested, separated by dry interpluvials during which the lakes were lower. It was believed that these phases could be recognized in evidence from lake shorelines, sediment sections and river terraces in other parts of Africa.

Recently there has been strong criticism of the use of essentially climatic units as stratigraphical units and as

Table 1

Oldest)

the basis of geological correlation between parts of Africa. This is expressed in the volume published as the result of the symposium on the systematic investigation of the African later Tertiary and Quaternary held at Burg Wartenstein under the auspices of the Wenner-Gren Foundation in July 1965, which was attended by many scientists of great experience in the field study of African Quaternary stratigraphy⁵. They recommended that an internationally approved code of stratigraphical nomenclature be accepted for the African later Tertiary and Quaternary which should "make clear the distinction between rock units, time-stratigraphic units, biostratigraphic units, and other units" (page 890). This distinction has not always been made up to now; for example, the term "Gamblian" has been used to mean: (a) certain strata at the type site (Gamble's Cave, marked GC on the map) and elsewhere in the Nakuru basin; (b) the pluvial period during which these strata were laid down; and (c) rocks in other areas believed to have been laid down during the same pluvial period. Such a nomenclature is likely to give rise to confusion and unsound correlations. advantages of climate as the basis of a stratigraphical classification were well summarized earlier by Flint (ref. 6, page 279).

There has also been some re-examination of the geological evidence for the earlier pluvial periods in East Africa (Kageran, Kamasian, Kanjeran) the existence of which was accepted with that of the later phases at the congresses of geology and prehistory held in the 1940s and early 1950s^{1,2,7}. It has been suggested that the geological evidence for the earlier pluvial phases, even in the type areas, is not always convincing^{6,9}. The evidence in the Nakuru-Elmenteita basin for the Gamblian, Makalian and Nakuran sequence of lake level fluctuations, however, has not been re-examined in detail since the 1930s and is

still generally accepted.

My recent work on the shorelines and sediments of the Nakuru-Elmenteita basin (1965-67) suggests that the use of the terms Gamblian, Makalian and Nakuran to indicate pluvial climatic units may also be open to doubt. concentrated on tracing the shorelines of the former lakes, looking for the few lake-shore cliffs which can be found and for coastal and shallow-water sediments such as pebbles and sections of sands and diatomaceous silts. I then measured their altitude as accurately and precisely as possible, using a Zeiss self-aligning level ('Koni 025'). In the early part of the work, for the levelling of the most important features such as the Menengai shoreline, Gamble's Cave and the Bahati plain, I levelled both ways, to and from the control points, to check the accuracy of the work, and found it to be quite satisfactory in every In the later work there was not sufficient time to level both ways, but I believe the results of this levelling are also reliable. Results in some cases did not agree with those of earlier workers3,4,10,11; I think this is because of the better apparatus that I used and above all because of the better height information, maps and air photographs which were available. The levelling in the 1920s was based on the railway height control points which are in many ways not satisfactory; I was able to use the Survey of Kenya network of trigonometrical stations and bench marks which have since been established in the area. On the map, the contours drawn with a solid line are taken from the recent 1:50,000 maps and give quite a good picture of the basin. They are unfortunately not drawn on these maps of the south of the basin and the form lines shown there with broken lines are taken from maps drawn in the early part of the century and give only

a rough indication of the topography.

I found (a) that the "Gamblian" phase is represented by a single shoreline slightly above 6,370 ft. (circa 600 ft. above Lake Nakuru) which may be linked to the lowest overflow from the basin which is at about 6,390 ft. The overflow is at the point OF on the Bahati plain (see map). The lake level seems to have been stabilized for some time

⁽Most recent) Nakuran post-pluvial wet phase—Lake Nakuru circa 145 ft. above present Makalian post-pluvial wet phase-Lake Nakuru circa 375 ft. above present

at the height of this outlet from the basin and the shoreline is thus not strictly an indication of a lake stillstand at a "pluvial maximum" climatic peak. It could reasonably be called the "Gamblian shoreline" if by this one means the shoreline contemporary with the beach sand found by Leakey in Gamble's Cave. The Gamble's Cave sand is at 6,344 ft. (Survey of Kenya datum) which is very close to the altitude of similar sand and pebble sections at the north end of the basin below the 6,370 ft. Menengai shoreline (3/6 at 6,343 ft., 1/7a at 6,341 ft.). The shoreline is best developed between points 1/7a and P on the map; it is untilted or at most very slightly tilted. I found (b) that the evidence for the "Makalian" shoreline, which according to Leakey included features at 373-379 ft. above Lake Nakuru, seems from my levelling to be spread over a height range of about 343-484 ft. above the lake, with no suggestion of a consistent tilt. Shoreline erosion and shallow water sedimentation seem to have taken place within these heights, but the evidence does not suggest a single important stillstand at about 375 ft. above the lake. (c) The lake stillstand of the "Nakuran" phase is based, according to the published accounts3,4, on only two shoreline features. One of these is a clear cliff line which according to my levelling is at 5,944 ft. (approximately 160 ft. above the lake); the other, reported as a "bank of stratified mud" (ref. 4, page 199), could not be found. (d) Evidence from the sediment sections, although not all were studied in detail, does not seem to demand an interpretation in terms of Gamblian, Makalian and Nakuran pluvial peaks; McCall studied recent lake sediments in this area and concluded that the three sediment divisions he recognized "appear to owe their existence primarily to factors other than climatic" (ref. 12, page 72).

It seems clear that there were considerably larger lakes (up to 600 ft. deep as opposed to the present depths of less than 10 ft.). in the Nakuru-Elmenteita basin during parts of Upper Pleistocene and Holocene times, and it is likely that these lakes resulted from wetter and possibly colder climatic conditions of which there is evidence from many other parts of Africa. There does not seem to be, however,

 $\langle :: \rangle \wedge$ NAKURU-ELMENTEITA BASIN MENENGAL Land above 6,600 feet 115 -6400- 6,400 feet contour Crater rim (BASIN EBURU

Fig. 1

sufficient evidence for the use of the terms Gamblian, Makalian and Nakuran to indicate climatic phases which may be correlated with possible pluvial or glacial phases from other parts of Africa and even Europe. Until absolute dates are obtained for material which can be linked to particular shoreline levels, any attempt at such correlations is of little practical value.

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Fossil Manganese Nodules from Sicily

From the stratigraphically condensed limestones of the West Sicilian Jurassic, which overlie a white Trias-Lias reefoid facies. Wendt recorded fossil ferromanganese nodules1.

The shape of the fossil nodules varies from place to place and from bed to bed. Some occur as small, roughly spherical, crenulate bodies, showing well developed concentric structure, others are larger, mamillated, potato shaped masses, or the nodules may fuse into an irregular pavement. The nodules and crusts are, in fact, physically indistinguishable from their modern counterparts; and it is interesting that this same geo-graphical variation in nodule form is also found in modern examples². Recent manganese pavements have been reported from the Blake Plateau³.

The fossil nodules are also comparable with recent ones in exhibiting lamination, which may disappear inwards, and, in some cases, alternate light and dark bands1; at one locality, instead of the usual limestone or organic remnant, pieces of reworked volcanic tuff have formed the nodule nuclei, thus furthering the comparison⁵.

Preliminary microprobe analysis shows that the Sicilian nodules contain most of the trace elements which characterize modern examples; however, in the fossil forms, the quantity of iron is persistently greater than the manganese (compare Mero's analyses2). This is possibly a result of post-depositional migration of the more mobile manganese^{6,7}. Calcium also is high because of the accretion of the nodules in a former limestone environment.

Mineralogically, apart from calcite, only goethite and occasionally haematite are posi-

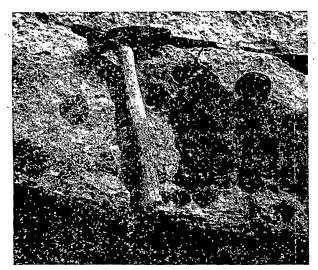


Fig. 1. Vertical section. Condensed bed with discrete, concentrically laminated ferromanganese nodules. Rocca Argenteria, Western Sicily. (Length of hammer handle, 36 cm.)

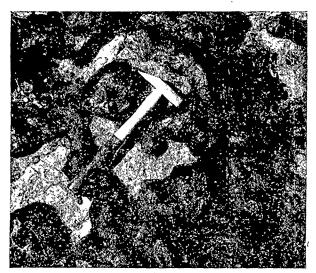


Fig. 2. Bedding surface. Condensed bed with fused ferromanganese nodules. Monte Kumeta, Western Sicily. (Length of hammer handle, 28 cm.)

tively identifiable by simple X-ray methods, the conclusion being that all the manganese oxides are colloidal or extremely fine grained—as is the case with many modern nodules². The fossil nodules described from the Cretaceous of Timor⁸, however, contain goethite with pyrolusite, hausmannite and probably cryptomelane.

The association of the fossil ferromanganese nodules with stratigraphically condensed beds is significant. Condensed beds must have been formed in an environment of minimal net sedimentation; in today's oceans the central deeps and current-swept topographic highs qualify as possible milieux, and it is in these two environments that ferromanganese nodules seem chiefly to occur now^{2,9,10}. The fossil nodules described by Molengraaf¹¹ from the East Indies occur in the presumed lithified equivalents of the red deep-sea clay; but there is little evidence for a deep-sea origin of the red condensed limestones of the Sicilian Jurassic. On the contrary, there is some evidence that these deposits were laid down in shallow water^{1,12}. Ferromanganese nodules themselves are no guide to depth; modern forms occur in a variety of shallow water environments13.

The condensed beds, in similar facies, encompass different time intervals from locality to locality (ref. 1 and personal communication from H. S. Torrens), showing that the conditions for their formation were not realized

solely during one period. The evidence therefore suggests that the condensed ferromanganiferous deposits from Sicily were laid down on transient topographic highs composed of the underlying Trias-Lias White Limestone, these topographic highs possibly being comparable with the non-magnetic seamounts now existing off the Iberian coast14.

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PHYSICS

Electron Energy Distributions in Moving Striations

PEKAREK'S theory of the wave of stratification1 has recently been extended to cover self-excited moving striations2. This theory, which assumes a Maxwellian energy distribution, implies that an essential characteristic of moving striations is a phase shift between the maxima in the variation of the electron density and electron temperature. Time-resolved Langmuir probe and microwave measurements^{2,3} seem to verify the theory.

The theory is linear, however, whereas the large

amplitude and non-sinusoidal shape of the observed variations imply a non-linear wave. Comparison with the familiar stationary striations, which are often found in the molecular gases, would indicate the existence of a double peaked electron energy distribution4. The concept of electron temperature is then inadequate, particularly because the departure from Maxwellian form is greatest in the high energy tail of the distribution which determines the production rate of ions and metastables.

For this reason, the electron energy distribution in moving striations has been obtained using the Druyvesteyn probe technique. To achieve this, time-resolved first derivative measurements were made, the second derivative being obtained by graphical differentiation.

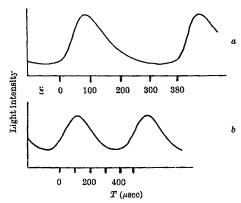


Fig. 1. Variation of light intensity. a, Discharge current 172 m.amp; b, discharge current 100 m.amp.

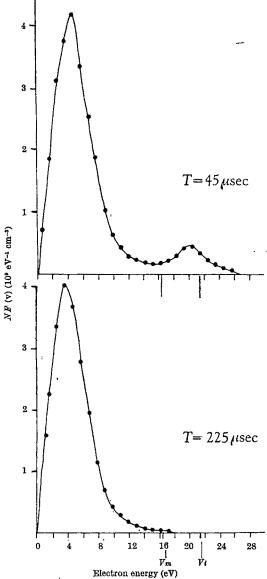


Fig. 2. Energy distributions in strictions with light intensity variation shown in Fig. 1a. Vm, Metastable potential (16.6 V); Vi, ionization potential (21.5 V).

The time-resolved first derivative characteristics were obtained by a sampling technique. A small sinusoidal signal was superimposed on the probe bias for a small part of each striation period and the fundamental frequency component of the probe current was measured. This quantity is proportional to the first derivative of the probe current-voltage characteristic. Measurements through a striation were made by varying the time delay of the signal pulse over a striation period. The fundamental component of the probe current resulting from the probe sheath capacitance is in quadrature to the component resulting from the first derivative and can be eliminated by phase sensitive detection. The arrangement for superimposing the a.c. signal on the probe bias was similar to that used by Boyd and Twiddy⁴. The d.c. bias was varied linearly by a motor and the output of the phase sensitive detector was taken to a chart recorder to give a continuous first derivative characteristic.

Measurements have been made in a hot-cathode discharge in neon (p=0.35 torr, 2R=5.6 cm, L=180 cm) in which two types of moving striation were observed, in both cases the phase velocity being directed from anode to cathode. The first type, present at discharge currents greater than 120 m.amp, had a frequency of 2.6 kc/s and a wavelength of 15.8 cm, corresponding to a phase velocity

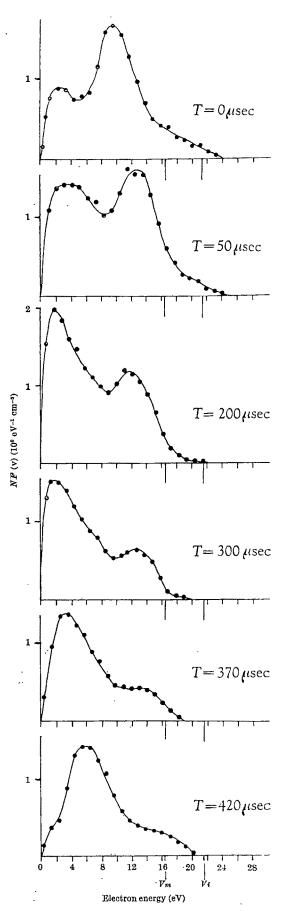


Fig. 3. Energy distributions in strictions with light intensity variation shown in Fig. 1b. Vm, Metastable potential (16-6 V); Vi, ionization potential (21-5 V).

of $4\cdot1\times10^4$ cm/sec. The dispersion relation for these striations was obtained by modulating the discharge current over a range of frequencies close to the natural frequency. The wavelength increased with frequency which showed these moving striations were backward waves. The group velocity was 3.9×10^4 cm/sec. The second type, present at a discharge current of 100 m amp, had a frequency of 2.1 ke/s and a wavelength of 8.5 cm, corresponding to a phase velocity of 1.8×10^4 cm/sec.

The spherical measuring probe was located 110 cm from the cathode and the a.c. signal applied to it had a frequency of 50 kc/s and a peak to peak amplitude of 0.5 V. This signal was applied for 12 usec in each striation period. The space potential was taken to be the point at which the first derivative was maximum.

Fig. 1 shows the observed variations of light intensity with time. Two energy distribution measurements made in the higher current striations are given in Fig. 2. The first measurement was made 30 µsec before the crest of the light intensity, the second 150 usec after the crest. These results show the two well defined groups of electrons which are characteristic of stationary striations, the higher energy group being produced by the acceleration of low energy electrons from the tail of the previous striation through a double space charge sheath4

Fig. 3 shows six measurements made in the striations occurring at the lower current. The first (T=0) was made 100 usec before the crest of the striation and the other distributions correspond to delays of 50, 200, 300, 370, 420 usec, respectively (striation period, 470 usec).

The second measurement (T=50) shows two groups of electrons, the high energy group, with mean energy of ~12 eV, having been produced by the acceleration of a low energy group through a potential step. The four subsequent measurements (T=200, 300, 370 and 420)show the depletion of this high energy group through the striation. Unlike the high energy electrons in the striations occurring at higher current this group is not completely exhausted but is accelerated through a further potential step to produce an energy distribution identical to that shown in the first measurement (T=0). A novel feature of this distribution is that the high energy tail, capable of direct ion-pair and metastable production, has been produced by the acceleration of electrons through two successive sheaths.

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Linear Polymers in the Turbulence Vortices

Long chain linear polymers in dilute aqueous solutions show several anomalies, particularly a reduction in turbulent skin friction. Attempts have been made to explain such anomalies in terms of visco-elasticity. It is, however, worth considering the possibility that the effects arise from a "spiral combing" of the macromolecules in the cores of the vortices which constitute the turbulence. If aligned at an angle to a cylindrical surface of slippagewith their front ends in the faster flow on the interiorthese macromolecules would act as "sea anchors" bridging

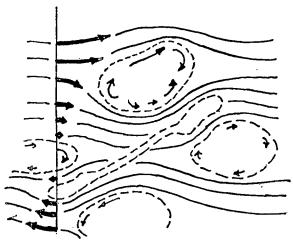


Fig. 1. Ends of a macromolecular tangle may get caught between other tangles.

the streamlines of different velocities, thereby increasing the effective local viscosity in these cores, and thus making these cores act more nearly as rigid bodies. In a plane shear flow such an alignment is improbable, for there a macromolecule, even if it happened to have been stretched, would tend to be turned by the vorticity of that flow until it is aligned with a streamline, and becomes free to slip with respect to other macromolecules so aligned. But the rather unusual circumstances necessary to produce such "combing" at a fairly large angle to the pathlines—namely, a brief spurt of a strong shear, followed by some reduction of the tendency of the fluid to sweep (turn) a stretched macromolecule towards a pathline—are indeed experienced by a macromolecule which is close to a vortex at the moment of its birth. As the diffusing vortex spreads over the macromolecule, this macromolecule first experiences a spurt of shear accompanied by the vorticity of an opposite sense; and by the time it is engulfed by the core (the vorticity of which would tend to sweep this macromolecule towards a pathline) the vorticity of the core is already diminished by the diffusion. It is therefore conceivable that an occasional macromolecule might be stretched by the spurt of the shear enough to have its end "caught" in the manner suggested in Fig. 1. Such "rigidized cores" would be less likely to diffuse, turn, stretch and break up than the vortices which constitute the turbulence in a homogeneous fluid; and so would more likely be formed in longer sections, and act as "rollers", namely, as more effective constituents of the vortex sheet formed by the turbulent layer. This explanation accounts also for other anomalies manifested by these solutions.

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Musical Flames

Interaction between flames and sound was first reported by John Leconte in 1858 and has since been studied by Rijke, Rayleigh, Tyndall, Andrade¹ and many others. It was the subject of a special session at the fourth symposium of the Combustion Institute in 1952 under the heading "Oscillatory Combustion".

Leconte had noticed that a gas flame at a concert responded to certain beats of the music. Subsequent work attempted to analyse and explain this effect in terms of the properties of sound waves and those of flames². Because very characteristic changes in the shape of a flame can be produced by sound waves of specific frequencies, flames have been used as sound detectors in

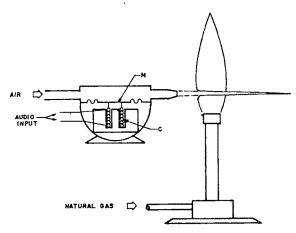


Fig. 1. The modulation of a bunsen burner flame by a jet of oxygen at right angles to the flame. The membrane, M, and the coil, C, are shown.

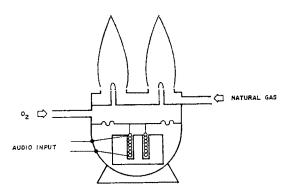


Fig. 2. A different arrangement of Fig. 1 using the acetylene and oxygen supply from a welding unit.

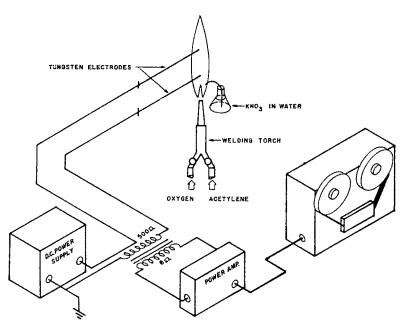


Fig. 3. Another method of modulating a flame using the output from the secondary winding of a transformer. Sufficient ionization is provided in the flame by a wick from a potassium nitrate solution.

acoustic research³. Experimenters have observed that the response of the flame is more energetic than the sound intensity which caused it; the flame thus acts as an amplifier, and feedback occurs⁴. Others have shown that flames can in fact accept and reproduce sound and music and act as loud-speakers, confirming the words attributed to Leconte² that we must "look upon all jets as musically inclined".

A very simple experiment (Fig. 1) uses a natural gas flame into which a jet of oxygen is blown at right angles. The flow of oxygen is modulated by motions of the membrane M, which in turn are imposed by the coil C (in practice a commercial loud-speaker membrane with its driving unit is easily adapted to form the flow modulation unit). Any electrical vibrations imposed on the coil membrane and oxygen flow will now be reproduced as audible sound by the flame. If these vibrations are taken from a tape recorder and constitute Beethoven's Fifth Symphony, a very fine rendering is obtained with a fidelity apparently limited only by the quality of the recording and the modulation unit. The "beats" which Leconte observed are clearly evident in the flame.

Many other arrangements are possible, of which we sketch only one (Fig. 2) conveniently operated from the acetylene and oxygen supply of a welding unit, with the same fine reproducing qualities.

In the experiments described, the modulation unit really produces the sound waves and the flame merely amplifies them, although the amplification, even on the laboratory scale described, is of the order of several hundred. We have found, however, that it is not necessary to modulate the flame physically; it can be done electrically as well, as was observed, on the shape of the flame and its wake only, by Zickendraht⁵. For this purpose a voltage is imposed on the flame between points near the base and near the end of the visible region. The voltage is taken from the secondary windings of a transformer, and it has to be biased to allow symmetrical oscillations. The desired input is fed to the primary transformer windings, and will be audibly reproduced by the flame. The experiment is sketched in Fig. 3. The acetylene and oxygen used do not contain sufficient ionizable species, but these are readily added by touching to the base of the flame an asbestos wick feeding an alkali salt like potassium nitrate in water solution. The arrangement shown will fill a

large room with music or speech.

The observations described show that the production of sound by the flame is associated with the surface in which the chemical energy is liberated, corroborating the concept of flame surface tension6. This is also the place where the strongest light emission takes place, and the latter also contains the imposed modulation. By optically forming an image of the light from this region of the flame in one of these experiments on a suitable photocell, amplifying its output and feeding it to a loud-speaker, the input from the tape recorder is again recovered in audible form. Using the experiments in Figs. 1 and 2 a delay will occur caused by the time the gas takes to flow from the modulation unit to the flame, but no delay is apparent with the experiment in Fig. 3.

The optical photocell arrangement can be used to show that even the weak flame of a candle has the properties described. If one talks at a candle flame, the monitoring photocell will reproduce the voice, albeit volume and fidelity are deficient in this case.

While this has considerable entertainment value, its implications may apply to a number of combustion phenomena,

including, for example, the instability of chemical rocket engines.

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Optical Activity in a Non-enantiomorphous Crystal Silver Gallium Sulphide

For the first time, optical activity¹⁻³ has been positively identified in a non-enantiomorphous crystal, that is, one which does not have left and right handed forms. observations are in agreement with the predictions of the theory of optical activity based on the gyration tensor.

The gyration tensor (an axial second rank tensor) is the lowest order tensor compatible with the observation that the sign of the optical rotatory power depends on the left or right handedness of the crystal axes but does not change when the direction of propagation is reversed. When the symmetry of this tensor is considered in relation to the point symmetry of the thirty-two crystal classes it is found that optical activity can only be present in fifteen of them $(1, 2, m, 222, mm2, 4, \overline{4}, 422, \overline{4}2m, 3, 32,$ 6, 622, 432 and 23). As far as can be ascertained optical activity has never been observed in the four non-enantiomorphous classes m, mm2, 4 and 42m. According to this theory the two bi-axial classes, m and mm2, would show rotation along the optic axes, provided that these are not in a plane of symmetry; unfortunately, no examples are known⁵. The two uniaxial classes, 4 and 42m, do not exhibit rotation along the optic axis because of the rotation-inversion symmetry. Optical activity should be present in other directions but it is masked by the much larger effects of birefringence. No evidence of optical activity has ever been reported in these two classes.

Silver gallium sulphide (AgGaS₂, class $\overline{42}$ m)⁵ is a yellow crystal with high refractive indices, transparent at wavelengths greater than 4800 Å. This crystal has been found to have the unusual property that the birefringence changes sign at about 4970 Å. At this wavelength the crystal is accidentally optically isotropic. This enables optical rotation to be measured with light of this wavelength in any direction. It can be shown from the form of the gyration tensor for class 42m that the optical rotatory power in a direction specified by direction cosines l_1 , l_2 , l_3 , with respect to the x, y and z axes, is

$$\rho = \pi g_{11}(l_1^2 - l_2^2)/\lambda_0 n$$

where g_{11} is an element of the gyration tensor, λ_0 is the free space wavelength and n is the refractive index. From this equation it can be seen that rotation along the x and y directions should be of equal magnitude and opposite sign. A direction lying in a symmetry plane should have no rotation.

These predictions have been verified. Plates of various thicknesses cut from a single crystal perpendicular to x or y directions show unambiguously a rotatory power of

522 degrees/mm at this wavelength, the signs of the rotation being opposite. No convention has yet been adopted to distinguish x and y and derive the absolute sign of g_{11} ; the magnitude of g_{11} is 3.88×10^{-3} . Plates cut perpendicular to the c axis show no rotation.

It is hoped that this observation will finally eradicate the notion that optical activity is exclusively related to

enantiomorphism.

I thank D. S. Robertson and H. A. Chedzey for growing the crystals and K. F. Hulme for helpful comments.

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Composition of Velocities

In pre-relativity kinematics the directed quantity velocity is defined by v = dx/dt, a postulate which inherently specifies the law of composition as the vector, or parallelogram, law. In relativity kinematics the velocity of a point mobile P is still defined as the directed quantity the components of which are those of dx/dt, but it is no longer a vector obeying the parallelogram law of addition,

a point which will be stressed by writing \hat{v} .

In equations \hat{v} appears through the matrix $T(\hat{v})$, the pure Lorentz matrix describing the transformation between co-ordinate systems attached to P and to the observer, such that every point of the system attached to P is seen to be moving with velocity ϑ . In the P system a typical point, such as P itself, being stationary, will observe only passage of time, an observation denoted by $[\mathbf{0}, d\tau]$. The corresponding interval will be seen by the observer as $[\mathbf{dx}, \mathbf{d}t]$, where by the very definition of the motion $\theta = \mathbf{dx}/\mathbf{d}t$. The connexion between the observations is then

$$[d\mathbf{x},dt] = T(\hat{v})[\mathbf{o},d\tau]$$
 (1)

Special relativity, however, in addition to defining velocity through equation (1), also specifies its law of composition by extending equation (1) to read

$$[\mathbf{dx}, \mathbf{dt}] = T(\theta) [\mathbf{dy}, \mathbf{d\tau}] \tag{2}$$

a relation holding between space-time intervals as measured in the two systems for a point mobile moving with respect to both of them. The fact that equation (2) is usually deduced from the simpler postulate that the system of equations connecting such observations is linear does not alter the fact that it represents an additional postulate over and above equation (1).

Writing $\hat{w} = dx/dt$, $\hat{u} = dy/d\tau$, equation (2) can be shown to be equivalent to the matrix equation1

$$T(\hat{v})T(\hat{u}) = T(\hat{w})R(\Omega) \tag{3}$$

which demonstrates explicitly the relativity law of composition of velocity \hat{u} followed by velocity \hat{v} to give a resultant velocity \hat{w} . The matrix $R(\Omega)$, having the structure of a spatial rotation operator, is usually interpreted as demonstrating that successive pure Lorentz transformations induce a rotation of the spatial coordinate frame followed by a resultant pure Lorentz transformation. Equation (3) has the advantage over equation (2) of showing explicitly the appearance of this operator, necessitated by considerations of mathematical congruity.

Equation (3), as shown by its equivalent equation (2), is sufficient for equation (1), but not necessary. If the assumption of a linear system of equations existing between $[d\mathbf{x},dt]$ and $[d\mathbf{y},d\tau]$ is abandoned the way is clear for alternative hypotheses as to the composition of velocities. One in particular, which shows extraordinary promise, is to retain the matrix T(t) to represent a velocity t but to postulate the law of composition of velocities as

$$[T(\hat{v})]^{2}[T(\hat{u})]^{2} = [T(\hat{w})]^{2}[R(\Omega)]^{2}$$
 (4)

Structurally, equations (3) and (4) are similar and the solution of equation (4), that is, expression of \hat{w}, Ω , in terms of \hat{v}, \hat{u} can be obtained from the known solution of (3) by observing that

$$[T(\vartheta)]^2 = T \left[2\vartheta / \left(1 + \frac{|\vartheta|^2}{c^2} \right) \right] \tag{5}$$

a similar relation existing for $R(\Omega)$. Once again the factor $[R(\Omega)]^2$ is needed for mathematical congruity. \hat{w} has been defined as the resultant velocity but this time it is meaningless to explain away $R(\Omega)$ as representing a rotation of some spatial co-ordinate framework, but more logical to suppose Ω to characterize some as yet unidentified property of the mobile itself. Because $R(\Omega)$ has the structure of a rotation matrix, Ω itself is inherently an axial directed quantity, as opposed to the parameters $\hat{u}, \hat{v}, \hat{w}$, associated with T-matrices, which are inherently polar.

Thus the motion of a mobile is described by a polar parameter \hat{w} and an axial parameter Ω through the matrix $[T(\hat{w})]^2[R(\Omega)]^2$. If these parameters change, so that the descriptive matrix becomes $[T(\hat{w}+d\hat{w})]^2[R(\Omega+d\Omega)]^2$ then it is logical to connect the change with the externally applied force. If that force is purely polar, \hat{f} , the change matrix multiplier will be of type T and this condition lead to

$$\hat{f} = \frac{1 - \frac{|\hat{w}|^2}{c^2}}{1 + \frac{|\hat{w}|^2}{c^2}} \frac{d}{dt} \left\{ \frac{\hat{w}}{1 - \frac{|\hat{w}|^2}{c^2}} \right\}$$
(6)

This equation has been derived from purely kinematical considerations. As usual, equality of inertial and gravitational mass is assumed so that equation (6) is converted into the dynamical generalization of Newton's second law by multiplying each side by the mass of the mobile m_0 . The energy integral is then

$$m_0 \int \hat{f} \cdot d\mathbf{x} = m_0 c^2 \log \left(1 - \frac{|\dot{w}|^2}{c^2} \right)^{-\frac{1}{2}}$$

$$= \frac{1}{2} m_0 |\dot{w}|^2 + \frac{1}{2} m_0 \frac{|\dot{w}|^4}{c^2} + \dots$$
 (7)

Calculations based on equations (6) and (7) for \hat{f} obeying the inverse square law give the correct values for the advance of the perihelion and the bending of light.

Use of equation (4) in place of equation (3) originally arose in an as yet unpublished investigation of the possibility of representing an electrostatic field vector by a T-matrix and using equation (3) to depict its transformation by a velocity T-matrix into a field containing an electrokinetic T-matrix and a magnetic R-matrix.

Although the structural requirements of the physical problem were met, quantitative agreement was only secured when equation (3) was replaced by equation (4), or rather a slight variant of it, where if & characterizes the electric field

$$[T(\hat{v})]^2 [T(\hat{u})]^2 = R(\Omega) [T(\hat{g})]^2 R(\Omega)$$
 (8)

 $T(\hat{g})$ being the electrokinetic operator rather than $T(\hat{w})$.

Physical theory founded on equations (4) and (8) gives results very different to those of special relativity. In particular contraction in dimension is isotropic and charge decreases with motion by the contraction factor

$$\gamma = \left(1 - \frac{|\hat{w}|^2}{c^2}\right)^{-\frac{1}{2}}$$

Thus in applying equation (6) to the motion of a charged particle, rest charge q_0 , rest mass m_0 , in an electric field ℓ the force is $q_0 \gamma^{-1} \ell$ and the energy integral is now

$$q_0 \int \hat{e} \cdot d\mathbf{x} = m_0 c^2 (\gamma - 1) = \frac{1}{2} m_0 |\hat{w}|^2 + \frac{3}{8} m_0 \left| \frac{|\hat{w}|^4}{c^2} + \dots \right|$$
 (9)

which is just the result of special relativity!

A similar calculation for a transverse magnetic field once more yields the relativity formula, so that the new theory is entirely consistent with the formulae used in analysing particle accelerators.

It remains to investigate Ω in the kinematic case. Noting that the left hand sides of equations (4) and (8) are identical suggests that there might be a kinematic significance to equation (8) when θ and \hat{u} are both velocities. \hat{w} is then \hat{g} rotated as prescribed by $R(\Omega)$, the moduli, or speeds, being equal.

moduli, or speeds, being equal.

This suggests that the true motion \hat{w} of a particle of non-zero Ω might appear "aberrated" into an apparent velocity \hat{g} . If this be so, particles of the same \hat{w} but different Ω would present different \hat{g} . Thus for a given $|\Omega|$ but random direction for the axis of Ω , \hat{g} would lie within a cone of half-angle $|\Omega|$ centred on \hat{w} , or in other words its direction would be uncertain, although bounded.

Finally, two particles having the same apparent velocity θ cannot have different Ω because their trajectories would then diverge. This is an exclusion principle.

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CHEMISTRY

Electrical Polarization and Microwave Absorption of Aluminium Acetylacetonate in Benzene Solution

STATIC dielectric constants and specific volumes have been measured for benzene solutions of aluminium acetylacetonate at 25°, 35°, 45° and 55° C. The dielectric constants and losses at frequencies of 9,133 and 24,560 Mc/s were also determined for benzene solutions of the aluminium chelate at 15°, 25° and 45° C. Total molar polarizations and dielectric relaxation times have been calculated from these data.

Aluminium acetylacetonate (J. T. Baker Chemical Co.) was repeatedly crystallized from benzene-petrolcum ether and then dried over phosphorus pentoxide under vacuum (melting point, 193.0°-193.5° C). The analysis

calculated for $Al(C_5H_7O_2)_3$ was carbon 55.55 per cent, hydrogen 6.52 per cent, and was found to be carbon 55.68 per cent and hydrogen 6.56 per cent by weight. Reagent grade benzene, free of thiophene (Fisher Scientific Co.), was fractionally distilled over lithium hydride and stored over 'Drierite' until immediately before measurement $(n_D^{200}, 1.50111).$

Static dielectric constants were measured with a heterodyne-beat apparatus (described in a report to be published) at 1 Mc/s. The dielectric constants and losses at the microwave frequencies were determined by using waveguide techniques described elsewhere1,2. The microwave results obtained from the solution studies were treated as described earlier3. The slopes of the straight lines thus obtained, a' corresponding to ϵ' and a''corresponding to ε", were plotted in Cole-Cole arcs to obtain the critical wavelengths, λ_m , the most probable relaxation times, τ_0 , the distribution parameters, α , and the arc intercepts at high frequency, a_{∞} . The values of these quantities are given in Tables 1 and 2. The concentration range in mole fraction of solute for the set of solutions examined is given in parentheses in Table 1. The total molar polarizations, $P_{2\infty}$, listed in Table 2, were calculated by the method of Halverstadt and Kumler⁵.

The absolute error in the $P_{2\infty}$ values of Table 2 was estimated to be ± 3 cc. An error analysis of these polarization values and the parameters from which they were deduced have not yet been published. The error in the to values of Table 2 was estimated to be ± 15 per cent.

Table 1. Slopes for the dependence of the diblectric constant and loss of aluminium acetylacetonate-benzene solutions (0-0-049) on mole fraction of solute

Frequency	1	5° C	28	5° C	45° C		
(Mc/s)	a'	a"	a'	a^*	a'	a"	
24,560	3.18	0.376	3.24	0.371	3.26	0.337	
9,133	3.52	0.329	3.48	0.260	3.41	0.174	
1	3.67		3.59		3.44		

Table 2. High frequency intercepts, critical wavelengths, relaxa -tion times, distribution parameters, and total moder polarizations of aluminium acetylacetonate in benzene solution

t (° C)	a_{∞}	λ_m (cm)	$\tau_0(\times 10^{-18}\mathrm{sec})$	α	$P_{z\infty}$ (cc)
15	2.87	1.56	8.3	0	
25	2.82	1.09	5.8	0.03	131.7
35					132.4
45	2.66	0.64	3-4	0	131.4
55					130.0

The static measurements confirm that aluminium acetylacetonate is non-polar. The change of polarization for a range of temperatures of 200° C is seen to be not more than 1 cc when these results are combined with the vapour phase total polarization value, 130.8 cc at about 240° C. The fact that aluminium acetylacetonate is nonpolar is significant because it has been found to exhibit considerable microwave loss in benzene solution (Table 1). For example, the losses observed at 9,133 and 24,560 Mc/s for a solution of about 0.05 mole fraction at 25° C were about ten times greater than those of the non-polar solvent, benzene. These losses are much too large to be accounted for by the presence of polar impurities in the starting materials or dissociation of the aluminium chelate in solution. Substantial dissociation in solution would have resulted in a measurable difference between the solution and vapour total polarization. Moreover, the infrared spectra of a number of metal acetylacetonates in solution show no evidence of absorption peaks resulting from dissociation products7. The observed dielectric losses therefore cannot be explained as the relaxation losses of rotating permanent dipoles. Any explanation of these losses must be in agreement with the decrease of loss with

increase of temperature, with the frequency dependence of the loss, and with the fact that the solution total polarization of the chelate does not differ significantly with the vapour phase value.

The molecular distortion mechanism suggested by Whiffen⁸ to account for the dielectric losses exhibited by benzene, carbon tetrachloride and other non-polar liquids cannot be invoked in the present case. This mechanism for the loss would lead to an appreciable difference between the solution total polarization and the vapour total polarization of aluminium acetylacetonate. contribution to the solution static polarization of about 11 cc is calculated from the extrapolated a_{∞} and the measured a_0 value (25° C). A solution total polarization 11 cc greater than that of the vapour would have been detected.

The thermal bending theory, considered as a possible explanation for the large differences between the total and electronic polarizations of a number of non-polar metal acetylacetonates, cannot explain these results. This theory postulates that the molecules bend considerably as a result of the energy imparted by thermal collisions and remain bent long enough to rotate like permanent dipoles in an applied static field, while still bent one way. The relaxation times found for the aluminium chelate in benzene solution (Table 2) are much too short to be consistent with such a picture.

The observed loss cannot be reconciled with the fact that it is the long wavelength tail of a vibrational, infrared absorption band which is being observed. If this were the case, an increase in the loss in the low frequency tail would be expected with increase in temperature because the line breadths of the infrared bands increase with tempera-

Recently, the structural details, as determined by X-ray crystallography, of thirteen acetylacetone chelates have been summarized10. In all cases, the acetylacetone portion of the chelate ring is planar within the experimental error of the data. The metal atom may, however, deviate from the plane of the chelate ring by as much as 0·1-0·5 Å, giving the chelate considerable flexibility at the metal. This fact suggests that a relatively low frequency vibration of the rings about the aluminium atom as a centre may be an important motion to consider in arriving at a plausible explanation of the observed loss of aluminium acetylacetonate in solution and its frequency dependence. At present, microwave absorption measurements are being made on solutions of other non-polar co-ordination compounds (for example, beryllium-, iron (III)-, and thorium (IV) acetylacetonate, and a number of transplanar complexes of copper and nickel). The tetrahedral, octahedral, cubic and planar complexes are being studied in the hope of throwing additional light on the problem.

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Catalytic Carbon-Carbon Bond Formation from Methylene Chloride over Evaporated Titanium Films

During a study of the reactions of alkyl chlorides at the surfaces of evaporated metal films we observed that a gas phase mixture of methylene chloride and hydrogen reacted in the temperature range 200°–300° C at the surface of evaporated titanium to yield a variety of hydrocarbon products from C_1 to a waxy polymer. Out of every hundred carbon atoms entering the reaction, not less than eighty entered products of C_2 or higher. We wish to direct attention to this reaction because a polymerization starting from a C_1 parent is unusual.

The reactions were carried out in a static system with initial methylene chloride and hydrogen pressures (measured at 0° C) of 6·0 and 60 torr, respectively. The apparatus and technique used were similar to those previously used for reactions over metal films (ref. 1 and unpublished results). Gas phase analysis was by means of mass spectrometry and vapour phase chromatography. Titanium films were in the range 5–10 mg, and the effective surface area under reaction conditions was estimated by the Brunauer–Emmett–Teller method to be about 500 cm². The reaction volume was 180 ml., and a standard reaction mixture contained $3\cdot84\times10^{19}$ molecules of methylene chloride.

The composition of the products in typical reaction conditions is given in Table 1. Usually, the reaction products fell into two categories: first, gas phase hydrocarbon products in the range C1-C5 were observed by analysis of the gase phase; and second, a colourless filmlike material which was presumably highly polymeric was recovered from the surface of the titanium after the reaction by treatment of the metal with dilute hydrofluoric acid. The material recovered in this way was found to be amorphous when examined with transmission electron diffraction in an electron microscope. nature of the experiment did not allow sufficient material to be produced for more detailed study. Examination of the titanium itself after the reaction by transmission electron diffraction gave a pattern that indexed as TiH1.0, using evaporated gold as a calibrating standard. Table 1 shows that the total number of carbon atoms which enter the polymer is in approximate agreement with the estimated weight of polymer. For example, if the polymer is assumed to be free of chlorine and of approximate composition $(CH_2)_n$, 330×10^{-7} moles of incorporated carbon corresponds to a polymer weight of about 0.5 mg. Not all the polymer could be recovered, but the total amount was estimated to be of the order of 1 mg.

The extensive range of products suggests that the mechanism of this reaction may well be a complex one. A detailed account will be published of the results of an investigation into the reaction mechanism. present purpose, however, we may summarize the following relevant observations. (a) Ethylene and propylene were formed with about the same activation energy (~6 kcal mole-1), and methane and the small amounts of ethane and propane were also all formed with about the same activation energy (~ 11 kcal mole⁻¹). (b) In a similar reaction with deuterium in the place of hydrogen, most of the ethylene was produced as C₂H₄. (c) Reactions at very low gas pressures (in the mtorr region) showed that surface hydrogen was not required for the process of hydrocarbon desorption, and the surface stoichiometry suggested that CH2 residues together with some more extensively dehydrogenated species were present on the surface. From these observations we tentatively suggest that the chief reaction path can be described by the following types of reactions. The notation is used where $(CH_2)_8$ means a methylene group chemisorbed at the titanium surface.

$$CH_2Cl_2 \rightarrow (CH_2)_s + 2(Cl)_s$$
 (1)

$$(\mathrm{CH_2})_s + \mathrm{CH_2Cl_2} \rightarrow \begin{bmatrix} \mathrm{CH_2}. \\ | & + & \mathrm{CH_2Cl_2} \\ \mathrm{surface}. \end{bmatrix} \rightarrow \\ (\mathrm{CH_3CH})_s + 2(\mathrm{Cl})_s \quad (2)$$

$$(CH_3CH)_{\delta} \rightarrow C_2H_4$$
 (3)

$$(Cl)_s \xrightarrow{H_s} HCl$$
 (4)

The residue $(CH_3CH)_8$ may react further with CH_2Cl_2 to give a C_3 residue. On this basis, the general propagation step is

$$(CH_3(CH_2)_nCH)_s + CH_2Cl_2 \rightarrow (CH_3(CH_2)_{n+1}CH)_s + 2(Cl)_s$$

The entities written within square brackets in reaction (2) are meant to indicate that a quasi free radical may be involved in the addition step, although this may only occur as the transition state is approached. We suggest that the saturates are formed by inter-residue hydrogen transfer reactions.

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Binuclear Carbonato-complex of Chromium (II)

With few exceptions, chromium (II) complexes can be simply classified according to their colours and magnetic moments. Most are blue or purple with magnetic moments which correspond to four unpaired electrons for each metal ion, but some salts of carboxylic acids are red or brown in colour and diamagnetic. Chromous acetate has been shown to possess a dinuclear structure with a short metalmetal bond. One species which seems to fall outside this classification is the product of the interaction of chromium (II) and carbonate ion. The simple colourless carbonate is soluble in excess aqueous carbonate media to give yellow solutions, from which yellow-brown complexes can be crystallized, such as Na₂Cr(CO₃)₂.10H₂O, Na₂Cr(CO₃)₂.H₂O, K₂Cr(CO₃)₂.3H₂O, (NH₄)₂Cr(CO₃)₂.H₂O (ref. 2). We have examined the yellow complex in aqueous solution by a spectrophotometric method and have shown that it contains two atoms of chromium per complex ion.

The visible spectra of chromous acetate and of the carbonate complex are shown in Fig. 1. By choosing the correct ratio of concentrations of acetate to bicarbonate, it is possible to prepare solutions with intermediate

Table 1. Typical distribution of products from the reaction of methylene chloride on evaporated titanium at 275° c

Product	CH4	CH ₂ Cl	Total C ₁	C_2H_4	C_2H_4	Total C ₁	C_3H_5	C_2H_8	Total C ₃	Total C ₄ -C ₅	Polymer (by) mass balance
Carbon appearing in indicated product (mole × 107)	60	11	71	55	19	74	22	8	30	Approx. 10	Approx. 330
Total CH _* Cl _* reacted = 51	6 × 10-7	mole.						•			

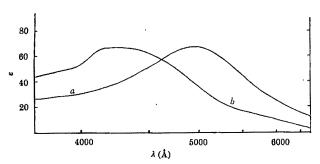


Fig. 1. Absorption spectra of chromium (II), (a) in acetate buffer medium, NaOAc 1.0 molar, HOAo 1.0 molar, chromium (II) 4×10^{-3} molar; (b) in bicarbonate medium, pH 7.2, potassium bicarbonate 1.0 molar, ammonium chloride 1.0 molar, chromium (II) 4×10^{-3} molar.

spectra, which must contain mixed acetato-carbonato complexes. (There is no isosbestic point.) At the wavelength 4340 A, the extinction coefficient of the pure carbonato complex exceeds that of the acetate, while at 5100 Å the reverse is true. If in a mixture of complexes the total chromium concentration is varied, the ratio of absorbances at these two wavelengths remains constant; therefore the relative proportions of complexes are constant, and the number of chromium atoms in each must be the same. Otherwise, with increasing total chromium, equilibria of the type in equation (1) would be displaced to the right or left, according to whether n is greater or less than two

$$n \operatorname{Cr}_{2}(\operatorname{OAc})_{x}^{(4-x)+} + 2y\operatorname{CO}_{3}^{=} \rightleftharpoons \\ 2\operatorname{Cr}_{n}(\operatorname{CO}_{3})_{y}^{2(n-y)+} + nx\operatorname{OAc}^{-}$$
 (1)

The number of carbonate ions in the yellow complex has not been determined, but it has been observed that, in mixtures of complexes with a constant ratio of free acetate and bicarbonate ions, the ratio of absorbancies at 4340 and 5100 Å is independent of the total concentration of these ions, for example, $[OAc^-] = 10 [HCO_3^-] = 0.2$ -1.4 molar. This suggests that replacement is a one-for-one process, the total number of ligand ions being the same in each dinuclear complex species.

Further speculation on the nature of the carbonatocomplex would be premature, but it may be pointed out that the spectrum is similar to that of chromous acetate although it is shifted to shorter wavelengths. Both spectra seem to consist chiefly of two broad bands (see Dubicki and Martin³ for an analysis of the acetate spectrum), giving a pronounced "tail" on the low wavelength side. It is also interesting to note that the carbonato complex is comparatively resistant to oxidation, even more so than the acetate. A dilute solution exposed to air takes several minutes to assume the green colour of aqueous chromium (III).

The stability of the complex with respect to dissociation can be judged from its reactions with different ligands. At pH 7.72 it is completely decomposed into monomeric chromium(II) by EDTA and 2,2'-dipyridyl in stoichiometric amounts. On the other hand, glycollic, tartaric, citric and iminodiacetic acids have no effect in concentrations up to 0.1 molar. Pyridine has no effect on the visible spectrum in concentrations even up to 1 molar.

Nitrilotriacetic acid (H₃L) produces partial dissociation into monomer, and further spectrophotometric measurements indicate the following equilibrium

$$\operatorname{Cr}_2(\operatorname{CO}_3)_y(4-2y) + 2\operatorname{L}^{3-} \rightleftharpoons 2\operatorname{CrL}^{-} + y \operatorname{CO}_3^{=}$$

with $K = [CrL^-]^2/[Cr_2(CO_3)_y(4-2y)+][HL^-]^2 = 0.6 \pm 0.2$ l./mole at pH 7.72 and 25° C in I molar potassium bicarbonate, 1 molar ammonium chloride.

It was further observed that K varies as the inverse square of bicarbonate concentration

$$\dot{K}[\text{HCO}_3^-]^2 = 0.45 \pm 0.2 \text{ molar}$$

At first sight this seems to imply that two carbonate or bicarbonate ions separate from every molecule of the dinuclear complex, that is y=2. This conclusion is not certain, however, because at the high bicarbonate concentrations used, there is the possibility of association between the mononuclear CrL- and bicarbonate ion. This is suggested by the fact that a number of nitrilotriacetato complexes ML- have been shown to give hydroxo derivatives with formation constants $\beta_{\text{MLOH}} = [\text{MLOH}^{=}]/[\text{ML}^{-}][\text{OH}^{-}]$ as high as 100 l./mole.

Further work on the acetate-carbonate system is in progress.

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Observations on the Dissolution Potential of Oxalic Acid

RECENTLY, Rastogi, Batra and Dass¹ have reported that potential differences are produced when crystals of an electrolyte just appear or when they are allowed to dis-The former has been called the precipitation potential and the latter the dissolution potential. One of the important conclusions of this study is that the dissolution potential of an electrolyte, in an aqueous solution of the same, is a function of the concentration and that its value increases as the dilution is increased. The dissolution potential was also seen to increase systematically in most cases as the temperature of the water is increased. This seems logical, for the dissolution potential must be related in some manner to the solubility of the electrolyte as the term implies.

Because this is a new effect, we wished to see whether there is any quantitative relationship between the solubility of an electrolyte and its measured dissolution potential. For this purpose measurements of dissolution potential were made for oxalic acid. Our method was essentially the same as described by Rastogi et al.1. A vacuum tube voltmeter (Philips model GM6000/90) with a least count of ± 10 mV was used. Experiments were conducted at four temperatures, and at five concentrations of oxalic acid with the temperature constant at 40° C. The experiments were repeated several times to check the reproducibility of the results. The data are summarized in Table 1.

Table 1. DISSOLUTION POTENTIAL OF OXALIC ACID AS INFLUENCED BY TEMPERATURE AND CONCENTRATION

Temperature of water (°C)	Dissolution potential (mV)	Concentration of oxalic acid	Dissolution potential (mV)*
31	210	0·1 normal	. 70
40	200	0.01 normal	110
50	200	0.001 normal :	160
60 .	220	0.0001 normal	190
		0.00001 normal	200

* Temperature kept constant at 40° C.

Table I shows that although there is a progressive increase in the value of dissolution potential with the decrease in concentration of the solute, there is practically no variation of dissolution potential with increasing temperature. If the dissolution potential is a function of the solubility it is imperative that the dissolution potential must also increase with increase in temperature because there is a nearly four-fold increase in the solubility2 of oxalic acid as the temperature is raised from 31° to 60° C. To us therefore the relationship between dissolution potential and solubility is not obvious.

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Rates of Adsorption of Wetting Agents and Detergents at 'Graphon' Solution Interface

THE sodium salts of α-sulpho fatty esters (R CH(SO₃Na)-COOR') behave as either wetting agents or as detergents depending on the length of the acid and alcohol chains (R and R'). In practice, if either R or R' is long and the other chain short, then the surfactant behaves as a detergent; if both R and R' are of the same medium chain length, then the surfactant functions as a wetting agent. The ability of a compound to act as a detergent was rated on the basis of 'Terg-O-Tometer' tests and wetting ability was assessed using the Draves test^{1,2}.

A physical chemical approach was sought to uncover the differences between wetting agents and detergents. Thermodynamic factors, such as co-areas at the air/water and oil/water interfaces and heats of adsorption at the solid/liquid interface (refs. 3 and 4 and unpublished work by A. C. Zettlemoyer), did not differ significantly. was recognized that a kinetic factor might distinguish between the two varieties of surfactants. measurement to make was the rate of adsorption to the 'Graphon' (a graphitized carbon black with B.E.T. nitrogen surface area 104 m²/g) surface by following the conductivity decrease with time after the graphitic powder was dispersed into the surfactant solutions (above their critical micelle concentrations, CMCs).

The α -sulpho fatty esters studied were prepared by

Stirton, Weil and their co-workers (refs. 3 and 4) and were purified; they are listed in Table 1.

Table 1. THE a-SULPHO ESTER SURFACTANTS STUDIED

Compound	\boldsymbol{R}	R'	0.01 carbon atoms $R+R'+2$
Wetting agents		_	
(1) Sodium hexyl a-sulphopelargonate (Na Hex aS Pelar)	7	6	15
(2) Sodium heptyl a-sulphopelargonate (Na Hep as Pelar)	7	7	16
(3) Sodium octyl a-sulphopelargonate (Na Oct as Pelar)	7	8	17
Detergents			
(4) Sodium methyl a-sulphomyristate (Na Me aS M)	12	1	15
(5) Sodium methyl a-sulphopalmitate (Na Me aS Palm)	14	1	17
(6) Sodium ethyl a-sulphopalmitate (Na Et as Palm)	14	2	18

Thus compounds 1,4 and 3,5 form exact pairs as far as the number of carbon atoms are concerned. Solutions of 5.8×10^{-3} moles (about 0.2 per cent) (well above their CMCs) were prepared with triply distilled water and bulbs containing 1 g of activated 'Graphon' (evacuated at 105° C in 10-5 torr vacuum for 4 h and then sealed off) were broken in 150 cc of the surfactant solutions, with a plastic enclosed magnetic stirrer in operation. All the experiments were done at a temperature of $25^{\circ} \pm 0.1^{\circ}$ C. All the The reduced conductances—that is, the ratio of measured (Ω) to initial conductance $(\Omega_0)-$ are plotted against time in Fig. 1. The plots indicate a clear difference between the two varieties of surfactants. The wetting agent

solutions reached a minimum conductance in about 3-7 min while the detergent solutions required about 20-25 min.

It is doubted that demicellization is the slow process in the case of detergents because demicellization rates of surfactants are reported to be almost instantaneous⁵. A more probable reason for this interesting observation is that the rates of diffusion of the detergent type species to the surface are slower than for the wetting type. For the detergent solutions, a slow ageing and thus a slow decrease in surface tension with time was also noted at the air/water interface (ref. 3 and unpublished work); no such ageing effect was noted for the wetting agent type.

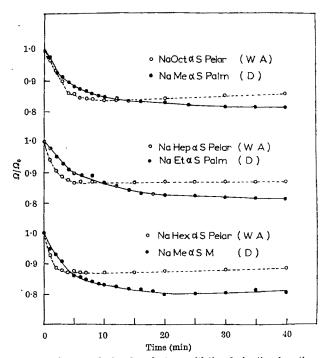


Fig. 1. Changes of reduced conductance with time during the adsorption of wetting agents and detergents at 'Graphon'/solution interface. O. Wetting agents (WA); •, detergents (D).

It is known that the adsorption of surfactant anions takes place with the hydrocarbon portion of the anion oriented toward the hydrophobic surface of 'Graphon' and the polar group projected into the bulk liquid phase. Another possible reason for the slow rates of adsorption of the detergent type species is because the chains R and R' are of different length; re-arrangement of the ions for the proper orientation at the interface may be delayed. In other words, the sticking coefficient of the detergent type species could be lower than those of the wetting agent type.

Diffusion measurements with radioactively labelled species would allow these two factors, diffusion rates and the sticking factor to be separated.

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BIOLOGY

Haemoglobin Variation in Anadara trapezia

THE most common respiratory pigment among the molluses is haemocyanin, which is widely distributed among the gastropods, cephalopods and amphineurans. Most bivalves lack a respiratory pigment, but, if present, the pigment is haemoglobin, which can be found in muscle tissue, in haemocoelic crythrocytes or dissolved in the haemolymph¹. In Anadara trapezia (Deshayes) (Mollusca, Bivalvia), an arcid clam, haemoglobin is present in haemocoelic crythrocytes.

Haemoglobin from some arcid clams has been shown to be heterogeneous. Yagi et al.² showed, by moving boundary electrophoresis (Tiselius apparatus), that the haemoglobin of Anadara inflata contained at least three components. Using starch gel electrophoresis, Manwell¹ showed that the haemoglobin from two arcid clams, Noetia ponderosa and Anadara transversa, was heterogeneous with two principal components present in approximately equal proportions. This communication reports what are probably genetically controlled variations in the haemoglobins of a population of A. trapezia from Sydney. A study of variations between geographically separated populations is in progress.

Specimens of A. trapezia were collected from Gunnamatta Bay in Port Hacking (about 20 miles south of Sydney) and kept in aerated aquaria until they could be bled. Haemolymph was withdrawn from the pallial sinuses of each animal with a hypodermic syringe and each sample was centrifuged at 3,000 r.p.m. for 10 min. The supernatant was discarded. The cells were washed three times with 3 per cent sodium chloride solution and were lysed by alternate freezing and thawing. After centrifugation the cell debris was discarded and the supernatant haemoglobin solution was retained for electrophoresis.

The haemoglobin solutions were immediately subjected to electrophoresis on 'Cellogel' strips (Chemetron, Milan) in a Kohn electrophoresis apparatus operating at 400 V. Satisfactory resolution of the chief haemoglobin fractions was obtained with three buffer systems: (a) the tris (hydroxymethyl) aminomethane (tris)-ethylenediaminetetraacetic acid (EDTA)-boric acid buffer (pH 9.5) of Gelman³; (b) the tris-EDTA-boric acid buffer (pH 9.2) of Aronsson and Grönwall4; and (c) the tris-hydrochloric acid buffer (pH 8.2) of Bates and Bower⁵. separation of the haemoglobin fractions was obtained in 1-1.5 h using the Gelman buffer, whereas 4 h were necessary for separation using the buffers of Aronsson and Grönwall and of Bates and Bower. Although the haemoglobin fractions were immediately detectable on the strips, they were permanently stained with a saturated solution of 'Amidoschwarz 10B' (Merck). The strips were then sealed, together with a little 50 per cent methanol, in polyethylene.

The haemoglobin samples showed either two or three principal bands, which were examined in detail, and up to three "trace" bands, which were omitted from this study. The principal bands, labelled Hb1, Hb2a and Hb2b, occurred in three patterns (as shown in Fig. 1, patterns 1, 2 and 3). All three patterns contained band Hb1. Pattern 1 contained band Hb2a, pattern 2 contained band Hb2b and pattern 3 contained both bands Hb2a and Hb2b.

The frequency distribution of the band patterns in the population of 188 A. trapezia suggests that the bands Hb2a and Hb2b are genetically determined by one pair

Table 1. Observed and expected frequency distribution (hardyweinberg) of haemoglobin electrophoretic patterns in a population of $A.\ trapezia$

	Pattern 1	Pattern 2	Pattern 3
Observed	8	114	66
Expected	8·93	114·96	64-11

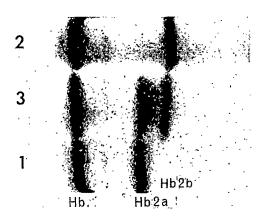


Fig. 1. Electrophoretic patterns (1, 2 and 3) of haemoglobins (Hb1, Hb2a, and Hb2b) from A. trapezia (tris-EDTA-boric acid buffer, pH 9.5).

of alleles. For, if pattern 1 is that of the homozygote Hb2a, Hb2a, pattern 2 is that of the homozygote Hb2b, Hb2b, and pattern 3 is that of the heterozygote Hb2a, Hb2b, the population should closely approximate the corresponding Hardy-Weinberg frequency distribution. It can be seen in Table 1 that this is the case, for the χ^2 value is 0·16 (P=0.7). Thus it seems that these band patterns are genetically determined by one pair of alleles.

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Blood Group Studies of the "Gene Bank" Sheep at Whipsnade Park

ROWLANDS¹ reported that the Zoological Society of London was establishing a "Gene Bank" composed of some rare breeds of domesticated animals which were in danger of becoming extinct. We therefore took the opportunity of investigating the blood groups of some of the sheep which were to form the nucleus of the "Gene Bank" sheep flocks.

Blood samples were collected with and without anti-coagulant. Using a haemolytic test^{2,3}, the red cells were tested for the presence of R and O blood group substances and the sera were tested for the presence of anti-R anti-body. Red cell potassium types^{4,5} were determined using a flame photometer, and the serum transferrin^{6,7} and red cell haemoglobin^{5,8} types were determined by starch gel electrophoresis. The sera and lysates made from washed red cells were stored at -20° C for about a year; they were then tested for electrophoretic variation in the recently discovered serum albumin⁹ and A esterase¹⁰ systems and also in the red cell carbonic anhydrase and "X" protein systems¹⁰.

Table 1 summarizes the results obtained. All the Cotswold sheep were of blood group R. In the other breeds, however, both R and O individuals were found, and in the Portland breed there was also one group i individual. All the group O sheep had anti-R present in their sera. The fast haemoglobin variant A was only found in four of the Woodlands Whiteface sheep; all other sheep were

Table 1. BLOOD GROUP PHENOTYPES IN THE WHIPSNADE "GENE BANK" SHEEP FLOCKS

Sheep breed	No. tested	R	R sys		i	A H	aemogl AB	obin B		Red cel Pota HK	ls ssium LK	Carl F	onic anh FS	ydrase S	X pr	otein —
Cotswold Lincoln Longwool Norfolk Horned Portland Woodlands Whiteface	12 11 5 6	8 6 1 4	0 6 5 3 2		0 0 0 1	0 0 0 0 3	0 0 0 0 1	8 12 11 5 2		0 1 11 1 4 Sera	8 11 0 4 2	0 0 0 0	0 0 0 0	8 12 11 5 6	0 1 2 5 1	18 11 9 0 5
Sheep breed	No. tested	AA	AB	AC	AD	Trans BB	sferrin BC	BD	cc	CD	DD	A est	erase A	F	Albumir FS	s
Cotswold Lincoln Longwool · Norfolk Horned Portland Woodlands Whiteface	8 12 11 5 6	2 0 0 0	4 0 0 0 0	1 0 1 0 0	0 0 0 0	1 2 5 0 0	0 4 5 0 3	0 1 0 1 0	0 4 0 0 3	0 1 0 1 0	0 0 0 3 0	0 6 0 1 0	8 6 11 4 6	0 0 0 0	0 0 0 0	8 12 11 5 6

The numbers listed under each phenotype indicate the number of sheep in the breed which were of that particular type.

of type B. The red cells of all the Norfolk Horned sheep were of high potassium type (HK), while those of all the Cotswolds were low potassium type (LK); individuals of both potassium types were found in the other breeds. A red cell carbonic anhydrase variant (CA-F) of faster electrophoretic mobility than the commonly occurring one was found in Merino, Columbia and Targhee sheep breeds in the USA (ref. 10), but it was not present in any of the Whipsnade sheep, which were all of the slow CA-S type. In fact, the CA^F allele has not yet been found in any of the breeds tested in Great Britain (Clun Forest, Welsh Mountain, Dorset Horn, Soay, Finnish Landrace, my unpublished results). The non-haemoglobin red cell protein, provisionally called "X", was found in one or more sheep in each of the breeds except the Cotswold. No unusual serum transferrin types were found, but four (TfA, B, C and D) of the commonly described six transferrin alleles were detected. A esterase activity (EsA+) was found in the serum of 50 per cent of the Lincoln Longwool sheep and in one Portland sheep; all other sheep were negative for this enzyme (EsA-). All the sheep were of the same albumin phenotype, presumably the S type described by Efremov and Braend's.

It is not possible in this article to do more than simply report the occurrence of these factors in the various breeds and to note that no unusual factors were detected. No conclusions as to breed origins or relationships can be drawn from such limited material. It should also be understood that the blood groups of these few individuals may not necessarily be truly representative of the breeds studied. The absence of any particular factor does not mean that it might not have been found had larger numbers of each breed of sheep been tested. I felt, however, that it might be of some interest to place on record those factors which were present in these breeds at the outset of the society's breeding scheme.

I thank Dr I. W. Rowlands and Mr E. H. Tong for allowing me access to the sheep, and Mrs V. A. Herbert and Mr L. Kilgour for technical assistance. I also thank Dr J. G. Hall for providing the transferrin reference samples and for checking my transferrin results.

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Modified Carbohydrate Metabolism in the Tropical Swamp Worm Alma emini

A TROPICAL papyrus swamp is a difficult environment for animal life, the mud in which the swamp worms (Oligochaeta, Glossoscolecidae) live is both anaerobic and In order to obtain atmospheric strongly reducing1. oxygen in these conditions, the dorsal surface of the hind end of Alma is specially modified as a respiratory organ which is exposed above the surface of the mud1. It is quite likely that Alma is biochemically adapted to life in the swamp environment.

Worms were collected from a swamp near Kampala and kept in macerated blotting paper soaked in tap water. Into this they discharged their gut contents. Alma were kept in very fine sand (decarbonized and sieved swamp mud) with access to air they survived for at least a month without food. During this time part of the body glycogen was broken down, but there were no significant changes in body fat. In anaerobic water, however, worms survived for only 3 days, but in swamp mud below an atmosphere of nitrogen they survived somewhat longer. The swamp worm is therefore primarily an aerobe.

Ethanol extracts of Alma kept in macerated paper with access to air were purified on resin and separated by paper and silicic acid column chromatography. Large amounts of succinic and malic acids were present (3.3 and 2.4 µmoles/g fresh weight) and some fumaric acid. Similar amounts were present in worms kept in swamp mud below air. In anaerobic water the malic acid almost disappeared within 12 h but the amount of succinic acid increased slightly. Malic acid probably acts as a hydrogen acceptor in anaerobic conditions while succinic acid may be a central molecule in anaerobic metabolism. Pyruvic and α-ketoglutaric acids were present in aerobic conditions and both decreased in anaerobic conditions.

Addition of isocitric, a-ketoglutaric, succinic and malic acids (in the presence of antibiotics) stimulated oxygen consumption in chopped Alma, in vitro, and fluoroacetic acid partially inhibited it. This suggests that the citric acid cycle is functioning. Citric, fumaric and oxaloacetic acids were without effect on in vitro respiration. But the respiratory quotient of whole worms measured in Warburg manometers was about 0.5, which is lower than that usually associated with utilization of fats. Analysis of the medium in which worms had been kept in aerobic conditions failed to show organic products. The large numbers of bacteria present probably destroyed any organic molecules formed by the worms: in the presence of 1 g/l. of penicillin and 0.5 g/l. of streptomycin, volatile fatty acids were found in the water. Gas chromatography of the free fatty acids on 5 per cent FFAP on 'Aeropack 30', 100/120 mesh (Varian aerograph) showed acetic, propionic and a small quantity of a branched C₅ fatty acid (either 2-methyl or 3-methyl butyric acid, but these acids could not be resolved with the available equipment). The results imply that in aerobic conditions there is a partial aerobic fermentation. It was not, however, possible to sterilize worms in order to repeat this observation in the absence of antibiotics.

In anaerobic conditions, acetic, propionic and the C_5 fatty acid were formed (ratio 1:10:0.8) together with traces of butyric acid and branched C6 fatty acid, both in the presence and absence of antibiotics. acids accounted for almost 100 per cent of the glycogen broken down in anaerobic conditions. Traces of acetoin were present, but other chemical tests for possible end products, including lactic acid, were negative. Succinic acid was only found in the medium if the worms had begun to disintegrate.

The swamp worm thus has a type of metabolism that resembles most closely the metabolism of certain parasitic worms, for example Ascaris lumbricoides. This resemblance may represent a convergent biochemical evolution in animals that have become adapted to low oxygen levels or quite anaerobic conditions. Certain aquatic pulmonate snails produce acetic and propionic acids in anaerobic conditions² and it seems highly likely that other interesting metabolic pathways exist in free-living invertebrate animals that have become adapted to a scarcity, or complete absence, of oxygen.

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Displacement and De-arousal

A RECENT communication by Delius¹ calls for some comment. Delius regards displacement behaviour as essentially made up of actions which are related to going to sleep. He therefore interprets displacement behaviour as a homeostatic mechanism in which "de-arousal" actions are called into play to counteract the excessive arousal occasioned by the conflict or thwarting situation to which the animal is exposed.

If this interpretation is correct, then clearly it must be applicable to displacement behaviour in general, and not only to a small number of selected cases. Displacement grooming in rats and cats are two of the cases cited by Delius in support of his views. Bolles² has shown that while rats may groom at almost any time, they are particularly prone to do so before going to rest and, to this rather limited extent, displacement grooming in Rattus norvegicus accords with Delius's views. I know of no study in eats comparable with that of Bolles, and while it is perfectly true that a cat does frequently groom before sleeping, grooming is also a normal accompaniment of waking up. In the absence of quantitative data, there is no justification for regarding grooming in cats as a de-arousal rather than an arousal action. In Cricetomys gambianus, however, grooming is an activity characteristic of arousal from the day's rest in preparation for the night's activity. It is therefore definitely not a de-arousal action and yet, in this species also, it is the predominant displacement activity³. In rats, cats and *Cricetomys* the factor which determines that grooming should be the chief displacement activity must therefore be sought elsewhere than in its relations to arousal or de-arousal. A comparison with two species in which grooming is not a very frequent activity is of some relevance. Xerus erythropus and

Suricata suricatta are both burrowing animals with rather coarse fur which is groomed much less extensively than in any of the three species previously considered. Both, however, are enthusiastic excavators. In Xerus, digging is predominantly an activity connected with burrowing, but in Suricata its predominant function is in food finding. In both species digging is the commonest displacement activity and I have not seen displacement grooming in These facts all suggest that the factor which decides what action shall be performed in a displacement context relates to its importance in the life of the animal and the frequency with which it is performed in its normal context, rather than its relation to going to sleep. If Delius's interpretation were correct, one would expect turning round in a circle to be the common canine displacement behaviour, for this action so commonly precedes settling to rest. I cannot, however, recollect ever having seen displacement circling in a dog.

Tinbergen4 long ago suggested that displacement behaviour has some therapeutic function, which serves to protect the central nervous system from over-activity and provides what in ourselves would be described as "relief of tension". Delius's view is, in a sense, an extension of this idea and an attempt to make it more precise. The attempt is laudable but I believe the interpretation put forward is mistaken. If we accept the disinhibition theory^{5,6} as providing at least a partial explanation of displacement behaviour, then it is easy to see why the actions performed should so often be those which are of very frequent cocurrence in the normal behavioural repertoire. The high frequency patterns are the ones which the animal is almost always ready to perform provided they are not inhibited by the activation of some other pattern with a higher urgency. They are therefore the ones most likely to appear if the currently activated patterns are suddenly switched off, with consequent cancellation of inhibitory effect on the high frequency pattern. Moreover, there is no need to invoke a necessary relation to de-arousal to account for the postulated tranquillizing effect of displacement behaviour. Once the latter is switched on, it is to be expected that it will, in its turn, inhibit the frustrated action and so bring about the required reduction in excitatory level. How beneficial this may be to the animal will, of course, depend on how extensive and how lasting is the effect produced. If it were very transitory, the status quo would merely be restored, but in a dynamic context even a fairly brief pause may permit changes in the external situation to occur and would at least allow the animal's central nervous system to re-assess the total situation from an altered "viewpoint"—that is, with a different excitatory/inhibitory balance in the relevant areas.

Clearly if the tranquillizing effect is indeed a reality and if a species is likely to show either of two displacement actions, one of which is a more effective tranquillizer than the other, then there will be selection in favour of the latter. If linkage with de-arousal were the basis for the greater tranquillization, then the situation postulated by Delius would result. The data available do not, of course, prove that this has never happened: the cases cited here, however, suggest that linkage with de-arousal has not been a factor of great importance in the evolutionary history of displacement behaviour.

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Effect of Colchicine on Meiosis of Hexaploid Wheat

HEXAPLOID wheat, *Triticum aestivum*, behaves cytologically as a diploid organism in that only bivalents are formed at meiosis. This diploid behaviour is principally the result of the activity of a gene or genes in the long arm of chromosome 5B (refs. 1-4). In the absence of this gene or genes multivalents are formed involving homoeologous chromosomes⁵.

Feldman⁶ has demonstrated that an increased dosage of this gene or genes results in asynapsis, interlocking of bivalents and some multivalent formation. The hypothesis put forward to account for these various behaviours was that in the absence of 5B both homologues and homoeologues are associated premeiotically; with the normal two doses of 5B, however, this association is restricted to homologues; with six doses of the long arm of 5B, or four doses at extreme temperatures, there is no premeiotic association, all chromosomes being randomly arranged before meiosis.

The phenomena of asynapsis and interlocking of bivalents were also observed by Barber' in Fritillaria meleagris treated with colchicine before meiosis. Because of the similarities observed in these dissimilar experiments colchicine was applied to hexaploid wheat shortly before the onset of meiosis. Plants of the variety 'Chinese Spring' grown in a glasshouse at approximately 20° C were used. A single application of approximately 0.25 ml. of either a 1 per cent or a 2.5 per cent solution of colchicine was injected into tillers above the tip of the immature spike by means of a hypodermic syringe.

Spikes were subsequently collected for meiotic study some days later, fixed in Carnoy's 6:3:1 and anthers were stained in acetocarmine.

From a spike fixed 7 days after treatment with colchicine anthers containing pollen mother cells (PMCs) with twice the normal number of chromosomes at metaphase I were obtained from three different florets. These dodecaploid cells apparently arose by means of C mitosis as described by Levan⁸. Thus the last premeiotic mitosis apparently took place about 7 days before metaphase I of meiosis in these cases. The uniformity of PMCs with eighty-four chromosomes in this material indicates that the last premeiotic mitosis is fairly well synchronized in all cells of the same anther.

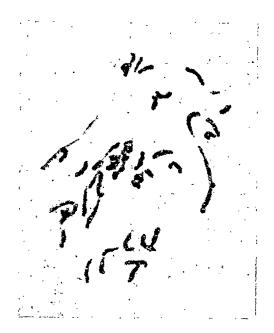


Fig. 1. Metaphase. of meiosis of a $6\times$ cell after treatment with colchicine, showing marked asynapsis and one trivalent.

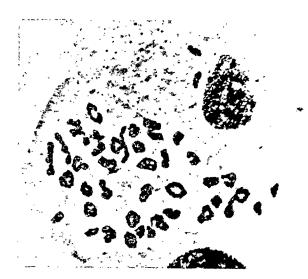


Fig. 2. Metaphase I of meiosis of a $12\times$ cell after treatment with colchicine, showing thirty-eight bivalents and eight univalents. Six of the univalents are obviously in three groups of two morphologically similar chromosomes.

In eight different spikes fixed 3-6 days after colchicino treatment anthers possessing PMCs with the normal forty-two chromosomes at metaphase I were observed. Thus in these cases colchicine had been applied after completion of the last premeiotic mitosis. In these cells, asynapsis and multivalent formation were observed. Multivalent formation was quite infrequent, only an occasional trivalent or quadrivalent being observed (Fig. 1). The mean pairing observed in these cells, as shown in Table 1, is slightly lower than that observed by Feldman's in plants with six doses of the gene or genes in the long arm of chromosome 5B.

Table 1. Mean and range of chromosomal pairing at metaphase I of meiosis in plants treated with colchicine

Type of cell	No. of cells	Univalents	Bivalents	Trivalents	Quadri- valents
6×	50	21·28 (12-36)	10·32 (3-15)		0·02 (0-1)
12×	50	8·32 (2-26)	37·38 (29–40)	0·04 (0-1)	0.20 (0-2)

From these observations it can be concluded that colchicine applied before meiosis inhibits premeiotic association of homologues.

The dodecaploid cells chiefly possessed bivalents (Fig. 2), with only an occasional multivalent as shown in Table 1. Multivalents find a ready explanation in these cells, for homologues are present in four doses. The scarcity of multivalents may be explained by the presence of colchicine. It is known that colchicine persisted in this material, for double dodecaploid cells undergoing C mitosis have also been observed in these anthers. Colchicine presumably suppresses quadrivalent formation by preventing the homologues associating into fours. Thus multivalent formation is limited to cases where more than two homologues happen to be in close proximity.

These cells, however, exhibit fairly regular bivalent formation. This is because the members of each bivalent were sister chromatids before C mitosis. Thus these automatically became premeiotically associated in formations referred to as "pairs of skis" by Nebel and Ruttle⁸. Synapsis and chiasma formation then ensued.

Bivalents were formed by these chromosomes which were associated because of failure of anaphase movement, and so it can be concluded that colchicine does not inhibit synapsis or chiasma formation. Also it can be concluded that colchicine cannot disrupt chromosome association once it has taken place but can only inhibit such association from being initiated. This clearly shows that pre-

meiotic association and synapsis are two distinct phenomena.

Colchicine is unable to disrupt chromosome association, and so the absence of such association in the hexaploid cells in this study indicates that the chromosomes of these cells were not premeiotically associated when colchicine was applied. This, however, does not necessarily mean that chromosomes are only associated immediately before meiosis, for there may be an association-disassociation cycle operating. This would involve normal association of homologues with disruption to some degree when the chromosomes go through the processes of successive mitoses. Such behaviour has been suggested by Feldman, Mello-Sampayo and Sears¹⁰ following their detection of a vestige of chromosome association in mitotic cells of hexaploid wheat.

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Effect of "Hardening" Radish Seeds

Subjecting seeds to one or more cycles of wetting and drying before sowing, in the process called "hardening", is said to affect the subsequent growth of plants in adverse environmental conditions. Various Russian workers, cited by May et al., have found that such treatment increases the resistance of plants to drought, heat and frost, but others2 have failed to confirm this. We have therefore grown radish plants from hardened and unhardened seeds, in wet and dry soil conditions at three levels of soil fertility, to find out whether hardening alters yields in different circumstances.

Radish seeds (Raphanus sativus L., variety 'Cherry Belle') were subjected to two cycles of wetting and drying, in which they were allowed to absorb water equivalent to about 25 per cent of their dry weight and then air-dried at about 72° F. A range of media, with increasing fertility and water-holding capacity, was compounded by mixing 1:2, 2:1 and 3:0 parts by weight of fertile clay loam and infertile grit, respectively. No fertilizer was added.

Twelve 5 in. plastic pots were filled with each mixture and sown with either treated or untreated seeds a week after the second hardening cycle. The emergent seedlings were thinned to one plant per pot. Within each group of six pots, three were kept moist by rewetting the soil to field capacity whenever 15 per cent of the available water had been lost, as shown by daily weighing, and three were not watered at all after an initial wetting to field capacity at the beginning of the experiment. The pots were housed in a growth room at about $68^{\circ} \pm 1^{\circ}$ F and a fluorescent light intensity of about 470 ft.-candles for 12 h daily.

In the moist soil, plants from hardened seeds grew better (Fig. 1) and produced significantly more dry weight than unhardened plants, irrespective of soil fertility, whereas there was no significant difference between hardened and unhardened plants in the dry regime except in the soil with the highest fertility and water-holding capacity.

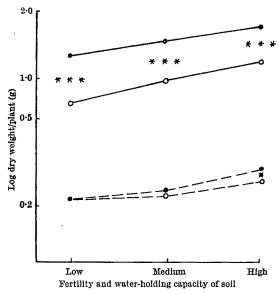


Fig. 1. Effects of seed treatments and soll moisture on growth of radish in three soll mixtures. \bullet — \bullet , Seeds hardened, moist soilwater regime; \bigcirc — \bigcirc , unhardened, moist; \bullet — \bullet — \bullet , hardened, dry; \bigcirc — \bullet — \bigcirc , unhardened, dry. \bullet , P=0.06; \bullet * \bullet *, P=0.001.

There was an increase in dry weight with increasing fertility of the mixture, irrespective of other treatments, but there were no striking interactions between any of the treatments. Similar trends were also shown for leaf areas, except that hardened plants in the least fertile mixture had significantly smaller leaf areas than unhardened plants in the more fertile soils.

These results suggest that, at least in some circumstances, "hardening" seeds may confer a greater advantage in favourable than in adverse growing conditions.

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GENETICS

Haemoglobin Variant in a Bushman: Haemoglobin Dβ-Bushmanαβ₂₂ ^{16Giy→Arg}

VARIANTS of human haemoglobin A with an electrophoretic mobility identical with Hb-S at pH 8.6, but not causing sickling, are designated HbD. This type of haemoglobin has been found in many widely separated geographical localities. In six cases the haemoglobins D were structurally identical with the substitution glutamic acid→glutamine at position 121 of the β-chain^{2,3}. These samples were obtained from a Punjabi, a Caucasian from North Carolina, an Italian family living in Chicago, a Portuguese, a Greek Cypriot and a white family living in Los Angeles in whom Hb-S was also present.

Two other samples of HbD had different substitutions. One obtained from a Turkish Cypriot was an α -chain variant, and the other came from a Gujerati and had an amino-acid substitution in the region β 18-30 (ref. 4). In northwest India, HbD has been found in frequencies of up to 2 per cent (ref. 5), while only sporadic cases of HbD have been reported from the African Continent^{6,7}.

HbD Ibadan found in a member of the Yoruba tribe has recently been shown to possess lysine in place of threonine at position 87 of the β chain (ref. 7). T. Jenkins et al. (unpublished work) found an HbD in a Kgalagadi (Bantu) schoolboy living at Kang in the Kalahari desert of Botswana (formerly the Bechuanaland Protectorate).

This article describes the amino-acid change in another example of HbD, which was found by one of us (T. J.) in a Kalahari Bushman family resident in the Ghanzi district of Botswana. The variant haemoglobin occurs together with normal adult haemoglobin in a 30 yr old man, his mother and all three of his children.

In the five individuals it was found, by starch block electrophoresis, to account for 34 per cent, 36 per cent, 37 per cent, 38 per cent and 39 per cent of the total haemoglobin. Tests for sickling were negative and the reduced form of the variant haemoglobin was completely soluble in 2·24 molar phosphate buffer, pH 6·9. Haemoglobin concentrations of individuals with the variant haemoglobin were slightly below normal limits (11–13 g/100 ml.), the packed cell volumes were within normal limits; thin blood films showed slight hypochromia and some degree of anisocytosis as well as some target cells.

No abnormal HbA_2 component was evident on electrophoresis, suggesting that the amino-acid substitution was in the β -chain. Hybridization experiments, using human haemoglobin variants of known chain abnormality (HbS and $HbJ\alpha$ Cape Town⁸) and with canine haemoglobin, confirmed that the substitution was in the β -chain in this sample of HbD.

A sample of freshly drawn red cells from one member of the family was sent to London, where further analysis showed that this abnormal haemoglobin was caused by a previously undescribed amino-acid replacement and it is therefore called HbDβ-Bushman.

The haemoglobin was purified by starch block electrophoresis using 0.05 molar barbiturate buffer, pH 8.6. After elution and concentration, further electrophoresis on starch block with 0.04 molar sodium phosphate buffer, pH 7.0, yielded pure HbDβ-Bushman. Globin was prepared from the pure haemoglobin and digested with trypsin. The soluble peptides of the tryptic digest were subjected to paper electrophoresis in pyridine, acetic acid and water (10:0.4:90) buffer, pH 6.4, followed by chromatography in pyridine, isoamyl alcohol and water (35:35:27). The peptides were located with 0.2 per cent ninhydrin and specific staining for tryptophan and arginine was carried out.

The following differences between the fingerprints of $HbD\beta$ -Bushman and HbA were visible (Fig. 1). (a) Peptide β T2, which stains positively for tryptophan, was absent in the $HbD\beta$ -Bushman fingerprint. (b) An additional peptide with similar electrophoretic mobility to β T2, but with slightly greater chromatographic mobility,

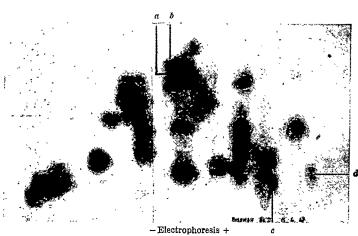


Fig. 1. Fingerprint of HbDβ-Bushman.

was present. It stained positively for tryptophan and arginine. (c) An extra basic peptide positive for arginine was visible. (d) β T3, positive for arginine, was reduced in amount.

These results can be accounted for if β 16 Gly has been replaced by β 16 Arg in the Bushman haemoglobin. The amino-acid sequence of HbA and HbD β -Bushman in this region would be as follows

In HbD β -Bushman a tryptic split would occur after Arg 16, giving rise to a peptide consisting of residues 9–16, positive for tryptophan and arginine, and having the same net charge as β T2 of HbA. The observed peptide spot 2 could be such a peptide. β T3 in HbD β -Bushman and HbA would consist of residues 18–30. In addition, a peptide comprising residues 17–30 might be expected in HbD β -Bushman, resulting from incomplete tryptic cleavage of N terminal lysine of this peptide. The position of the observed spot 3 in the Bushman fingerprint is that expected for Lys- β T3 (residues 17–30). The presence of this peptide would explain why β T3 (residues 18–30) is reduced in amount in the fingerprint of HbD β -Bushman.

	Tabl	e 1	
	Peptide 2, molar ratio		Peptide 3, molar ratio
Thr Ser Ala Val Leu Try Arg	1·0 0·7 2·2 1·1 1·1 + 1·0	Asp Glu Gly Ala Val Leu Lys Arg	2·2 2·7 0·8 3·0 1·1 1·1

Tryptophan was detected by Ehrlich's reagent and assumed to be one residue. The figures for peptide 2 have been corrected for a small amount of α T5 impurity. From the electrophoretic mobility of peptide 3, one of the aspartic acid residues must have been present as asparagine. The figures for peptide 3 have been corrected for a small amount of α T4 impurity.

Amino-acid analyses of peptides 2 and 3 are consistent with this conclusion. These two peptides of the HbD β -Bushman were eluted from a heavily loaded fingerprint, stained with 0.02 per cent ninhydrin. The peptides were hydrolysed with 6.7 normal hydrochloric acid at 110° C for 18 h and subjected to amino-acid analysis (Table 1).

Peptide 2 when compared with β T2 has one extra arginine residue, one less glycine residue and one less lysine. This confirms that β 16 Gly has been replaced by Arg in HbD β -Bushman, and that peptide 2 comprises residues 9–16. In addition, the amino-acid composition of peptide 3 is that expected of Lys- β T3.

The results of the fingerprinting of the HbD β -Bushman and the amino-acid analysis are therefore consistent with the haemoglobin being $\alpha_2\beta_2$ 16Gly \rightarrow Arg.

The fingerprint prepared from the haemoglobin variant HbD β found in a Gujerati, described by Benzer *et al.*⁴, also shows reduced yield of the peptide β T3, suggesting this haemoglobin is similar to the one described here; however, in the former a new peptide was located in the neutral region of the peptide map, while no such peptide was present in our case.

It is interesting that the analogous substitution has been found in the δ chain, in the variant Hb-B₂ (α₂-δ₂16Gly-Arg (refs. 10 and 11)). The fingerprints obtained from HbD-Bushman and HbB₂ are similar. In the HbB₂, a peptide comprising residues 9-17 was observed because of incomplete tryptic splitting after Arg 16 (ref. 11). This was not observed in the Bushman fingerprint, presumably because complete hydrolysis had occurred after Arg

The amino-acid β 16 has been shown to be in the A helix of haemoglobin (residue A 13) lying near the surface of the haemoglobin molecule^{12,13}. This position is known to be tolerant to substitution, because it is occupied by acidic, neutral or basic amino-acids (but not arginine) in various normal haemoglobins and myoglobins¹³. The substitution of arginine in this position would therefore not be expected to give rise to gross haematological abnormality. Further work is necessary before the mild haematological changes found in the carriers of this haemoglobin can be definitely related to its presence.

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Blood Groups in Natives of Easter Island

CONSIDERING their geographic location, the islands of the Pacific Ocean should have been inhabited first by Asiatic and/or American peoples. Heyerdahl¹ showed that this is a possibility when he sailed from the Peruvian coasts

to the Polynesian Islands by a very primitive means, Because of the proximity of the islands to America, determination of the blood groups of the inhabitants should clarify the situation. Until Graydon and Simmons' study² of blood samples of pure natives obtained by Heyerdahl, only the classical groups were known³⁻⁷.

Easter Island (Pascua, Rapa-Nui), to which we sailed in 1963 on the Transporte Aka-Pinto of the Chilean Army, is a small volcanic island in the Pacific Ocean, 182 km², located off the Chilean coast, 2,040 miles from Valparaiso and 2,300 miles from Tahiti (see Fig. 1). It has 1,170 inhabitants, in 200 families who are very much mixed with Polynesians, Europeans and Chileans. There are only sixty pure natives, who believe they are descended from the Polynesian King Hotu-Matua⁸.

Our subjects were 233 natives of which 137 were men and 96 were women. Fifty of them were children and only 35 were pure natives. Blood specimens were taken, from a vein, in acid citrate dextrose and blood groups were determined on the island, except in the case of a few Easter Islanders, whose blood was determined on board ship. Determination of the haptoglobins and abnormal haemoglobins was carried out on the mainland. Techniques used were standard ones. Sera were given by Ortho except for the anti Dia that was sent by Layrisse from Venezuela.

In the general population there is a high frequency of A (66 per cent, gene frequency 0.42), and A₁ is in 92 per cent of the population. N and s predominate on M and S, respectively, with s usually related to N. There is a high frequency of R2(cDE) and absence of Kell and Dia; a low frequency of Fya (40.3 per cent) and an extraneous mixture index of 8 per cent for A2, 2.5 per cent for B and 0.5 per cent for dd, and there is a large proportion of Ro (cDE). For 35 "pure natives" the results agree with those of Graydon and Simmons². B is absent, as in the general population, and in comparison, gene O is more frequent (0.61 versus 0.54) in the pure natives as are genes M (0.48 versus 0.39), R2 ($cD\bar{E}$) (45.71 per cent versus 24.63 per cent) and Fy^a (0.53 versus 0.23). R^o (cDe) is absent from the "pure natives", compared with its presence in 6.16 per cent of the general population.

The inhabitants of Easter Island have been identified both physically and genetically with the rest of the Polynesians¹⁰, but the origin of the primitive inhabitants of the island is unknown. The islands were possibly then, as they are now, a meeting place of routes to Micronesia, Indonesia and Polynesia in the west and America in the east. The genetic factors which the islanders have in common with the American Indians suggest that these were the primitive inhabitants of Easter Island. They lack B, AB and dd, and have a high percentage of R2 (cDE)

Table 1. BLOOD GROUPS IN THE GENERAL POPULATION OF EASTER ISLAND					
Population Location Polynesians Easter Is.		Total No. tested	No. and per cent of phenotypes O A B AB	ıcies	
A-B-O blood groups		233	No. % No. % No. % No. % r p 72 30·90 155 66·52 5 2·14 1 0·44 0·56 0·49	q 2 0∙02	
Subgroups of "A"			A ₁ A ₂ No. % No. %		
		70	64 92 6 8		
M-N phenotypes			M MN N No. % No. % No. %		
		178	33 18.54 79 44.38 66 37.08 0.41 0.58	•	
Distribution of Rh-Hr blood groups	8	ccddee No. %	ccDee CcDee CCDee CcDEe ccDEe ccDEE CCDEe No. % No. % No. % No. % No. % No. % No. %	CcDEE CCDEE No. % No. %	
Total population "Pures"	35	0 0 0 0	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	7 1 0-68 0 0 1 0 0-00 0 0	
Duffy system	82	Fys + No. %	Fya - Fyb +: No. %	fуb	
Total population "Pures"	67 15	27 40·3 12 80	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		
Diego factor		Dia+	Dia Dib		
	183	No. % 0 0	No. % 183 100 0-00 1-00)	
Kell-Cellano group		K+ No. %	K→ No. %		
	183	0 0	183 100 0.00 1.00)	

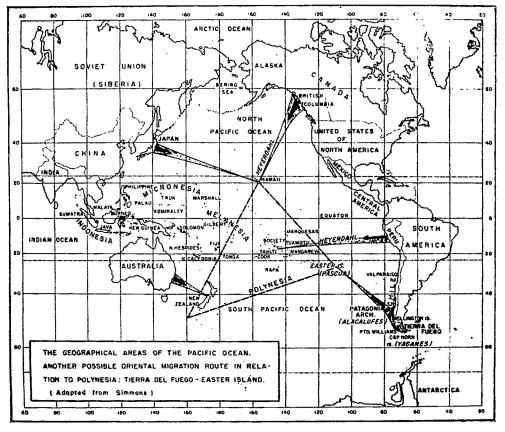


Fig. 1

Fya and s. Easter Islanders are mostly A and N and pure Indians have only O with the gene M predominating. Factor Dia is absent in Polynesians, but is found in all Indians to a greater or lesser extent, except in the Fuegians¹¹. There is possibly a greater genetic relationship between these Indians and the pure natives of Easter Island, particularly the Alacalufes or "sea nomads" who live in the Archipelago of Occidental Patagonia (Fig. 1), the closest to Easter Island. They could have brought in the genes O, M, R2 (cDE) and Fya which are also very frequent in the pure islanders who also lack chromosome R^{0} (cDe). The Yaganes, on the other hand, have a larger proportion of R_{1} (CDe), R^{0} (cDe) and N, but these last two genes could come from a mixing with Europeans that was revealed when B, A2 and a possible case of glucose-6-phosphate dehydrogenase deficiency were found.

The present inhabitants of Easter Island might also have been a mixture of so-called "pure" or primitive with other tribes from North America (British Columbia) as Heyerdahl suggested. Possibly these were the ancestors of the present Blackfeet and Bloods, who contributed the gene A, the percentage of which is actually the highest in the world13. Peruvian incursion towards the end of the last century, migrations and epidemic diseases have much reduced the population. This, and mixing with Europeans, Chileans and Polynesians (the absence of haemoglobin S excludes mixing with Negroes), makes identification of the present population with the primitive inhabitants of Easter Island very difficult.

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IMMUNOLOGY

Antibodies to IgM in Normal Human Sera

THE sera of some normal human subjects contain antibodies to human IgG1, and have proved useful in investigating the genetic markers of human IgG (the Gm and Inv factors). But the IgM proteins of different human sera have also been found to differ serologically2, and investigations by means of precipitin systems have led to the designation of possible subclasses3,4. set out to determine whether normal human sera contain antibodies to human IgM, for such reagents might help the delineation of the structure of the immunoglobulin. We have screened more than two thousand normal human sera for antibodies to IgM; forty-two were found to have such activity.

Table 1. AGGLUTINATORS IN NORMAL SERUM FOR WALDENSTRÖM'S MACRO-

MINDON	CONTEND ON HOR	WAN DIE	TIMOOTIE
4 47	No. of sera		tive sera
Antigen	screened	No.	Per cent
Bi	60	5	8.3
Pi	55	4	5.3
Qu	159	4 7	4.4
Lo	120	4	3.3
Ca	375	9	2.4
Во	164	4	2.4
St	325		1.2
Vi	190	2	1.0
\mathbf{Pr}	437	$\frac{4}{2}$	0.2
Ka	302	2	0.7
Au	140	0	0.0
Gi	29	0	0.0

For use in the screening procedure, IgM proteins from twelve patients with Waldenström's macroglobulinaemia were isolated by either euglobulin precipitation or starch block electrophoresis, depending on the physical characteristics of the individual protein. The euglobulin precipitations were repeated three to five times, and the precipitate was washed extensively with distilled water after the first and last precipitation. All preparations were then chromatographed on a column of 'Sephadex G-200' to remove remaining contaminants. The purity of each preparation was checked with appropriate antisera by both immunoelectrophoresis and gel double diffusion in agar. Each of the twelve proteins was then coated on type O Rh-positive red cells with the use of trivalent metal ions^{5,6}. Sera to be tested for antibodies to IgM were obtained from routine blood donors.

For antibody testing, one drop of serum was added to one drop of a 2 per cent suspension of cells on a glass tile at room temperature. The tile was gently agitated for 2–5 min and read for agglutination. Saline solution and sera known to contain no IgM agglutinators served as negative controls, and rabbit anti-IgM antiserum monospecific for IgM was used as a positive control to ensure that the cells were coated. Cells coated with human serum albumin were used as cell controls. Simultaneous screening for IgG agglutinators was carried out with human IgG and anti-D (Ripley) coated cells.

For inhibition experiments, one drop of an appropriate dilution of agglutinator was incubated with serial dilutions of inhibitor for 15 min at room temperature. The cells were then added and agglutination was tested as in the screening procedure. Sera with agglutinator activity were chromatographed on a column of 'Sephadex G-200', 2.5×100 cm, in 0.17 molar tris saline buffer, pH 8. A sample (1 ml.) of serum was applied to the column; fractions (3 ml.) were collected, and the protein content was estimated by determining the absorbancy at E_{280} m μ with a Zeiss PQ II spectrophotometer. Selected fractions were then tested for haemagglutinating activity.

Macroglobulin antibody-antigen complexes were prepared by incubating a mixture of a known saline anti-Rhoserum (1:1) with Rh-positive cells for 2 h at 37° C. The cells were then washed three times with saline. Samples, 0.5 ml., of the packed washed cells were incubated with 0.1 ml. of agglutinator serum at room temperature and at 37° C for 30 and 60 min and were then centrifuged. A sample (0.05 ml.) of the supernatant fluid was removed and tested for agglutinating activity. The remaining mixture was incubated overnight at 4° C and similarly tested. A further sample of sensitized cells was lysed in distilled water and washed three times or until the supernatant fluid was clear. The stroma was incubated with the agglutinator at 37° C for 1 h and then centrifuged; a portion of the supernatant was removed and tested for agglutinating activity. The rest of the mixture was incubated overnight at 4° C, and was then similarly tested.

The number of sera which were screened for anti-IgM antibodies and the number positive with each of the twelve coats are listed in Table 1. It can be seen that the number of sera positive against each coat varied from none to 8.3 per cent. The two highest percentages (5.3 per cent for coat Pi and 8.3 per cent for coat Bi)

may reflect the fact that fewer sera (50 and 55, respectively) were screened against these antigens. Only one of the agglutinators had a titre greater than 1:2; serum Fe had a titre of 1:32 against the Ca coat. Two sera had activity against both IgG and IgM. Two other sera reacted with more than one IgM coat; both of these were active against both Bi and Ca. All the other agglutinators reacted with only one coat of the panel.

Five agglutinators to Ca, including serum Fe, were selected for further study. Four reacted with cells coated with isolated Ca heavy chain in titres equal to those obtained with cells coated with the whole molecule. To determine whether the agglutinators of the various coats could be used for defining genetic variations in IgM molecules, the inhibition of these sera by the twelve isolated macroglobulins was tested at protein concentrations ranging from 0.0025 mg to 1 mg/ml. In each case, only the homologous paraprotein produced inhibition. Of seventy-two normal sera screened for their ability to inhibit the agglutination reaction with Ca and Bi coats, only two (2.8 per cent) inhibited agglutination of the Ca coat, and only three (4.2 per cent) the Bi coat. The inhibiting property was lost when the serum dilution was greater than 1:2. Again, the inhibitors appeared to be specific for the homologous coat.

The agglutinator of serum Fe was characterized by fractionation on a column of 'Sephadex G-200'. The fractions obtained were immediately tested for agglutinating ability. As shown in Fig. 1, the material from the 19S peak agglutinates IgM-coated cells, whereas the protein from the 7S and 4S peaks does not. The agglutinator proved to be labile, and concentration procedures were sufficient to destroy the activity. Dialysis of serum Fe against 0·1 molar mercaptoethanol overnight at 4° C, with subsequent removal of the reducing agent, destroyed the activity. Serum Fe dialysed at 4° C without reducing agent retained activity. The reduced serum, however, blocked the agglutination of untreated Fe.

In view of the limited specificity of these sera, the possibility that they could represent anti-antibodies was explored. Rh-positive cells heavily coated with anti-Rh saline agglutinin were incubated with an agglutinator serum at room temperature and at 37° C for 60 min and overnight at 4° C. Stroma from sensitized cells were used in similar experiments. In all instances, these attempts at absorption failed to remove the agglutinating activity.

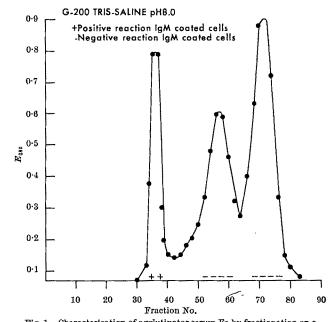


Fig. 1. Characterization of agglutinator serum Fe by fractionation on a 'Sephadex G-200' column. The agglutinator was present only in the 19S peak.

Thus these anti-IgM agglutinators do not seem to be antiantibodies to IgM.

Normal sera have long been known to contain antibodies to various antigenic determinants on the IgG molecule1. The agglutinators described here are distinguished by their low incidence and very low titre. They are remarkable in that their specificity seems to be limited to the IgM coat used for their detection. The specificity in all instances seemed to be directed primarily towards the heavy chain of IgM, as shown by the direct reaction with isolated heavy (µ) chain and the failure to react with red cells coated with IgG or light chains. At present, the most plausible explanation for the restricted activity seems to be configurational changes in the molecule produced during binding to the red cell by the metallic ions. Individual macroglobulins vary in amino-acid composition7 and the heavy chains of the immunoglobulins are probably divided structurally into a variable and an invariable region, as has been shown for the light chains, and so it seems possible that the variable regions contain antigens available for reaction. The low percentage of normal sera capable of inhibiting such reactions suggests that the reagents used in this investigation are of limited value for detecting genetic antigens of macroglobulins. It is possible, however, that the antigen(s) involved represents uncommon determinants. Further work is required to elucidate this point.

The possibility that these agglutinators are anti-antibodies seems to be remote for two reasons. First, they were blocked by reaction with free homologous antigen, and second, macroglobulin antibody-antigen complexes failed to remove agglutinator activity. second point, however, can be disputed in the light of recent reports that at least some human anti-antibodies have Gm specificity. The nature of the agglutinator is interesting, because it seems to be a macroglobulin. It is present in the first peak obtained by 'Sephadex G-200' chromatography and is sensitive to mercaptoethanol. Neither of these criteria is absolute inasmuch as polymer IgG molecules and monomer IgM molecules Unfortunately, absorption by antisera to IgM is not possible because such sera themselves in high dilutions ($\hat{1}:100,000$) agglutinate the test cells.

In summary, we have established the presence of agglutinators for IgM in human sera. They seem to be macroglobulin molecules and to have limited specificity. The question of IgM genetic determinants remains unanswered.

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Mixed Agglutination between Established Burkitt Cell Lines and Fresh Biopsy Specimens

When the serum from patients with Burkitt-type lymphoma is incubated with living suspensions consisting mostly of Burkitt tumour cells, the cell surfaces become coated with immunoglobulin as detected by the fluorescent antibody technique¹. Evidence suggests that this surface reaction is correlated with the occurrence of a virus like particle, and that this particle is also associated with a cytoplasmic antigen detected by the fluorescent antibody technique2, but there are good reasons to distinguish between this and the membrane fluorescence phenomenon demonstrable on the living cell.

A working hypothesis has been that the gamma globulin factor which combines with this cell surface may be antibody to a tumour specific antigen, analogous to that found in certain murine lymphoma systems. While testing this hypothesis it became important to compare surface antigens on living fresh biopsy cells and those on the established culture cell lines.

The finding that a biopsy specimen from a 17 yr old patient with Burkitt lymphoma was heavily coated with gamma-M immunoglobulin provided the experimental approach. If this immunoglobulin coating were antibody against a surface antigen present on the lymphoma cell (but not the normal spleen cells of the patient), sufficient free antibody combining sites should exist to co-agglutinate with uncoated cells bearing free surface determinants of the same specificity. This proved to be so, for when these biopsy cells were mixed with a long-established line of Burkitt cells, giving the membrane fluorescent reaction, mixed agglutination was observed.

The technique employed was as follows: one of the cell lines to be examined, usually the cultured cell line, was incubated in 0.025 molar fluorescein diacetate in balanced salt solution (BSS) for 20 min at room temperature, as described before³. These cells showed intense cytoplasmic fluorescence in ultraviolet or blue light because of splitting of the compound in the living cell. Fluorescence was sustained as long as the cell lived. After incubation the cells were washed twice with BSS and then mixed with the second type of cell to be examined. Some (0.1 ml.) of the fluorescent cells in a concentration of approximately 5×10^5 /ml. were mixed in a 1·0 ml. plastic tube with 0·1 ml. of the second cell type, usually a biopsy cells uspension at a concentration of 1×10^6 cells/ml. A favourable ratio of fluorescent cultured line cells to biopsy cells seemed to be between 1:5 and 1:10. This mixture was sealed and incubated at 37° C for 30-45 min. A small drop containing gravity sedimented cells was taken from the bottom of the tube with a small bore Pasteur pipette, put on a glass slide and covered with a glass cover. The mixed cells were examined with a dark field condenser, orange tungsten light and ultraviolet light mixed in an orthoplan microscope (×540). In these preparations, the biopsy cells without fluorescein were orange and usually small (8–11 μ in diameter). The cultured lines were seen as uniformly fluorescent and large (14–18 μ in diameter).

It was extremely important to perform each test with as near 100 per cent living cultured line cells marked with fluorescein as possible, because it proved unreliable to attempt to distinguish the cultured line from the biopsy cells entirely on the basis of size. The presence of a significant number of nonliving and therefore nonfluorescent cultured cells gave irregular results. Agglutination of the cells was scored for 200 single cells; never clumps. A fluorescent cell was considered positive when one or more nonfluorescent, usually smaller, cells adhered to the surface of the single fluorescent cell. No systematic attempt was made to grade the agglutination between single cell adherence and complete rosette formation; the average number of adherent cells in a positive test was usually

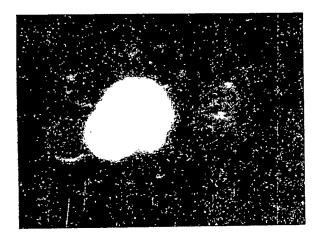


Fig. 1.

between two and five. In an attempt to block the test, a gamma-G globulin preparation from a Burkitt serum known to produce membrane fluorescence when incubated with the same cultured cell line4 was incubated for 20 to 30 min at room temperature before incubation with the second cell line, and washed twice in BSS before the mixture was made. Fig. 1 shows a positive cultured line cell with a rosette of smaller biopsy cells around it.

This test has been performed on four different biopsy specimens of African origin. Dau and Mar were from patients with histologically confirmed Burkitt lymphoma. Phy was taken from a patient with a lymphosarcoma of less distinctive histology. Ngi biopsy specimen was from a haemangiopericytoma of the mandible. The cultured cell lines Jijoye (HR-5), EB-3, Annah and B35M show The lines a positive membrane fluorescence reaction. Ogun and Kudi show no membrane fluorescence.

Preliminary data are presented in Table 1, and show that mixed cell agglutination occurred between the

Table 1. MIXED CELL REACTION BETWEEN BIOPSY AND CULTURED CELL LINES FROM THE BURKITT LYMPHOMA*

	Reacting cell types			Percentage of labelled cel
	Fluorochrome U	Inlabelled	No. of	with one or more
	labelled cell	cell	tests	unlabelled cells attached
(1)	Jijoye t	Dau¶	11	30 (19-35)†
\-,	Dau	Jijoye	1	15
	Ogun§	Dau	3	0
	Kudiş	Dau	ž	
	Annah‡	Dau	ī	5
	B35M1	Dau	î	3
	EB-31	Dau	ô	7
	Day alone	1744	7	'n
	Jijoye alone		1321127211111222222	0 5 3 7 0 0 0 0 0 0
	Ogram	*****	ĩ	ŏ
	77 41	-	î	ň
	Lamob	******	î	ň
	מיא		î	ň
	700E M		Ť	ň
(2)	Jijoye	Mar**	5	97
12)	Ogun		ő	ő
(3)	Jijoye	Phytt	ő	ซอั
40)	Ogun		ő	ő
110		Ngi‡‡	ő	ŏ
(4)	Jijoye	7481++	6	ŏ
(5)	Ogun	**	2	U
(5)	Jijoye preincubated with active fraction of			
	active traction of	There	1	0
	Mutua§§ serum	Dau	Ť	
		Phy	1 1 1	25
	Jijoye	Phy		75
	Jijoye	Dau	1 1	29
	Jijoye		1	0

*The details of this method are described in the text. Fluorochrome tabelling was performed by preincubating the cells with fluorescein diacetate. The unlabelled cells were added in approximately fivefold excess. After 30 min of incubation at 37° C, the cells were scored for mixed agglutination.
† Average and range.
‡ Burkitt line, positive in membrane fluorescence test.
§ Burkitt line, negative in membrane fluorescence test.
¶ Biopsy cells from Daudi (KCC 750), heavily coated with γM and lightly with γ6.

¶ Biopsy cells from Daugi (ACC 750), heaving occasion with γG .

** Blopsy cells from Margaret (KCC 759), 17 yr old African girl with Burkitt lymphoma, lightly coated with γG .

†† Biopsy cells from Phylis (KCC 755), girl with probable lymphosarcoma, slightly coated with γG .

‡‡ Biopsy cells from Ngigi (KCC 758), African patient with haemangiopericytoma of the mandible. No detectable immunoglobulin coating.

§§ Mutua (KCC 454), African patient with Burkitt lymphoma from whose serum was obtained highly purified γG fraction having strong property of inducing the membrane fluorescence reaction.

heavily \(\gamma M\)-coated biopsy specimen of Dau and Jijoye. The lightly YG immunoglobulin-coated biopsy cells of Phy and Mar reacted to a lesser extent with Jijoye. Ngi, which had no detectable antibody coat, did not react with Jijoye. Thus those cell lines showing strong membrane fluorescence reactions when exposed to positive Burkitt lymphoma sera were the only ones which agglutinated with the coated biopsy cells. The biopsy cells with no immunoglobulin coating failed to react with the same cell lines. Furthermore, the reaction was blocked by preincubation of the Jijoye cell line with the vG fraction from a patient with Burkitt lymphoma. It seems reasonable to assume that the antigen like properties of the biopsy and the cultured cell surfaces are identical.

This preliminary observation is presented at this time in order to make possible more extensive examination of this reaction and with a larger number of biopsy cell types. If this observation is confirmed by examination of additional biopsy specimens, and the surface property is identified as a true antigen, the technique may make possible studies of the antigenic surface mosaic of individual tumours, of the specificity of the serum factor, and of the relationship between various tumours of differing

host and geographical origin.

This work was supported in part by grants from the National Institute of Child Health and Human Development, the American Heart Association, the Swedish Cancer Society, the Jane Coffin Childs Memorial Fund, and the National Cancer Institute; the work was performed while one of us (R. T. S.) was at the Tumor Biology Institute in Stockholm, under the auspices of the Commonwealth Fund. Miss Bodil Lidin gave technical assistance.

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CYTOLOGY

Effect of Phytohaemagglutinin on the Surface Charge of Human and Murine Lymphocytes

Non-immune murine lymphocytes are cytotoxic to allogeneic target cells in the presence of phytohaemagglutinin (PHA)¹. In a similar system using cells of human origin this "allogeneic inhibition" occurs without PHA (ref. 2). The role of PHA in the mouse experiments is unknown. but it has been suggested that it acts by promoting intercellular contact, thus permitting the close apposition of dissimilar groups of histocompatibility antigens3. This would imply that there is some factor in the mouse experiments which tends to inhibit close intercellular contact but which does not operate in experiments using human cells.

A recent communication⁴ has suggested that cell surface charge effects may modify certain interactions between lymphocytes and target cells. The magnitude of potential

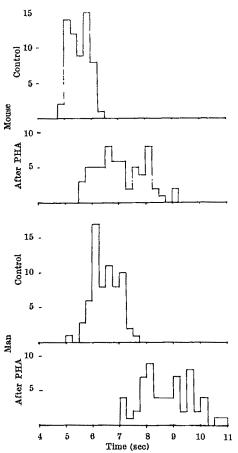


Fig. 1. Frequency distribution of electrophoretic mobilities of murine and human lymphoid cells before and after exposure to PHA, expressed as time taken to travel 31.6μ . The distribution is plotted as "number of observations" with a class interval of 0.25 sec.

energy barriers to contact between these cells may well be regulated by the sum of charges at their cell peri-Experiments were therefore carried out to compare the surface charges of human and murine lymphoid cells and to assess the effects of treatment with PHA. Normal human lymphocytes were obtained from the peripheral venous blood of adult volunteers by the method of Coulson and Chalmers. The resulting cell suspensions were treated with hypotonic saline for 30 sec to lyse red cell contaminants and the suspensions were left to stand for 1 h at 37° C to allow granulocytes to adhere to the walls of the vessel. The preparations obtained by this method contained at least 97 per cent lymphocytes, less than 5 per cent of which failed to exclude lissamine green.

Mouse lymphoid cells were obtained from the minced and filtered spleens of adult CBA inbred mice. These cells were treated in the same way as human lymphocytes and contained at least 95 per cent lymphoid cells with about 85 per cent viability.

Each suspension was diluted in medium 199 to a final concentration of one million cells/ml. and to 1 ml. of each preparation was added 0.1 ml. of reconstituted PHA (Burroughs Wellcome). Both treated and untreated suspensions were incubated for 30 min at 37° C. After incubation the cells were washed and resuspended in 0.145 molar aqueous sodium chloride adjusted to pH 7.4with 0.145 molar sodium hydroxide.

Electrophoretic mobilities were measured in a cylindrical micro-electrophoresis apparatus described by Bangham et al.⁵. The cells were timed over a distance of 31.6μ at 25° C. Sixty observations were made on each preparation and the results from each batch compared by means of Student's t test. The results were also plotted as a fre-

quency distribution histogram with a class interval of 0.25 sec and are shown in Fig. 1.

These results indicate that CBA mouse spleen cells have a higher electrophoretic mobility than human lymphocytes (0.05 < P < 0.1) and that treatment with PHA reduces their mobility (P < 0.05). The mobility of normal human lymphocytes is also substantially reduced by PHA (P < 0.001). Comparison between mouse lymphoid cells treated with PHA and control human cells reveals no significant difference (P > 0.2).

Electrophoretic mobility is largely a measure of net surface charge,, so it is concluded that mouse lymphoid cells bear a higher net negative charge than human peripheral blood lymphocytes and that PHA abolishes this difference. This effect could provide a mechanism for the facilitation of allogeneic inhibition by PHA. Treatment of murine lymphoid cells with PHA, by reducing their high surface charge, would diminish electrostatic intercellular repulsion to levels which permit direct contact between cell walls exhibiting dissimilar antigenic groupings. Human lymphocytes, with their lower surface charge, would make contact with target cells more readily, thus dispensing with the need for PHA. Sundaram et al.8 have revealed that lymphoid cells stimulated with antigen have a reduced electrophoretic mobility which is further reduced by incubation with the same antigen. This would explain the pronounced cytotoxic effects of immune mouse lymphoid cells on target cells in the absence of PHA (ref. 9). The failure to demonstrate allogeneic inhibition recently reported by Chernyakhovskaya et al. 10 is at variance with previous reports by Hellström and may be explained by the omission of PHA from their culture system.

The mode of action of PHA as a potent mitogen is poorly understood. It is antigenic¹¹ but there is no evidence that its mitogenic effect is related to its antigenicity. Similarly, the relationship between an antigenic molecule and its appropriate lymphoid cell receptor remains a subject for speculation. An analysis of the effects of PHA and antigen on the physico-chemical properties of the lymphocyte cell wall should provide a better understanding of immune mechanisms at the cellular level.

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Cell Division after Removal of the Division Line in Stentor

Can a cell which has already entered on division go on to divide even when the presumptive or visible fission line or furrow is removed? This question can be answered conclusively for the large ciliate Stentor coeruleus, which is remarkably suitable for micro-surgical operations. One might expect that the ciliate would then be unable to divide. Uhlig² reported this finding after deleting the fission line from dividing S. coeruleus, and when Suzuki³ excised mid-cell disks bearing the visible fission line from dividing Blepharisma, these ciliates also failed to divide, although removal of the presumptive fission line did not prevent division, as I have confirmed in Stentor.

To obtain abundant dividers, older static cultures of Stentor were supplied with ample Paramecium bursaria and divisions occurred on the following day. Stentors entering on division can be identified as early as 6 h before furrowing by the presence on one side of the cell of an oral primordium, as shown in Fig. 1a. This produces the new feeding organelles for the eventual posterior daughter cell, also indicated in Fig. 1a.

The principal variable in the experiments was the stage at which the presumptive or manifest division line was removed. The later the stage at which the cells could still divide, the more significant, for deletions at early stages might allow time for reconstruction of some hypothetical divi-

sion organelle or constriction ring. Evidently the locus of the fission line is pre-established or determined at an early stage. Excision of the anterior ends of the dividers even 5 h before furrowing resulted in unequal division yielding small anterior daughters and the cells divided where they would have done, had they not been out (unpublished results). Thus the presumptive division line can be removed by deleting mid-cell parts.

Progression toward fission has been staged by clearly recognizable steps in the development of the oral primordium1. When this development is arrested, the appearance of the fission line and furrowing is correspondingly delayed, and if the primordium regresses division is cancelled4. Up to stage 6 of primordium development the division line may be called presumptive for it is not visible. During stage 6 formation of the new set of feeding organelles is essentially completed; the micronuclei divide and the macronucleus is compacted into a single mass from a chain of nodes. There is no indication that division of either type of nucleus triggers or participates in cytokinesis; rather, the cytoplasm signals the nuclear behaviour1,5. A Stentor can be enucleated as early as stage 6 without preventing either division or final oral differentiation for the posterior daughter cell1.

In late stage 5 and early stage 6 the incipient furrow is first indicated by a line of colour contrast around the equator of the cell produced by predivision of the opaque, underlying, glycogenoid carbohydrate reserve granules. Where this line intersects the longitudinal pigment bands the blue-green pigment granules are displaced in late stage 6, leaving a colourless, visible division line. It may be inferred from silver staining studies on other ciliates, as described by Fauré-Fremiet, that the ciliary rows or kineties lying between the bands are also severed along this line. Furrowing in the division line then begins, first on each side of the anterior end of the oral primordium, to cut this anlage into the posterior daughter cell, and then extends right and left around the equator. Gathering and bunching of the cut ends of the pigment bands clearly indicate that fission is an active constriction of the cell cortex sharply at the fission line.

Selected dividers were quieted in methyl cellulose solution and operated on by a hand-held glass needle¹. Transection of the cell on each side of the equator isolated a mid-cell fragment, initially disk-shaped, carrying almost all of the prospective or visible fission line or furrow except for its forward bend around the oral primordium. This

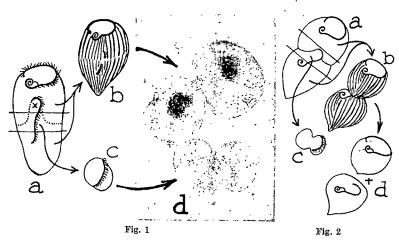


Fig. 1. Operation on a stage 4 divider and consequences. (a) Two cuts on each side of the presumptive division line (dotted) isolated into a middle fragment (c) almost all this locus except the part running forward to include the anterior end of the lateral oral primordium (x). (b) The two remaining major portions of the cell were fused together with a line of heal indicated by breaks in the pigment bands. (d) Both fragment and cell remainder divided, yielding four daughter cells.

Fig. 2. Same as Fig. 1 for a late stage 6 divider beginning to furrow. (a) All the furrow was excised into a fragment which constricted only half way (c). (b) In 30 min the cell remainder formed a new furrow and constricted half way, and after 45 min the cell pinched in two and daughter cells were separated.

can be seen in Fig. 1a. These pieces were observed to determine whether they could divide. To test the remainders—cell minus fission line—for division, the anterior had to be healed to the posterior part. This fusion frequently required sacrificing the mid-piece which had to be cut initially and distorted to bring the two other parts together before it was excised.

One positive case is sufficient to demonstrate what the Stentor cell can do. Figure 1 shows how a late stage 4 divider was cut and that 2 h later both the middle piece carrying the presumptive fission line and the cell remainder pinched in two as in normal division, each producing two separate daughter cells. Another case of the same operation performed on a divider in late stage 6 after furrowing had begun is shown in the protocol sketches of Fig. 2. Although the furrow was removed, the cell nevertheless formed a new furrow in the line of heal and divided promptly into two separated daughter cells. The midpiece carrying the original furrow constricted only half way and therefore did not yield daughter cells.

The presumptive or manifest division line or furrow was successfully removed from fifty dividers at stage 4 to 7 inclusive. In eight instances all of the equatorial disk was removed except for the oral primordium, which was left intact as a connexion between the anterior and posterior portions of the cell to assure their healing together. In twenty-one cases the anterior end of the primordium where the fission line begins was also excised or cut into the middle fragment to test whether the presence of this "initiator" region, shown in Fig. 1, is necessary for constriction after removal of the greater part of the division line. It was found to be unnecessary; the division line simply began in another place, and this is in accord with an earlier study.

We deleted the prospective or manifest fission line or furrow and graded the behaviour of fifty cells which continued to divide. The most complete performance, exemplified by the illustrated cases, consisted of full constriction in the line of heal and separation of two daughter cells. This form of division occurred in five Stentors with deletions made, one at stage 4, one at stage 5, and two at stage 6 with furrows, respectively, as shown in Figs. 1 and 2. Next was division without separation, in which constriction in the line of heal was complete and the cells all but separated yet remained attached and then melded back into one cell as the constriction relaxed. This occurred in the case of one cell

operated on at stage 4 and in seven cells cut at stage 6, two of which had begun furrowing. In twenty-seven cases with deletion of the original division locus at stages 4 to 7, the specimens showed definite, but partial, constriction. They were pinched in two up to half way, but the daughter cells remained joined by a wide stalk. Finally, in seven instances of cells operated on at stages 5 to 7, there was no apparent constriction when the time for fission arrived nor thereafter. Considering that the operation involved major deletion and injury, the performance of the specimens in cytokinesis after excision of the division locus, fission line or furrow was remarkable.

The twenty-eight middle pieces which carried the original presumptive or visible division line or furrow, and the fate of which was followed, showed the same grades of performance. In these fragments, in contrast to the cell remainders, the earlier they were cut the more significant. Ten went on to divide and separated tiny daughter cells, including one from a stage 4 divider as in Fig. 1c and two from stage 5 dividers which could go on to produce a visible division line and furrow within the isolated fragment. Four divided without separating daughter cells, twelve showed incomplete constriction, and two gave no indication of constrictive division.

These results seem to present new possibilities for a theory of cytokinesis. Current hypotheses derived from studies on egg cleavage, do not apply to Stentor and other ciliates. Because the macronucleus divides amitotically and the micronuclei endomitotically, there are no dominating mitotic spindle or asters to play an important part in the cytokinesis. Successful fission in equatorial Stentor fragments seems to exclude the cortical expansion hypothesis of furrowing; if anything, the cortex should be pulling away from the furrow in healing over the two extensive wound surfaces of a central disk fragment, yet complete furrowing can occur. It is highly improbable that the furrow in Stentor is an inward growing partition, because cells could refurrow and fully constrict only 45 min after the original furrow was excised, as seen in Fig. 2, and in cells relaxed or deconstricted after division without separation of daughters there is no indication of new cortical growth.

The normal division locus has been presumed to be a temporary differentiation gradually prepared for constriction, which is then indispensable for division. After the prospective or visible fission line or furrow is removed, however, other parts and perhaps any level of the Stentor cell can constrict transversely when the time comes for fission.

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Staining of Mast Cell Acid Glycosaminoglycans in Ultrathin Sections by Ruthenium Red

STUDIES from our laboratories have shown that ruthenium red, empirical formula $[Ru_3O_2(NH_3)_{14}]Cl_6\cdot 4H_2O$, M.W. 858.5 (ref. 1), reacts specifically with mast cell granules2. These organelles are stained red and rendered electron dense, probably as a consequence of a reaction between the stain and granule acid glycosaminoglycan2. After vital staining or staining at fixation, the electron density is usually intense but irregular and sometimes varies from one experiment to another. The variation might be caused by differences in the content of glycosaminoglycan, blocking substances or poor penetration of the stain3. With vital staining it is also obvious that ruthenium red can function as a poly-cation histamine releaser, although at temperatures greater than $4^{\circ}\,\mathrm{C}$ this stain did not degranulate mast cells as markedly as did astra blau, a copper containing polyammonium

Most of the difficulties of staining glycosaminoglycans with ruthenium red for electron microscopy would be eliminated if ultrathin sections could be stained. Staining material embedded in epoxy resin did not increase appreciably the electron density of mast cell granules. but staining specimens embedded in the water-soluble glycol methacrylate (GMA, ref. 4) (Röhm and Haas, Darmstadt) was useful.

Peritoneal mast cells were collected from rats³, and fixed for 1 h in 4 per cent methanol-free formaldehydes or glutaraldehyde in 0·1 molar cacodylate buffer, pH 7·4, with or without 0.001 molar p-chloromercuribenzoates (Sigma Co., St. Louis, USA). Dehydration and embedding in GMA were carried out as described in refs. 7 and 8.

Sections 1µ thick were stained with 0.001 molar ruthenium red in 0·1 molar phosphate or tris-maleate buffer. pH 7.4, for 5-15 min at room temperature. To study the effect of increased electrolyte concentration on stain selectivity we added I molar sodium chloride to the tris-maleate buffer in some cases. Consecutive ultrathin sections on grids were stained in the same way and briefly rinsed in distilled water. Other ultrathin sections were stained conventionally with 4 per cent uranyl acetate and lead or examined unstained.

Ruthenium red in phosphate buffer stained mast cell granules intensely, but nuclei and other peritoneal cells remained unstained or showed at the most a faint tinge under both the light and electron microscopes (Figs. 1-3). When tris-maleate buffer was used as solvent for the stain. however, selectivity was not as good (Fig. 4), for nuclei also became electron dense. When the electrolyte concentration was increased to 1 molar by adding sodium chloride to the stain and tris-maleate buffer, staining of nuclei was considerably suppressed, while mast cell granules still reacted quite intensely. If the idea of critical salt concentration" which implies that the capacity of polyammonium salts to precipitate giveosaminoglycans is determined by the electrolyte concentration, is also valid for the interaction of ruthenium red with mast cell granules, the results suggest that the acid glycosaminoglycan demonstrated was heparin. agrees with the results of vital staining with ruthenium red2. Thus, embedding in GMA did not seem to interfere with the binding of ruthenium red to granule heparin.

The marked variation in granule electron density seen after vital staining2 was not apparent after sections had been stained. The variability observed in the former case might thus be caused by variation in the degree of penetration of the stain through the perigranular membranes. This will be further investigated.

When the uranyl acetate and lead were used there was no specific increase in granule electron density

These results demonstrate that the hydrophilic embedding material GMA makes possible the use of a conventional histochemical technique in ultrastructural studies. In addition, it is possible to overcome the penetration problems connected with high-molecular reagents. Staining of consecutive sections also makes it possible to compare the effects of various light and electron microscope techniques on the same specimen.

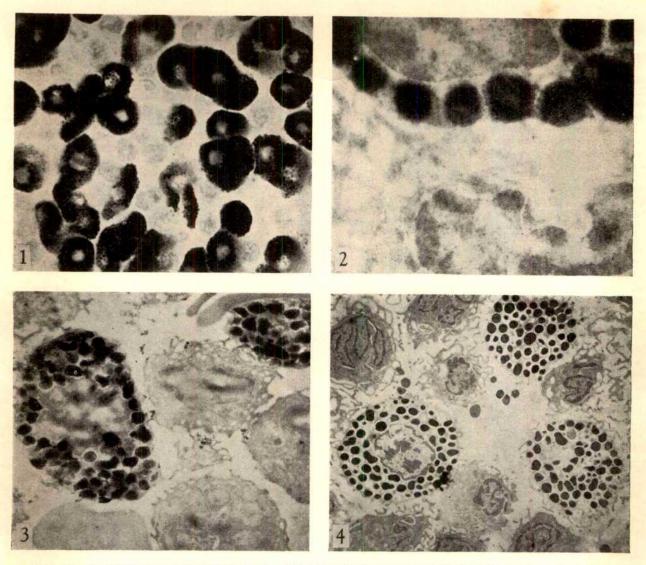


Fig. 1. Rat peritoneal cell suspension fixed in formaldehyde. A GMA section (1 μ thick) was stained with 0.001 molar ruthenium red in phosphate buffer for 5 min. Mast cell granules stained intensely, contrasting with pale nuclei and other cells. (×1,050.)

- Fig. 2. Ultrathin section from same specimen as in Fig. 1, stained in the same way for 15 min. There is a similar contrast between mast cell granules and other cell organelles as in Fig. 1. There is no additional electron staining in this or other figures. (×14,500.)
- Fig. 3. Peritoneal cell suspension fixed in glutaraldehyde. The ultrathin section was stained as in Fig. 2. Mast cell granules are rendered selectively electron dense. (× 6,750.)
- Fig. 4. Cell suspension fixed in glutaraldehyde. This section was stained for 5 min with 0-001 molar ruthenium red in tris-maleate buffer.

 Mast cell granules are markedly electron dense but nuclei have also taken up stain. (×4,750.)

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PHYSIOLOGY

Cerebral Composition and Function in Experimental Brain Injury in the Rat

NEURONAL activity is dependent on the ability of nervous tissue to control the concentration of electrolytes across the nerve cell membrane. The most important component of this process is the active extrusion of sodium from the neuronal cytoplasm against an osmotic gradient. A nerve impulse is normally initiated by the production of an area of increased permeability on the cell surface which allows the outward flow of potassium ions and the inward flow of sodium ions1. After the passage of the impulse, energy is required to restore the electrolyte distribution associated with the resting polarized state1. Nerve impulses can also be initiated by altering the electrolyte content of the fluid surrounding the neurone. Electrolyte disturbances occur in oedematous brain following trauma and cold injury and have been studied in detail by Pappius and Gulati, but in such conditions the mechanism of water accumulation and other ionic changes are not known². The following experiments were carried out on anoxic-ischaemic brain damage in rats³ in order to investigate the role of changes in the electrolyte and water content of the brain in the production of the hemiplegia and convulsions which occur in this condition, and their relationship to cerebral protein synthesis and energy production.

Brain damage was induced in adult and immature white Wistar rats of either sex by ligation of the right common carotid artery in the neck followed by intermittent exposure of the animal to 1 atm. of nitrogen for 30 min as described by Levine³. This resulted in hemiplegia in approximately 70 per cent of animals. Convulsions occurred in twelve of the thirty-nine hemiplegic adults and in none of the immature

animals. Previous experiments had indicated that only the most severely hemiplegic rats developed convulsions and these had a mortality rate of almost 100 per cent during the 48 h after inducing brain damage. Rats were killed immediately after the anoxic episode and at intervals up to 108 h afterwards. The brains were rapidly removed and the forebrain separated from the hindbrain by sectioning the midbrain. The cerebral hemispheres were separated by a median sagittal cut and weighed separately. The hemispheres were then dried to constant weight at 105° C. The dried brains were dissolved in 0·1 normal nitric acid and the sodium and potassium contents determined by flame photometry and the chloride contents determined by a mercuric nitrate method⁴.

Figure 1 shows the water and chloride content of the anoxic-ischaemic hemisphere at different intervals after induction of the brain injury. During the first half-hour little change from the control values was observed, although some animals did exhibit moderate increases in cerebral water content. Animals which developed convulsions at this time had cerebral water and chloride levels within the normal range. One and a half hours after injury no abnormality was found in the brain chloride of convulsing and non-convulsing animals. The mean water content of the right forebrain in the entire hemiplegic group at this time was, however, 1.3 g/100 g wet weight higher than the mean control value. The corresponding increase in the animals with convulsions in this group was 1.9 g/100 g wet weight. After 1.5 h the

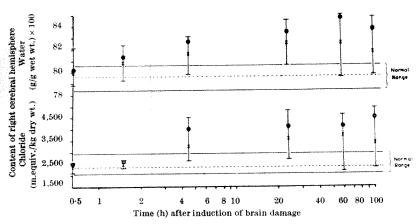


Fig. 1. Water and chloride content of anoxic ischaemic hemisphere at intervals after induction of injury. I, Range of values (between six and ten observations); ×, the mean of this range; and •, the mean value for animals in each group which had fits. The normal range is shown and the interrupted line indicates the mean of the normal.

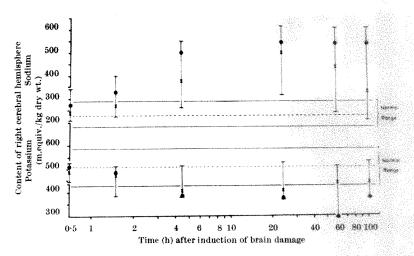


Fig. 2. Sodium and potassium content of anoxic ischaemic hemisphere at intervals after induction of anoxic ischaemic encephalopathy. The notation is the same as in Fig. 1.

mean water and chloride contents of the test hemispheres were consistently higher in convulsing animals than the mean values for the hemiplegic group taken as a whole. The maximum mean water and chloride content of the injured area of the brain was attained at 24 h and 60 h, respectively, after which both of these declined. There was a wide scatter of results, but consistently high values for both water and chloride content occurred between 4.5 and 108 h in the animals which had convulsions (Fig. 1).

The sodium and potassium contents of the hemisphere also showed maximum deviations from the normal at 24 h. From 1·5 h onwards the rats with fits had more marked changes in cerebral sodium and potassium concentrations than the mean values for the group as a whole (Fig. 2). No significant change was seen in sodium and potassium content in the first 0·5 h, but from 1·5 h after brain injury there was a consistent drop in potassium and increase in sodium content of the anoxic ischaemic hemisphere. The values for these electrolytes also showed a mean maximum change at 24 h and a slow return towards the normal range thereafter. After 1·5 h there were bigger changes in animals with convulsions than in the hemiplegic group as a whole.

Out of forty-nine immature rats treated with the Levine procedure, only eight showed water and electrolyte changes in the right cerebral hemisphere. All of the latter animals were more than 7 days old. The abnormalities were quantitatively similar to those occurring in the adult rats.

By comparing the increase in cerebral water content with increases in sodium and chloride in individuals with brain damage, the presumptive sodium and chloride content of the cedema fluid was calculated. There was no significant variation in these values at different intervals after brain damage and with different degrees of severity of oedema or functional disturbances. Table 1 shows that the mean sodium content for the cerebral oedema fluid for all adult hemiplegic animals was 285.0 m.equiv./l. and the mean chloride content was 1,630·0 m.equiv./l. Table 1 also shows that the most severely affected rats (that is, those with convulsions) had sodium and chloride concentrations in their oedema fluid which were similar to those of the group as a whole. There was a significant decrease in the chloride content of the

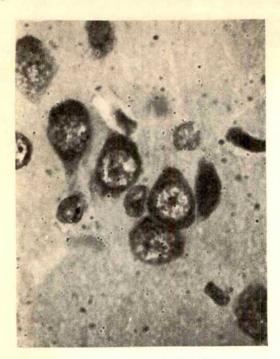


Fig. 3. Autoradiograph of left (control) cerebral cortex of anoxic ischaemic rat tissue during anoxic episode following an injection of tritiated glucose. There is a great reduction in the number of radioactive grains compared with controls (haematoxylin and eosin, ×960).

oedema fluid in immature hemiplegic rats. These values for the calculated sodium and chloride content of the oedema fluid are much greater than the concentration of these electrolytes in the plasma. This indicates that the electrolyte changes in the brain are not merely the consequence of capillary damage with a release of plasma into the tissues. A more likely explanation is that the electrolyte changes are part of a widespread intracellular process and that capillary damage plays only a contributory part. Also, the sodium and chloride concentrations in the plasma of immature rats are higher than those found in adult rats (personal communication from M. J. Seller), whereas their cerebral oedema fluid contains equal or lower concentrations of these ions compared with the adults.

The results suggest that animals with anoxic-ischaemic cerebral damage show evidence of a breakdown of active ionic transport in the brain after the animal has been returned to an atmosphere of air. This disturbance appears to be maximal about 24 h after the end of the anoxic episode and at this time the rats with the more marked functional disturbances showed the greatest oedema and electrolyte changes. Nevertheless, in the early hours after the induction of the brain lesion there was no correlation between functional disturbances and cerebral electrolyte abnormalities-even animals with marked hemiplegia and convulsions showed normal values for cerebral water and electrolytes. Thus other factors must produce this type of cerebral disfunction. Anoxic anoxia alone can lead to fits and yet rats which have convulsions in an atmosphere of nitrogen have no cerebral oedema5. The mechanism of anoxic convulsions is presumably depression of regions

of the brain with the highest oxygen requirement caused by the acute cessation of oxidative phosphorylation in these selectively vulnerable neurones. Fits could result if inhibitor activity were abolished before motor activity. It seems probable that a failure of oxidative phosphorylation can also persist after an episode of anoxia and following return to a normal atmosphere. Suggestive evidence for post-anoxic inhibition of the tricarboxylic acid cycle was provided by measurement of brain lactate and adenosine triphosphate (ATP) at intervals up to 24 h after the production of the Levine type of brain damage*. Normal rat brain contained 4.2 µmoles of lactate/g of fresh brain and the anoxic ischaemic brain contained 8.0 µmoles of lactate/g of fresh brain between 0.5 and 24 h after There was a simultaneous decrease in cerebral ATP. The mean normal value was 2·2 μmoles/g of fresh tissue and the post-anoxic values varied between 0.3-1.4 µmoles/g of fresh tissue. Although there was a decrease in total glucose uptake by post-anoxic brain7 the accumulation of lactate indicated that glycolysis was less severely inhibited than the tricarboxylic acid cycle. Confirmatory evidence for a prolonged block in tissue respiration is the marked decrease in in vitro oxygen uptake by post-anoxic brain8. One mechanism by which the tricarboxylic acid cycle is impaired is loss of succinic, isocitric, lactic and malic dehydrogenases in both neuronal and glial elements of anoxic brain9,10. This does not seem to be caused by an early increase in lysosomal activity, for there is an initial fall in acid phosphatase, but it could be a consequence of a failure of protein and nucleotide synthesis11. In the absence of accelerated enzyme removal, the cessation of protein synthesis precedes enzyme deficiency because of the time taken for normal protein catabolism.

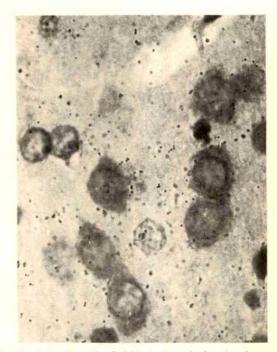


Fig. 4. Autoradiograph of right (test) cerebral cortex from same animal as in Fig. 3; a similar reduction in grain density has occurred compared with the left cortex (haematoxylin and eosin, ×960).

Table 1. CALCULATED SODIUM AND CHLORIDE CONTENTS OF OEDEMA FLUID IN THE ANOXIC-ISCHAEMIC HEMISPHERE OF ADULT AND IMMATURE HEMIPLEGIC RATS

		Sodium			Chloride		
Group	No. of observations	Mean	S.E. of mean	Significance (t test)	Mean	S.E. of mean	Significance (t test)
Adult hemiplegic with	8	296-0	47-4	t = 0.275 P = 0.7 - 0.8	1,557-0	255.5	t = 0.541 P = 0.5-0.6
All adult hemiplegic	20	285.0	24-3	t = 1.315	1,630-0	159.7	t = 2.92
Immature hemiplegic	6	213-5	60.5	P = 0.2 - 0.3	581.5	332-0	P = 0.01 - 0.001
Concentrations are measu	red in m.equiv./l.						

This will vary in different cell types and in different areas of the brain. Nevertheless, de novo protein synthesis is decreased as soon as the animal is placed in an atmosphere of nitrogen. In the present experiments, adult rats (body weight 150-180 g) had their right common carotid arteries ligatured and were injected with 150 µc. of tritiated D-glucose or L-lysine. The rats were then placed in an atmosphere of nitrogen for 10 min. The animals were then killed and autoradiographs were prepared as described before¹². Autoradiographs of both hemispheres (Figs. 3 and 4) showed a decrease in grain numbers compared with normal animals. The grains represent radioactive glucose or amino-acid incorporated into protein. Two hours after the end of the anoxic episode, however, the control (left) hemisphere showed the distribution and number of grains similar to normal controls (Fig. 5), whereas evidence of very little protein synthesis was seen in the anoxic ischaemic hemisphere of the same animal (Fig. 6).

An additional factor which could produce deficiency of respiratory enzymes is early loss of intracellular cerebral dehydrogenases into the plasma after brain damage¹².

Severe endothelial swelling can occur immediately after the induction of the Levine lesion but is not invariably seen under the electron microscope in this condition¹³. Thus tissue oxygenation could be impaired when swelling of capillary endothelial cells was of a sufficient degree to produce occlusion of the vascular lumen.

This evidence suggests that an acute episode of cerebral anoxia—and thus a period when aerobic respiration is inhibited—can lead to more permanent changes in the brain which persist for many hours after the return of the animal to a normal atmosphere. These biochemical lesions could produce a sustained deficiency of aerobic energy production and could therefore be related to the flow of sodium into the brain.

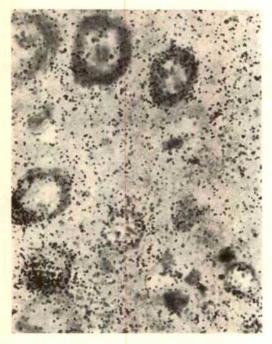


Fig. 5. Autoradiograph of left (control) cerebral cortex of anoxic ischaemic rat 6 h after the anoxic episode and 1 h after injection with tritiated glucose. Normal grain density and distribution (haematoxylin and cosin, × 960).

It seems unlikely that oedema alone can permanently damage the brain. Administration of sufficient water to adult rats to produce cerebral oedema and convulsions does not lead to any histological change 24 h afterwards¹⁴.

Nevertheless, the water content of the brain in rats injected with water and which had convulsions was similar to that found in the right hemisphere of convulsing animals 24 h after applying the Levine procedure¹⁴. Thus fits in rats 1 day after an anoxic episode could be partly caused by their cerebral oedema, but in these animals there is also a deficiency in aerobic metabolic processes which in itself can produce convulsions.

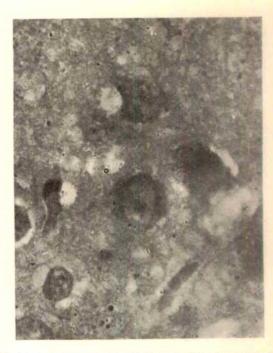


Fig. 6. Autoradiograph of right (test) cerebral cortex from same animal as in Fig. 3. There is a striking reduction in grain counts in the absence of definite histological change (haematoxylin and cosin, ×960).

A characteristic feature of the oedema described in these experiments is the initial delay in its appearance followed by progression in the first 24 h. A relatively small failure of the energy dependent sodium extrusion mechanism could lead to a continuous accumulation of sodium and water within the brain. Although protein synthesis is inhibited as soon as anoxia begins, enzyme loss is delayed for a variable interval after this until the existing enzyme protein is catabolized. Thus the functioning of the tricarboxylic acid cycle would progressively deteriorate after the end of the anoxic episode. could result in an increasing failure of sodium extrusion. The failure of anoxia to produce oedema and electrolyte changes in the brain of rats before the end of the first week of life could be a result of the diminished tendency of the rat brain to develop oedema before the age of 11 days after osmotic disturbances¹⁵. Another important possibility is the resistance of the immature animal to anoxia. This seems to be partly caused by a high glycogen content of the myocardium, the relatively greater utilization of anaerobic glycolytic metabolic pathways and their poikilothermic behaviour when deprived of oxygen¹⁶. It is not until the second week after delivery that a differential concentration of sodium and potassium occurs across the neuronal membrane and this seems to be related to an increased utilization of mitochondrial aerobic respiratory mechanisms17. The lower trans-neuronal differential electrolyte concentration suggests that a breakdown in energy dependent transporting mechanisms would result in a smaller flow of electrolytes. This is a possible explanation of the lower chloride content of the oedema fluid in the immature rats compared with the adults.

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Response Characteristics of Neurones in an Insect Brain

Units have been found in the tritocerebrum of the locust which respond in a complex fashion to visual stimulation. These responses differ in several respects from those of units in the protocerebrum, optic lobes and ventral nerve cord1-8.

Adult locusts (Schistocerca gregaria Forskål) from crowded laboratory culture were used. The head was waxed into a rigid frame which did not obscure vision, and the wings and metathoracic legs were removed; the animal was otherwise intact and no anaesthetic was used. A silver wire was implanted dorsally in the abdomen as the indifferent electrode. An aperture was cut in the frons, and the right tritocerebrum was exposed. The median ocellar and both antennal nerves were cut, the latter to abolish antennal muscle contraction. A varnished tungsten microelectrode was inserted into the tritocerebrum immediately beneath the antennal lobe, and recordings were made in the neuropile lying anterior to the descending tracts running to the circumoesophageal connectives. Action potentials were amplified and recorded together with stimulus markers on magnetic tape, and these data were subsequently analysed with a gated amplitudesensitive counting device. All experiments were carried out in photopic conditions with a background luminance of 1.3-2.0 log₁₀ cd/m². Stationary spots of light were projected on to a large screen placed 42 cm from the eye. These spots were between 0.3 and 2.0 log units brighter than the background illumination. Stimulus intensity was controlled by neutral density filters and the diameter of the spots varied in the range of 30' to 25° subtended at the eye. For experiments on moving stimuli a black disk subtending an angle of 4° at the eye was moved manually at approximately 40°/sec, being held at the end of a long glass rod. During a sequence of movements along an axis, successive movements were in opposite directions; for example, forward/back/forward. . . . some instances the disk was attached to a pendulum. In the initial control sequences, an interval of at least 2 min elapsed between successive movements; during an experimental sequence the interval was about 2 sec. We have been able to record the discharge of individual units for more than 24 h and experienced little difficulty in holding units for about 5 h.

The units we have studied responded to movement anywhere in the visual field of the contralateral eye, and this article is based on a detailed analysis of eighteen out of more than fifty such units recorded from twenty All units were tested briefly for multimodal responsiveness using clicks and puffs of air applied to the body surface. None showed a response to these stimuli.

The rate of "spontaneous" discharge of all units was low, varying from 2 to 60 impulses/min, with a mode of about 10/min. Only half the units gave any response to stationary spots of light, and these gave weak "on" and 'off" discharge to most stimuli irrespective of the size and intensity of the spot. This finding suggests that a spot of light elicits both excitatory and inhibitory effects which are interacting and finely balanced. The shortest latency of the "on" response was 40 msec. It is difficult to make meaningful comparisons with latency measurements obtained by other workers, but 40 msec is longer than the latency of a response to a test flash in the optic lobe of Locusta² and is in the extreme upper limit for protocerebral units in Sphinx⁶. Multimodal neurones in the optic lobe of Locusta, however, have visual latencies varying between 30 and 200 msec (ref. 5).

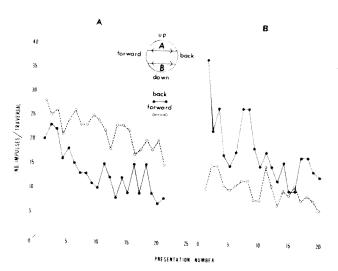


Fig. 1. Response of a unit to movement of a black disk in the visual field, which here and in Fig. 2 is represented by the inset circle. A line within the circle shows the line of traversal of the disk, the direction of movement being indicated by the arrows. The forward/back line is horizontal and parallel to the long axis of the insect's body. The responses to movement (of approximately 60° at the eye) along the line indicated by A in the inset are plotted in graph A. The disk was moved forward and backward repeatedly. The number of spikes elicited by each successive movement is plotted, a separate curve being constructed for each direction. Forward movements along line A constantly evoked a larger response than backward movements. When the disk was moved along line B parallel to but displaced 12° downward from line A, the preferred direction was backward (graph B). Directional effects were thus not homogeneous throughout the field. Although the responses waned, the directional effects were maintained throughout a sequence of presentations.

All units gave a high frequency burst to movement and most responded more vigorously when the disk was moved in one direction across the visual field than when it was moved in the reverse direction. None of the units was inhibited by movement, in contrast to those described by Horridge et al.5 and Collett and Blest?. When the movements were repeated the response rapidly waned, although the directional effect was sometimes maintained. the unit the responses of which are plotted in Fig. 1A fired more vigorously when the disk was moved forward along a horizontal line than when it was moved backward along the same line. A difference in response was maintained through many successive forward/backward presentations during which the magnitude of both responses declined.

The directional effect was usually maintained for movements along parallel lines, but exceptions were not rare.

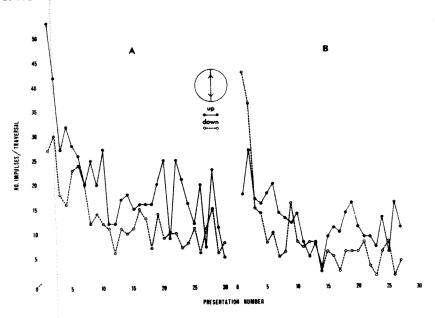


Fig. 2. An example of primacy reversal. A, When the disk was moved first upward and then downward, the to and fro movement being repeated thirty times, there was a clear preference for up. At the end of the sequence the stimulus was withdrawn for 5 min and a second sequence (B) then commenced, the initial movement being down. This was now the preferred direction for the first two pairs of movements, after which the up preference was re-established.

For example, after the sequence of movements which evoked the discharge plotted in Fig. 1A, the disk was displaced 12° downward and moved forward and backward along a line parallel to the first. The directional effect was reversed and maintained although the responses

gradually waned (Fig. 1B).

The initial "directional" effect was frequently found to be determined by the order of stimulus presentation, that is, it was not a directional effect but a primacy effect. When a sequence of to and fro movements along a given line was performed, a directional effect was frequently observed at least for the first two or three movements at When the sequence was the start of the sequence. repeated after a pause of a few minutes, but with the first movement in the opposite sense to the first movement of the original series, the directional effect was frequently reversed. In twelve units which were tested in both the up/down and back/forward directions, twenty of the twenty-four cases showed primacy reversal of this sort. The effect seems to be similar to that exhibited by a fibre in the optic nerve of crayfish. An example is given in Fig. 2. In this experiment, two sequences of movements along a vertical line were made. In one sequence the first movement was upward (Fig. 2A); there was a clear directional effect, upward being the preferred direc-When, however, the initial movement was downward (Fig. 2B), this was the preferred direction for the first two pairs of movements. As the response declined the original preference was restored. Thus the magnitude of the initial response is largely determined by the order of presentation of stimuli (for example, upward first or downward first) rather than by an inherent directional selectivity. It thus seems unwise to classify a unit as directionally selective in the absence of any test for primacy reversal.

Once the response to a given movement had waned a discharge could usually be evoked by moving the disk in some other part of the visual field or by withdrawing the stimulus for some time before moving it again along the original axis. In the latter case, a rest of several minutes was usually sufficient to restore the response, though

occasionally this was not achieved even after a pause of several hours. It is unlikely that this prolonged effect was caused by a deterioration of the preparation because a vigorous response could be elicited by movement in some other part of the field.

Others^{5,7} have shown that some units in the optic lobes are excited by movement in one direction, and are inhibited by movement in the opposite direction. Primacy effects would ensue if complementary cells, with properties similar to those described by these authors. possessed mutually inhibitory connexions—the first cell stimulated by movement in its preferred direction depressing the subsequent response of the other to movement in the reverse direction. The units described in this communication may be synaptically connected to arrays of such complementary cells. Alternatively, both primacy and "directional" effects could be produced by a complex response decrement in a non-directionally selective system of neurones.

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Pattern of Cortical and Thalamic Connexions of the Somatic Sensory Cortex

The constituent cells of any architectonic subdivision in the somatic sensory and visual regions of the cerebral cortex respond to peripheral stimuli of a different nature from those which excite cells in other subdivisions. These individual differences have been thought to be principally dependent on afferent thalamic connexions or on cortical association connexions with other subareas1,2, but other factors may also be important, particularly because the various subareas also differ in their callosal connexions3,4, and because cortico-fugal influences may modify the activity of thalamic neurones. As a consequence, in any interpretation of these functional differences it is advisable to take all the extrinsic connexions into account. The patterns of thalamic and cortical connexions of the somatic sensory cortex have been examined in the cat and monkey using experimental neurohistological techniques. distribution of degenerating fibres, following lesions in the thalamus and in the somatic sensory and other cortical areas, has been studied by the Nauta method6 and with the electron microscope.

In the cat, large lesions which destroy the first and second somatic sensory areas of one hemisphere cause degeneration in several parts of the forebrain. In the ipsilateral cortex these are: the motor cortex (area 4); a portion of area 6; and one of the parietal fields, area 5. In the contralateral cortex, only the first and second somatic sensory areas show degeneration and this is restricted to those regions in which the face, trunk, tail and proximal parts of the fore and hind limbs are represented7,8. In both the first and second areas, regions related to the more distal segments of the limbs do not receive commissural afferents from any part of the opposite cerebral cortex. Neither of these latter regions lies within the confines of a single cytoarchitectural field10. In the thalamus, degeneration is found in the nucleus ventralis posterior and the medial division of the posterior

nuclear group 11,12.

The organization of these connexions was examined by placing small lesions in different parts of the first and second somatic sensory areas and in other cortical regions. With regard to the commissural connexions of the first somatic area, it was found that regions in this area which are related to the distal parts of the limbs neither send nor receive commissural fibres. With this exception, the callosal connexions of the first area are organized so that any part projects to a symmetrical point, only, in the contralateral first area, and to a region in the opposite second area which is related to the same part of the periphery. In the second somatic sensory area also, the region related to the distal segments of the limbs does not send or receive commissural fibres; those regions which are related to the trunk, tail and proximal segments of the limbs give off commissural fibres, but these terminate entirely in symmetrical regions of the opposite second area. Regions related to the face, however, project both to symmetrical regions of the contralateral second area and to a small portion of the opposite first area containing the bilateral representation of the face^{7,8}. Electron microscopy has shown that degenerating commissural axons in both the first and second somatic areas terminate on spines attached to dendrites of small and medium size in all cortical layers but especially in the deeper parts of layers I and III and in layer IV.

The association connexions of the somatic sensory areas can be considered as both intrinsic and extrinsic. Lesions in one of the larger functional subdivisions of the first area cause degeneration throughout the same subdivision but not in other subdivisions, while damage to any part of the second area results in degeneration throughout the whole second area. The functional sub-

divisions of the two areas are reciprocally connected and electron microscopy shows that the fibres joining them terminate on dendritic spines chiefly in layers IV to VI with an occasional terminal in the more superficial layers, especially layer I. The subdivisions of the first and second areas are also reciprocally connected with their counterparts in the motor cortex and each has a small projection to a part of area 6 in the region of the supplementary motor areas. Only the first somatic area projects to area 5 and this projection is organized so that hindlimb, trunk and tail regions project to those parts of area 5 situated in the splenial and lateral gyri, while face and forelimb regions project to those parts in the anterior and middle suprasylvian gyri; the latter region has been described as a third somatic sensory projection area13. With the exception of the motor cortex, no other cortical area sends fibres to the somatic sensory regions.

Both components of the postero-ventral nucleus project in a topographically organized manner on the first and second somatic sensory areas14, and all three of the cytoarchitectural divisions of the first area receive the terminations of thalamo-cortical fibres. Electron microscopic studies of the first area following thalamic lesions indicate that most of these fibres terminate on spines or shafts of dendrites of medium size in layer IV and in adjacent parts of layers III and V. A few degenerating terminals also appear on dendritic spines in the more superficial parts of layer III and in layer I. Cortico-fugal fibres in turn project from both the first and second somatic areas back to the nucleus ventralis posterior and also to the medial division of the posterior thalamic nuclear group. The projection to the former is organized so that only parts of the cortex receiving fibres from a specific portion of the nucleus send fibres back to it. Following lesions in either the first or the second somatic sensory areas, degenerating terminals have been seen with the electron microscope ending axo-axonically and axo-dendritically in the nucleus ventralis posterior.

In the monkey, in which the association and callosal connexions of the first somatic sensory area are arranged in a similar manner to those of the cat, it has been possible to restrict lesions to single cytoarchitectural fields. Ipsilaterally, the parts of these fields situated within one of the functional subdivisions of the first area are reciprocally connected with one another and with parts of the second somatic and motor areas which are related to the same portion of the periphery. Each field has, in addition, an organized projection to area 5 and sends a small number of fibres to the supplementary motor area. The regions functionally related to the distal segments of the limbs⁸ include portions of areas 3, 1 and 2 and do not send or receive commissural fibres; other parts of the three fields project to both the first and second somatic areas of the opposite hemisphere, but the projection to the first area is restricted to a symmetrical region in the

homotypical field only.

The observations recorded here demonstrate that in each hemisphere portions of the first and second somaticsensory areas in which a given part of the periphery is represented are reciprocally connected with one another, with a similarly related part of the motor cortex, and in an organized manner with the postero-ventral nucleus of the thalamus. In contrast, the callosal connexions of the two areas display a certain lack of uniformity: the first somatic area sends fibres to both the contralateral first and second areas; the second area sends most of its fibres to the opposite second area only; the distal limb regions of each area lack interhemispheric connexions entirely. This pattern of connexions bears many similarities to that which has already been demonstrated in the visual cortical areas of the cat. In both cases, the relevant thalamic nucleus projects to all the known functional and architectural subdivisions of the respective areas 15,16. In the somatic sensory cortex all these subdivisions are interconnected by cortical association fibres and the same seems

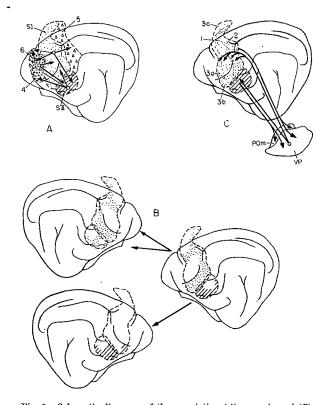


Fig. 1. Schematic diagrams of the association (A), commissural (B), and thalamic (C) connexions of the somatic sensory areas in the cat. Somatic areas are drawn after Woolsey', and in A and C cytoarchitectural details have been added following Hassler and Muha-Clement's whose numbering scheme is retained (SI, first somatic sensory area; SI, second s group).

to be true for the subdivisions of the visual cortex which have been studied^{2,17}. In addition, both cortical areas are connected to the same restricted portion of area 6 (ref. 17). The callosal connexions of both areas do not terminate throughout the corresponding region of the contralateral hemisphere^{9,17}. In the somatic sensory areas, those parts which are commissurally interconnected are the regions related to part of the body close to the midline, and there is evidence that the restricted parts of the visual cortex which are commissurally connected are "best excited by visual stimuli near the midline"18. Finally, there is good evidence from this and other studies that the somatic and visual areas are reciprocally connected with their respective thalamic relay nuclei and also send fibres to one other part of the thalamus—the posterior group in the case of the somatic areas and the nucleus lateralis posterior in the case of the visual areas^{17,19}. It would be interesting to know whether the cortical connexions of other sensory systems are also similar and, if so, whether there is, perhaps, some basic pattern common to all.

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Comparison of the Rate of Habituation of the Flexor Reflex between Both Hind Limbs tested sequentially in the Rat

WE have developed a technique for measuring the electromyographic discharge and isometric tension in the biceps femoris muscle of the conscious rat1, which has enabled measurements of latent period, duration of electromyographic discharge and tension to be made during habituation of the flexor reflex in response to repeated uniform stimuli applied to the web space of the ipsilateral hind limb. Establishment of habituation of the reflex has been assessed from: (a) the number of stimuli required before the animal ceased to respond to the standard stimulus; (b) the progressive increase in latency of the reflex response; (c) the progressive reduction in total duration of electromyographic discharge; and (d) the decrease in tension developed. Habituation of the flexor reflex has been demonstrated with this technique in both intact rats and rats tested 4 days after spinal transection.

We have now compared the rate of habituation of the flexor reflex recorded separately from each hind limb of the same rat. Assessment of habituation was made using all four criteria, but for simplicity of quantitative presentation, values for criterion (a) only are given here, although the significance of assessment by criteria (b), (c) and (d) is referred to.

In three intact rats the right limb was tested first, and in a further three rats the left limb was tested first; in each case the other limb was tested 1-2 days later. There was no significant difference in the rate of habituation calculated from changes in latency, tension developed and duration of electromyographic discharge when a comparison was made between the right and left limbs; nor was there any significant difference in the mean number of stimuli required to achieve complete loss of reflex response. When a comparison was made between the first and second limbs tested, the rate of increase in latency and decrease in tension developed and decrease of duration of electromyographic discharge was significantly lower in the second than in the first. The second limb tested required significantly more stimuli than the first to achieve complete loss of the flexor reflex response (see Table 1).

A similar experiment was performed on six rats which had undergone spinal transection at the level of T.10

Table 1.

			quired to achieve		
	No. of	complete loss of	reflex response		
	observa-				
	tions	Right leg	Left leg	t	\boldsymbol{P}
Intact rats	в	251 ± 51	255 ± 47	0.06	> 0.9
Spinal rats	6	154 ± 34	187 ± 35	0.70	> 0.5
		First leg tested	Second leg tested		
Intact rats	6	176 ± 12	327 ± 50	2.90	< 0.05
Spinal rats	6	170 ± 24	171 ± 38	0.02	> 0.0

4 days previously. There was no significant difference in the rate of habituation calculated from changes in latency, tension developed and duration of electromyographic discharge when a comparison was made between the right and left hind limbs, nor was there any significant difference in the mean number of stimuli required to achieve complete loss of reflex response. There was no significant difference in the number of stimuli required before the animal ceased to respond, nor was there any significant difference in the rate of change of latency, tension developed and duration of electromyographic discharge when comparison was made between the first and second limbs tested (see Table 1).

When the number of stimuli required to achieve habituation in the second limb was expressed as a percentage of that required by the first limb, the mean value obtained for the intact rats was 187 ± 27.0 per cent, and for the spinal rats 101.8 ± 10.9 per cent; these percentages differed significantly (P < 0.001).

In intact rats habituation of the flexor reflex to repeated application of the uniform stimulus occurred at a lower rate in the second limb tested than in the first, which indicated that previous "experience" of the stimulus was important in the establishment of habituation. In spinal rats the rate of habituation of the flexor response was the same in both hind limbs, which suggests that utilization of previous "experience" of the uniform stimulus involves, at least in part, supra-spinal centres. These observations indicate that there is a supra-spinal mechanism which delays the establishment of habituation in the intact rat. This mechanism would therefore have an opposite action to the frontal areas of the cortex, because in the intact animal the frontal areas of the cortex must act to facilitate the establishment of habituation, because frontal lesions impair the process of habituation (refs. 2 and 3 and our unpublished work).

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Irreversible Inhibition of Acetylcholinesterase by Dibenamine

WE are investigating the idea1 that the muscarinic receptor and the anionic site of acetylcholinesterase (AChE) are identical by comparing the kinetics of inhibition of AChE and the receptor by certain irreversible inhibitors.

Belleau and Tani2 have recently shown in a qualitative manner that N,N-dimethyl-2-chloro-2-phenethylamine (C₆H₅.CHCl.CH₂.NMe₂), which spontaneously produces the ethyleniminium ion in aqueous solution, is an irreversible inhibitor of AChE at the anionic compartment. We have found that a related compound, dibenamine $((C_6H_5.CH_2)_2N.CH_2.CH_2Cl)$, slowly inhibits the enzyme through its ethyleniminium ion, the reaction in acid solution involving a group on the enzyme with approximate pK_a 5.8, whereas in alkaline solution a fast reaction occurs with a group on the enzyme with approximate pK_a 9.2.

The reaction between dibenamine $(0.24-0.95 \times 10^{-4})$ molar) and bovine AChE at 25° C in 0.2 molar sodium chloride and gelatine media at pH 9.5 was followed by withdrawing samples at predetermined intervals and assaying the enzyme activity by the pH-stat method using 0.002 molar acetylcholine bromide as substrate. A linear plot of $\log (a-x)$ versus t for the reaction, where (a-x) represents the enzyme activity remaining at time t,

for different concentrations of enzyme with a fixed inhibitor concentration showed that the reaction followed first order kinetics of the form, $v = k(Db)(AChE) = k_{obs}$ (AChE). The second order rate constant, k, however, progressively decreased with increasing dibenamine concentration, which suggested that an inhibitor-AChE complex was formed before "intramolecular" alkylation of the enzyme in accord with equation (1). Green and Smith4 noted a similar relationship for the reactivation by oximes of AChE inhibited by organophosphorus com-

Inhibitor + AChE
$$\stackrel{K}{\rightleftharpoons}$$
 AChE Inhibitor complex
$$\stackrel{k_5}{\longrightarrow}$$
 Inhibited enzyme (1)

A plot of 1/k versus [dibenamine] gave a straight line with slope $1/k_3$ and intercept K/k_3 which gave approximate values for K and k_3 of 1.56×10^{-5} mol. $1.^{-1}$ and $4.52\times$ 10-2 min-1.

The second order-rate constant k for the reaction between fixed concentrations of inhibitor and enzyme was influenced by the reaction media at low ionic strength at pH 6.5 but only to a slight extent at pH 9.5 (Table 1). These observations may be interpreted in terms of interaction between ions of opposite charge during the reaction at pH 6.5, which would implicate dibenamine in the reaction as its ethyleniminium ion (Db), and, errone-

ously, as shown here, a reaction between the uncharged dibenamine base and the enzyme at pH 9.5.

The reaction between enzyme and dibenamine (19 ml., 0.75×10^{-4} molar) at pH 10.0 was prevented by allowing the dibenamine solution to stand in contact with thiosulphate ion (0.2 molar) for 10 min before addition of the enzyme. In another experiment, the enzyme was omitted and the unreacted dibenamine base extracted with ether. The residue recovered from the ether was dissolved in dilute acid (19 ml.) and this solution was used to inhibit the enzyme in the usual way. The rate of inhibition was comparable with that observed in a control extraction experiment where sodium chloride (0.2 molar) replaced the thiosulphate ion. These experiments firmly establish that the active alkylating species present in dibenamine solution at alkaline pH which is destroyed by the thiosulphate ion is the ethyleniminium ion and not the free base with which thiosulphate is also known to react?.

Table 1. EFFECT OF IONIC STRENGTH ON THE INHIBITION RATE CONSTANT, & 1.mol.-1min-1+

			Tonic str	ength* (I)		
	0.0	0.0005	0.01	0.0195	0.1905	0.5
pH 6·5 pH 9·5	141	413	93.5	$\begin{array}{c} 77.2 \\ 476 \end{array}$	502	33.6

* Sodium chloride.
† Dibenamine concentration, 0.71 × 10⁻⁴ molar.

Certain \(\beta \)-halogenoethylamines related to dibenamine are known to alkylate the adrenergic receptor6 and acetylcholinesterase² as their readily formed ethyleniminium ions, but dibenamine gives a very low yield of its ion in aqueous organic solvents7,8 and has not previously been directly observed as an alkylating agent in a biological system. The following scheme (equation (2)) is proposed to explain the observed first order kinetics, where Db is the reacting species, which is based on the premise that the concentration of Db remains constant within narrow limits during the reaction.

Applying the general case of two consecutive first order reactions, then with a reactive intermediate, that is, k_2 (H₂O) $\gg k_1$, its concentration will be very low and may be considered essentially constant because of the existence of a steady state. This view is supported by studies on certain other β -halogenoethylamines which cyclize slowly and give low constant concentrations of ethyleniminium ions for long periods8,10

The rate-pH profile for the reaction using fixed concentrations of enzyme and inhibitor was a sigmoid-shaped curve with a pronounced hump at the foot. A plot of $1/k_{\rm obs}$ against [H+] in the usual way gave the pK_a s of the two groups on the enzyme involved in the reactions which

were approximately 5.8 and 9.2.

The rate-pH profiles obtained for the hydrolysis of substrates by AChE together with other work show that two basic groups¹¹ with pK_a 6.3 (imidazole) and 5.5 and and acidic group¹² with pK_a 9.35 (phenolic hydroxyl of tyrosine) are involved in the catalytic process. The pHrate data in the acidic region can be interpreted either as alkylation of imidazole or as prevention by a protonated imidazole group to binding of the ethyleniminium cation at the anionic compartment of the active site—a situation analogous to that noted for charged competitive inhibitors11. Reaction between the bound ion and an unionized group elsewhere at the active site could then occur.

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Effect of Gastric Juice and pH on Inorganic Iron in Solution

THE absorption of ionized iron in humans is influenced by gastric acidity¹⁻³. Ferric ions in solution usually form complex mixtures in which the metal is co-ordinated to a number of hydroxyl ions and in some conditions there is a high degree of polymerization4. Ferrous ions do not undergo such marked changes but tend to be oxidized to ferric forms when the pH approaches neutrality4. Filtration on 'Sephadex' gel has been used in this laboratory to examine the behaviour of iron salts at different pHs and after mixing with gastric juice.

Freshly prepared aqueous solutions of 59Fe-ferric citrate with iron concentrations of 2 µg and 200 µg/ml. and unlabelled ferrous sulphate solution with an iron concentration of 200 $\mu g/ml$. were examined. The solutions of ferric iron were also examined after mixing with an equal volume of freshly aspirated gastric juice which had been neutralized to pH 7 with 0.1 normal sodium hydroxide. Samples (I ml.) were eluted from a column of 'Sephadex G-25', 15 mm in diameter after equilibration with 0·1 molar buffers at pH 2-6·8 and also with 0·1 normal hydrochloric acid. Fractions (2 ml.) were collected. The activity of iron-59 in the fractions was

measured by scintillation counting in a well crystal and the chemical iron content was determined using tripyridyltriazine5.

At pH 1.3 ferric iron behaves as a relatively unpolymerized ionic species. Its peak concentration appears in a corresponding low molecular weight fraction after gel When pH increases above 3.5 ferric iron filtration. appears in fractions which gradually approach the void volume of the column (Fig. 1), in this case corresponding to a molecular weight of about 5,000. This behaviour results from the increasing condensation of ferric ions at increasing pH to form complex ions of high molecular weight. The behaviour of ferrous iron differs in that no change in molecular size seems to occur between pH 1.3 and 6.8. Ferrous iron appears in the fractions in which the smallest ferric ions are found. It shows the same behaviour at concentrations of 2 µg and 200 µg/ml., and the positions of radioactive peaks correspond to those of peak concentrations of chemically determined iron.

When ferric iron mixed with gastric juice is passed through the column at $p{\rm H}$ 6.8 some of the iron appears in the void volume, which indicates that it is bound to a high molecular weight component of the gastric juice (Fig. 2). This portion is smaller and often less discrete at pH 5.5 and is absent at pH 4.3 or lower. The proportion

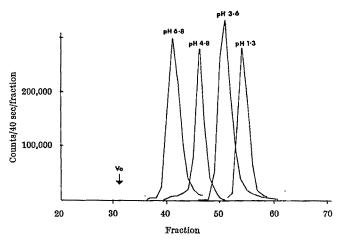


Fig. 1. Radioactivity of fractions obtained after gel filtration of $^{59}{\rm Fe-ferric}$ citrate at different $p{\rm Hs.}$

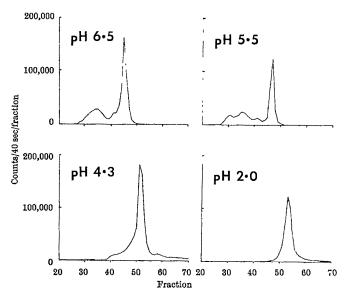


Fig. 2. Radioactivity of fractions obtained after gel filtration of a mixture of ao Fe-ferric citrate and fresh gastric julce at different $p{\rm Hs.}$

of iron in the "bound" peak is approximately the same at both iron concentrations. When gastric juice was first incubated at 37° C for 1 h before neutralization there was little binding of iron.

At a neutral pH ferric ions seem to be larger than ferrous ions. This may be a factor in determining availability of iron for absorption in the small intestine, and it may partly explain the effect of gastric acid on iron absorption and the greater absorption of ferrous iron. Gastric juice does not seem to bind iron in an acid medium and the physiological significance of this phenomenon is uncertain. While no direct inference can be made from these observations regarding the state of iron in the jejunum in vivo, its behaviour in relation to the pH usually found in the upper gastrointestinal tract and its reaction with gastric juice are relevant to the study of this problem. After a meal gastric contents at about pH 2 leave the stomach and traverse the duodenum as a Alkaline secretions in the duodenum do not increase the pH of this bolus to more than 3-4. At the upper end of the jejunum the bolus is mixed with intestinal secretions and acidity fluctuates between pH 4 and 7 (ref. 6). If the behaviour of iron in vivo is similar to that demonstrated in vitro, then intraluminal pH may determine both its availability and the site of absorption.

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RADIOBIOLOGY

Dinitrophenol decreases the Radiation Sensitivity of L Cells

BECAUSE the repair of radiation injury would seem to consume energy, chiefly in the form of ATP, we have studied the response to radiation of L cells, severely depleted of intracellular ATP. We have found that 2,4-dinitrophenol (DNP) can reduce intracellular ATP by about 90 per cent, that L cells treated with DNP for up to 8 h lose none of their reproductive integrity and that treated cells are less sensitive to radiation, particularly at a high dose.

L-929 cells were used as monolayers on Eagle's medium (MEM) supplemented with calf serum (10 per cent serum for monolayers, 17 per cent serum for cell survival studies) in glass bottles. For cell survival experiments, monolayers were trypsinized, counted with a haemacytometer, diluted with fresh medium, and incubated for 24 h at 37° C in an atmosphere of 5 per cent carbon dioxide–95 per cent air. After this attachment period the medium was removed from some of the plates and replaced with glucose-free Hanks solution containing 5×10^{-5} molar DNP, pH adjusted to $6\cdot8-7\cdot0$ with $7\cdot5$ per cent sodium bicarbonate.

After 30 min of contact with DNP, the cells were irradiated with spaced doses of X-rays (150 kVp, 15 m.amp, 6 mm Al HVL, dose rate 100 rads/min; irradiations were performed in conditions of full backscatter). The DNP remained in contact for 2 h after irradiation and was replaced with fresh medium. Control plates, set

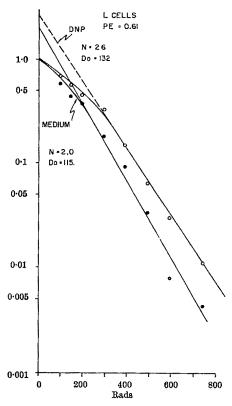


Fig. 1. Post-irradiation survival curve for control and DNP treated cells.

up from the same inoculum, were irradiated at the same time as the plates treated with DNP. After 12 days incubation, the colonies were fixed, stained and counted. Plating efficiencies ranged from 0.42 to 0.65.

Figure 1 shows that treatment with DNP increases both n and D_0 compared with control cells irradiated in medium and the effect is to prolong the shoulder segment. There was some increased survival after the lower doses of X-rays, but the effect is more pronounced with larger doses. For this experiment, the average cellular multiplicity was 1·1 for both treated and control cells.

Table 1 shows the result of a toxicity study with DNP. For this experiment, DNP was left in contact with the cells for various times. Up to 8 h of contact there was no evidence of toxicity; after 8 h there was slightly more survival than in cells maintained in medium.

Table 1. TIME-TOXICITY RESULTS FOR L OELLS

Treat- ment	Time of contact	Plates	Cells plated	Colonies	Average
Control (medium) DNP DNP DNP	2 h 4 h 6 h	3 3 3	60 60 60	32, 36, 34 35, 40, 43 38, 40, 43 42, 39, 40	34±2* 39±4 40±3 40±2
DNP	8 h	3	60	44, 46, 40	43 ± 3

The average cellular multiplicity was 1.04 to 1.1 for both controls and DNP treated cells.

* Standard deviation.

Fig. 2 shows that DNP reduced the intracellular concentrations of ATP. For this experiment, cells were gently scraped from the monolayers with a rubber policeman and suspended in fresh medium at a concentration of about 1.5×10^6 ml. The suspension was stirred gently, aerated and maintained at 37° C. At various times after collection, 1 ml. samples were taken from the suspension, extracted with perchloric acid (PCA)—final concentration 0.5 N—neutralized with potassium hydroxide, and the concentration of ATP was measured by the firefly luciferase method using a liquid scintillation counter¹. When several samples had been collected, the suspension was centrifuged and the medium was replaced by DNP. Additional samples

were taken, the suspension was again centrifuged, and DNP was replaced by fresh medium. As Fig. 2 shows, although DNP produces a rapid decrease in the concentration of ATP, there is a rapid increase after removal of

DNP acts on ATP by uncoupling mitochondrial oxidative phosphorylation. As our results show, DNP is not only non-toxic (at least a 5×10^{-6} M, pH 7, and up to 8 h contact), it promotes increased survival after irradiation. Consequently, short periods of ATP deficiency are compatible with viability, normal colony growth and increased resistance to the effects of ionizing radiation.

Perhaps part of the explanation of our results is in the recent work of Elkind et al.2, who propose that repair of radiation injury in cultured mammalian cells, measured by the paired dose method, may not be a biochemical process in the usual sense. This idea stems from their previous work, which showed that "repair" occurred at low temperatures and in anoxic conditions3. They consider their current data compatible with the concept that repair may be a consequence of a passive (possibly physico-chemical) rather than an active enzyme process.

We consider our results entirely compatible with the data of Elkind et al. We found that immediately after treatment with DNP, synthesis of DNA, RNA and protein in the cells virtually stops4. Consequently, the increased survival after irradiation is paralleled by (and is possibly a result of) a depression of macromolecular synthesis. This agrees with the idea that the repair process is enhanced when cells are treated with cycloheximide,

which inhibits protein synthesis.

Probably the most biologically significant effect of radiation is to produce physico-chemical alterations of the DNA bond structure. As a result, endonucleases and exonucleases would attack the altered site (the "cut" partner of the "cut"—and "patch" group of enzymes) and produce breaks in the DNA molecule. If the breaks were single stranded, then repair could progress by a re-synthesis of the absent segment, using the unbroken DNA strand as a template. If both strands be broken, however, the lesion is probably irreparable7.

The active factor seems to be the nucleases rather than the radiation. Perhaps if the "cut" system of enzymes is held back for a period of time after irradiation—by such agents as cycloheximide or DNP-some relaxation of the physico-chemical distortion of the DNA bonds can occur. After removal of the inhibitor, then, the number of sites requiring "cutting" would be decreased. If, on probability

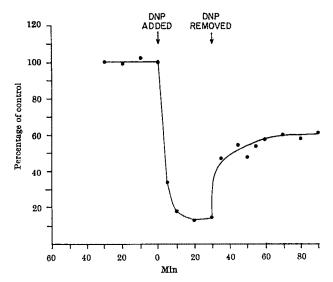


Fig. 2. The effect of DNP on concentrations of ATP in L cells. The results, originally in units of p moles/10 $^{\circ}$ cells, are given as percentage of control.

grounds, some of the potentially double stranded breaks were converted into single stranded breaks, greater postirradiation survival would result.

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PATHOLOGY

Acquired Radioresistance of Tumour Cells

RADIORESISTANCE of tumours, which appears as a result of repeated irradiation, was described in 1904 by Lassueur. Certain characteristics were later examined: tumour ploidy²⁻⁶; the state of the mitochondria⁷ and ribosome⁸; the content of sulphydryl groups ,10; the capacity for anaerobic glycolysis11; catalase activity12; and changes in the immune relationships between tumour and organism13. The mechanism of the enhancement of tumour radioresistance, however, remains unknown. In experiments with Ehrlich ascites tumours in mice we have obtained radioresistant strains. This was achieved by intraperitoneal reinoculation preceded by irradiation of the tumour with 300 r. in vivo for 3 consecutive days and a single dose of 1,000 r. in vitro. We made between 50 and 120 passages in these conditions of repeated irradiation. The difference in radiation response of rhythmically irradiated and initial strains of Ehrlich tumour was determined after check-up irradiation with 1,000-3,000 r.

When the inoculation material was irradiated with doses of 1,000 and 2,000 r. and then introduced intraperitoneally the control tumour grew much less than the tumour which had been irradiated repeatedly. In particular, inoculating 106 cells did not result in any development of the control tumour, while the chronically irradiated one showed a marked growth (Fig. 1).

Similarly, when doses of irradiation vary from 1,000 to 3,000 r. and the number of inoculated cells remains constant, the number of animals in which tumours develop is greater when the repeatedly irradiated cells are inoculated (Fig. 2).

The DNA content of repeatedly irradiated strains of an Ehrlich tumour in the fortieth and seventy-first generations was less than that of the initial tumour for 72 h after the check-up irradiation with 1,500 r. Apparently the marked polyploidy of the control tumour is the result of an accumulation of cells with premitotic contents of DNA. To a certain extent this is confirmed by the sudden decrease in the mitotic index, which was between two and ten times lower than that of the repeatedly irradiated strain. The decrease in the mitotic index in the control tumour after irradiation with 1,500 r. may also account for the smaller number of tumour cells in the peritoneal liquid compared with those in the repeatedly irradiated tumour (Figs. 3 and 4). All this implies an increase in the

radioresistance of repeatedly irradiated tumours. Radioresistance was recorded fifteen to twenty passages earlier in vitro than in vivo.

In considering the factors connected with the creation of increased radioresistance we wish to emphasize some of

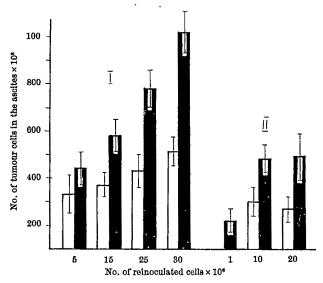


Fig. 1. Growth of control () and chronically irradiated () tumours: taken on the tenth day 1,000 r. (I) and 2,000 r. (II).

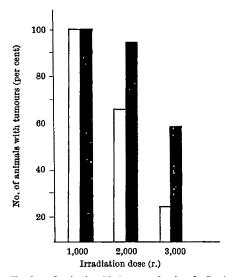


Fig. 2. Number of animals with tumours developed after inoculated 20×10^4 cells irradiated at different doses. \Box , Control; \blacksquare , repeatedly irradiated.

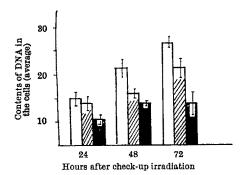


Fig. 3. Contents of DNA in the cells of control and those of chronically irradiated tumour after check-up, dose of 1,000 r. White columns, control; hatched columns, fortieth generation; black columns, seventy-first generation.

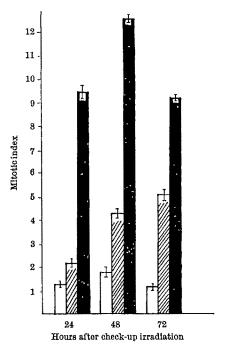


Fig. 4. Mitotic index of control and chronically irradiated tumours during three days after check-up, irradiation 1,500 r. White columns, control; hatched columns, fortieth generation; black columns, seventy-first generation.

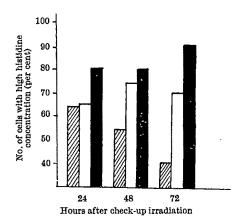


Fig. 5. Number of cells (per cent) with high histidine concentration during three days after check-up, irradiation with 1,000 r. Hatched columns, control; white columns, fortieth generation; black columns, seventy-first generation.

our results. Cytochemical investigation of histidine revealed two types of cells in Ehrlich tumours: the first were strongly stained and the others were only slightly stained. These two types are referred to as cells with "high" and "low" histidine concentrations. After irradiation with 1,500 r., cells with a high histidine content accumulated in the radioresistant strain while the initial control tumour showed fewer such cells (Fig. 5).

Thus the relationship between the increases in histidine content and radioresistance becomes more evident.

We found that the content of sulphydryl groups in soluble proteins of radioresistant strains is 42 per cent greater than in cells of the control strain (Fig. 6). (Sulphydryl groups were estimated by amperometric titration.)

Caspersson and Révész⁹ similarly found an increased content of sulphydryl groups bound to protein (PSH) in cells of radioresistant tetraploid strains of Ehrlich ascites tumours, which were obtained by means of selection. They also found a correlation between the contents of DNA and PSH. We observed an increase of the PSH groups in a

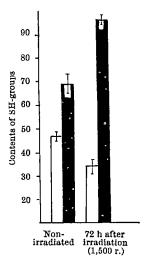


Fig. 6. Content of protein sulphydryl groups in control and chronically irradiated tumours after check-up, irradiation 1,500 r. ☐, Control; ☐, 106th generation.

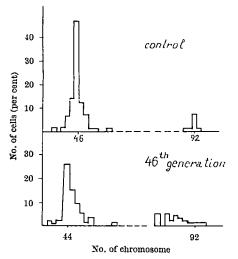


Fig. 7. Chromosomal distribution in chronically irradiated tumour cells.

radioresistant tumour obtained by repeated irradiation; its ploidy was unchanged.

We have also examined by electrophoresis the fractions of soluble proteins in tumour cells. The content of A-globulins was greater in the radioresistant strain than in its initial (control) strain. The content of the A fraction was twice as great in the radioresistant as in the control strain. The content of B-globulins was unaffected. On the third day after irradiation with 1,500 r. the fraction of A-globulins in the radioresistant strain decreased while it increased in the control strain. The content of B-globulins had markedly increased in the radioresistant strain.

The accumulation of B-globulins in the control strain after irradiation seems to be related to an increase in the total protein content of the cells as a result of the inhibition of mitosis, while in the radioresistant strain the decrease of the A-globulin content reflects the almost unaffected mitosis.

The mechanism of the increase of B-globulins in the radioresistant tumour after irradiation may be more complex and may depend in particular on the accumulation of lipoproteids14. Lipoproteids are highly radioresistant8 and take an active part in the restitution processes.

The relationships between the enhanced radioresistance of tumours and chromosome changes are being widely discussed and data are contradictory. Repeated irradiation both in vivo and in vitro of Ehrlich ascites tumours

followed by intraperitoneal inoculation has revealed a continuous increase in cells with low chromosome numbers. while the stem strain shows a trend towards a decrease in chromosome number.

Our control Ehrlich strain is a hyperdiploid with a stem line of cells containing forty-six chromosomes and very few polyploid forms. When repeatedly irradiated in vivo the stem strain contained chiefly cells with forty-four chromosomes beginning from the forty-sixth passage (Fig. 7), while in vitro a similar stem strain was obtained from the thirtieth passage with a dose of 900-1,000 r., and from the eleventh passage with a dose of 2,000 r. In both cases no significant changes in the number of polyploid forms were observed.

A similar tendency to accumulate cells with low chromosome numbers also takes place after repeated irradiation of ascites ovarian tumours in rats. Thus it seems that the characteristic of developing radioresistance is not the polyploidy but an increase in cells with low chromosome numbers.

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MICROBIOLOGY

DNA Homology of Two Mycoplasma **Species**

RECENT work has involved classifying bacteria according to their DNA base composition and homology (for example. ref. 1). This work has used the hybridizing techniques^{2,3} developed for hybridizing RNA with a DNA of high molecular weight which was immobilized in an agar gel. An attempt has also been made to determine whether there is any homology between the DNA of two mycoplasma species, Mycoplasma gallisepticum and Mycoplasma gallinarum, and the DNA of a bacterium Haemophilus gallinarum4. Evidence was presented which demonstrated the reversion and transformation of avian mycoplasmas to H. gallinarum⁵ but no DNA homology was found. Neither could DNA homology be demonstrated between the DNAs isolated from $Streptococcus\ MG$ and Mycoplasmapneumoniae-another pair of organisms which have been suspected of being genetically related -although the two DNAs had similar base compositions. follows is a report of an attempt to determine whether two mycoplasma species, Mycoplasma mycoides variety capri and Mycoplasma laidlawii, are genetically related.

The organisms from the cultures used—the identity and purity of which were confirmed by the appropriate cultural, chemical and serological tests by Dr R. H. Leach (Central Public Health Laboratories, Mycoplasma Reference Laboratory)—were M. mycoides variety capri

P.G. 3 (obtained from Dr D. G. ff. Edward, Wellcome Research Laboratories) and M. laidlawii 545B (obtained from Dr P. Pease, Department of Bacteriology, University of Birmingham). They were grown in the media previously described7,8 and, to obtain DNA labelled with carbon-14, 2^{-14} C-thymidine (20 $\mu c./l.$ of medium) was added. The DNA from the two organisms grown in the non-radioactive and radioactive media was isolated and purified as previously described7.8. The yield (mg/l. of culture medium) and specific activities for the DNA samples isolated were: M. mycoides variety capri DNA, 2.8 mg/l., <0·1 c.p.m./µg; M. mycoides variety capri DNA-14C, 2·7 mg/l., 411 c.p.m./µg; M. laidlawii DNA, 1·1 mg/l., <0·1 c.p.m./µg; M. laidlawii DNA-14C, 1·2 mg/l., 44</p> c.p.m./µg. The base composition of the samples is given in Table 1.

Table 1. BASE CONTENT (MOLES/100 NUCLEOTIDES) OF DNA ISOLATED FROM M. mycoides var. capri AND M. laidlawii

	Adenine	Gua- nine	Cyto- sine	Thy- mine	Guanine + cyto- sine (%)
M. mycoides var. capri DNA	37·8	12·7	12·3	37·2	25·0
M. mycoides var. capri ¹⁴ C-DNA	38·0	12·5	12·7	36·8	25·2
M. laidlawii DNA	33·4	15·8	16·7	34·1	32·5
M. laidlawii ¹⁴ C-DNA	33·5	16·0	16·5	34·0	32·5

The labelled DNA preparations were sheared in an ultrasonic disintegrator (Measuring and Scientific Equipment Limited) until their molecular weights, as determined by sedimentation, were about 300,000. The nonradioactive DNAs were denatured and trapped in agar as described by McCarthy and Bolton³ and the hybridization between this and the denatured labelled DNA was performed according to their method. Hybridization was also attempted between the mycoplasma DNAs and calf thymus DNA in order to check whether any nonspecific hybridization was taking place. Pure Clostridium welchii DNA (ref. 9) was also trapped in agar and hybridization attempted between this and mycoplasma DNAs, because Cl. welchii DNA has a base composition similar to M. laidlawii DNA. The results are given in Table 2.

Table 2. DEGREE OF DNA HOMOLOGY

Radioactively labelled DNA (30 µg)	DNA trapped in agar $(300 \mu g)$	labelled DNA bound (aver- age of two de-	Percentage of labelled DNA bound, relative to homologous hybridization	Percentage of guanine + cytosine of DNA
M. mycoides var. capri P.G. 3	Calf thymus M. mucoides	1.6	5-2	42.0
capit r.c. s	var. capri	31.0	100.0	25.0
	M. laidlawii	5.0	16.2	37.5
	Cl. welchii	2.5	8.1	31.0
	Agar blank	1.2	4.0	-
M. laidlawii 545B	Calf thymus M. mycoides	1.0	2•8	42.0
	var. capri	7.0	18.9	25-0
	M. laidlawii	37·0	100.0	32.5
	Cl. welchii	2.6	7.0	31.0
	Agar blank	0.5	$\mathbf{i} \cdot \mathbf{\check{z}}$	

The results show a limited amount of heterologous hybridization between species of mycoplasma, but several factors have prevented any attempt to show whether this is more than that expected from non-specific hybridization. The DNAs of the mycoplasma species investigated all have extreme adenosine+thymine compositions. Presumably the more extreme the DNA composition, the more non-specific hybridization will be found to occur. The DNAs of the mycoplasma species used here differ in their guanine+cytosine content by 7.5 per cent and thus one would not expect heterologous hybridization to be very high. Sueoka has shown that a difference of 10 per cent in the guanine+cytosine content of the DNAs from two bacterial species indicates that they have few DNA molecules in common because of the unimodal distribution and narrow range of guanine+cytosine content which bacterial DNAs have been shown to possess¹⁰. It was not possible to obtain DNA in sufficient quantity or labelled with carbon-14 from two mycoplasma species which had DNAs with similar base contents. The homologous hybridization of the DNAs used is

rather low. Whether this is because the hybridization had to depend on chiefly adenine-thymine base pairs or whether it is the result of other factors is not known, but it necessarily increases the uncertainty with which the heterologous hybridization results can be interpreted. Moreover, the amount of labelled thymidine which was incorporated into M. laidlawii DNA was not very high but, however, the figures do serve to confirm the trend noticed for the hybridization involving the labelled DNA from M. mycoides variety capri which shows a significant increase for the heterologous hybridization compared with the hybridization obtained with a nonrelated bacterial DNA with a similar base composition.

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Removal of Microbial Pollutants from Waste Effluents by the Redbeard Sponge

RECENT studies1 have shown that the redbeard sponge (Microcionia prolifera Verrill), a common inhabitant of the Atlantic shorelines, is able to use bacteria, notably E. coli, as food organisms. Sponges effectively filter these bacteria out of the sea water and concentrate large quantities of them in their bodies before digestion. The filtering capacity of the sponge was found to depend on the initial concentration of bacteria present in the surroundings, and it was shown that the more bacteria present, the more efficiently the sponge utilizes them. This suggests that the redbeard sponge can be used to combat microbial pollution in estuaries coming from faecal contamination.

In a recent series of papers²⁻⁵, it has been shown that lower fungi like Candida albicans not only survive if exposed to sea water but retain their viability and infectivity to mice almost indefinitely in this environment. This suggests that the seas could be a reservoir for the dissemination of these potentially harmful organ-Because of this, we undertook a study (to be published) to see whether or not redbeard sponges would also use Candida albicans or Cryptococcus neoformans as food organisms, and thus remove these pathogens from the marine environment. These studies indicated that the sponges are able to ingest and utilize these lower fungi in the same fashion as the E. coli in our earlier experiments. Our in vitro studies have shown what seems to be chitinolytic activity in the sponge tissues which effectively lysed the fungal cells. Because all these studies were performed in laboratory conditions, using artificial inoculationsthat is, cultures of bacteria or fungi-before further extrapolations could be carried out as to the effectiveness

of the sponges in the abatement of pollutants, it was necessary to test the effect of some waste effluents known to contain a large microbial population. For this reason, effluents originating from duck farms were selected because they are notorious for their large content of E. coli and other microbes. Our experiments and results on the purification of untreated duck farm wastes by the sponges are presented in this paper.

The localities of collection, the picking of the sponges, the cleaning procedures employed and the maintenance of the animals, were the same as described earlier1. The sponges were manually cleaned of gross contaminating organisms and were kept in glass, non-toxic bonded 10 gallon aquaria containing 19.2 l. of water at constant

temperature (12° C) under constant aeration.

Untreated duck farm effluent was collected at the Carman River Duck Farm, New York, in polyethylene containers. The pH of the effluent was 6.4 at 16°C and its salinity 10 p.p.t. The amount of E. coli was immediately determined. After complete mixing, standard serial dilutions of the duck farm offluent were made using a buffered diluent, and selected dilutions were processed through a 'Millipore' filter apparatus. After filtering, the 0.45 micron filters were placed in 'Millipore' Petri dishes on the surface of sterile absorbent pads soaked with 'M-Endo Broth MF'6. This procedure was repeated for two subsequent trials. All the Petri dishes were incubated at 37°C, and, after 24 h, colonies which exhibited the typical E. coli-like appearance were counted on each set of plates and then averaged to give the number of E. coli cells/ml. of duck farm effluent.

Quantities of the duck farm effluent ranging from 500 to 2,000 cc were added to the aquaria each containing approximately 3.5 lb. wet weight of sponges. Four aquaria without sponges served as controls to test for the inherent bactericidal activity of the sea water7,8. The final concentrations of E. coli in the different aquaria (organisms/cc) are shown in Table 1. The same table

Table 1. VARIABLE PARAMETERS

Variable parameters Tank No.	Organisms per cc initially added	Salinity p.p.t.	<i>p</i> H*
Tank No. 1			
(500 cc DFE added) Tank No. 2	22,530,000	26-0	6.8
(1,000 cc DFE added)	45,060,000	25.5	6.8
Tank No. 3			
(1,500 cc DFE added) Tank No. 4	67,590,000	25.0	6.8
(2,000 cc DFE added)	89,120,000	24.5	6.8
Control No. 1	00,120,000	24.0	0.0
(500 cc DFE added)	22,530,000	26.0	6.8
Control No. 2			
(1,000 cc DFE added)	45,060,000	25.5	8.8
Control No. 3 (1,500 cc DFE added)	67 KAO AAA	25.0	6.8
Control No. 4	67,590,000	20.0	0.9
(2,000 cc DFE added)	89,120,000	24.5	6.8

^{*} As a result of the buffering action of sea water (pH 6-8) the drop in pH caused by the addition of DFE (pH 6-4) is negligible. DFE, Duck farm effluent.

shows the salinity values obtained in terms of electrical conductivity measurements performed after the effluent was added to the aquaria. The changes are proportional to the dilutions. Every 24 h for 6 days samples were taken from the water surrounding the sponges and from the control tanks and E. coli counts were performed as described.

The results of the experiments are compiled in Table 2. It is evident that, as in our original experiments, E. coli originating from the duck wastes multiplied rapidly, but after about 24 h there was a drastic decrease possibly as a result of their uptake and digestion by the sponges. During a period of 5 days the count observed dropped from 174 million to approximately 100/cc. In the control experiments, the decrease averaged three orders of magnitude less than in the aquaria containing sponges. earlier observation that the filtering ability of the sponge depends on the concentration of the available microorganisms was again borne out as indicated by comparing the results obtained from tanks 1 and 4, which showed initial counts of 22 and 90 million/cc, respectively. In tank 4 the disappearance of the E. coli was approximately 1.6 times faster than from tank 1 between days 2 and 4 under the same conditions (except for dilution) and at the same sponge weight. The different salinities in these experiments do not seem to be responsible for the faster death rate of the bacteria in the less saline media because the four control tanks did not exhibit such a change. One may therefore conclude that the value mentioned is indeed a real expression of the ability of the sponges to digest greater numbers of bacteria at a rapid rate if quantities of them are available.

The experimental results obtained by using untreated duck farm effluent as a means of introducing food organisms for the sponges do not differ in any essential feature from those of the earlier published data1. Because duck farm effluent probably represents the most noxious type of household or industrial waste, at least in the sense of its high microbial count, the ability of the sponges effectively to clarify such effluents gives further support to the speculation about their possible use as naturally occurring anti-pollution agents. Although these experiments were conducted in laboratory conditions, the use of raw effluent instead of pure cultures is a further step towards the investigation of the sponges in their natural environment.

The dilution tolerance of these animals seems to be quite high and, indeed, the maximal values were not reached. In view of this, it seems reasonable to state that a relatively small mixing of the sewage with sea water would be required if sponges are to be used for microbial decontamination purposes. Sponges could be grown on dykes in the estuaries where sewage effluents enter. The animals would then possibly filter out the bacterial contaminants and the water passing through their bodies would be free of micro-organisms. In addition, the sponges

Table 2. DETERMINATIONS OF BACTERIAL CONCENTRATIONS

Number of E. coli/cc of water	0	1	2	3	Days 4	5	6	7
Tank No. 1* (500 cc DFE added) Tank No. 2	22,530,000†	97,000,000	2,700,000	46,000	740	480	No count performed	100‡
(1,000 cc DFE added)	45,060,000	120,000,000	6,500,000	97,000	1,160	560	No count performed	No growth
Tank No. 3 (1,500 cc DFE added)	67,590,000	135,000,000	9,300,000	135,000	1,440	680	No count performed	100‡
Tank No. 4 (2,000 cc DFE added)	89,120,000	174,000,000	11,200,000	175,000	1,810	800	No count performed	No growth
Control No. 1 (500 cc DFE added)	22,530,000	117,000,000	93,000,000	40,000,000	23,000,000	5,100,000	No count performed	1.900,000
Control No. 2 (1,000 cc DFE added)	45,060,000	137,000,000	107,000,000	46,000,000	23,000,000	7,500,000	No count performed	2,700,000
('ontrol No. 3 (1,500 cc DFE added)	67,590,000	147,000,000	126,000,000	51,000,000	27,000,000	8,200,000	No count performed	2,700,000
Control No. 4 (2,000 cc DFE added)	89,120,000	189,000,000	139,000,000	57,000,000	31,000,000	10,800,000	No count performed	3,400,000

^{*} Approximately 3.5 lb. wet weight of sponge.
† Organisms in initial dilution are calculated from pure duck farm effluent count (786,000,000/cc).
† Represents one colony grown from a 10-2 dilution.
DFB, Duck farm effluent.

would not only eliminate contaminating microbes from their environment but they would also remove organic matter from the seas. Most of the bacteria entering the estuaries are killed gradually by the toxic effects of sea water and their subsequent decomposition eventually leads to the organic enrichment of the water. If they are taken up by the sponge, however, a high proportion of their material is directly utilized by the animals to build up their own bodies, thus eliminating some of the constituents of the organic load presented by the microbes. These factors indicate the importance of further studies, which should be conducted in actual field conditions to ascertain the long range feasibility of utilizing the redbeard sponge for the control of microbial pollution.

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BIOCHEMISTRY

Differential Response of Protein Synthesis in Ehrlich Ascites Tumour Cells and Normal Thymocytes to 2,4-Dinitrophenol and Oligomycin

RECENT work with intact yeast¹ and bacterial cells² suggested that some step in protein synthesis requires energy in a form similar to or identical with the high energy intermediates of oxidative phosphorylation rather than ATP directly. We present here data which indicate that a similar energy form is used for some phase of protein synthesis by intact mammalian cells, such as the thymic lymphocyte. The same requirement, however, cannot be demonstrated for a highly undifferentiated neoplastic cell -the Ehrlich ascites tumour cell (ATC). This alteration in the way in which metabolic energy is usually coupled to protein synthesis may represent the loss of an important control mechanism for regulating protein synthesis in this tumour cell.

The rationale for the experimental approach used in this study is the same as that described for yeast cells1. This can be summarized as follows: if uncouplers (2,4dinitrophenol) or blockers (oligomycin) of oxidative phosphorylation inhibit protein synthesis without affecting the intracellular concentration of ATP in anaerobic conditions when ATP is derived exclusively from glycolysis, some step in protein synthesis must require a high energy form similar to or identical with the high energy intermediates of oxidative phosphorylation.

Ehrlich tetraploid ascites tumour cells given by Dr H. G. Hempling, maintained by weekly intraperitoneal inoculations of 0.2 ml. of a 1:5 dilution of ascitic fluid, were collected 7-10 days after inoculation, and isolated by

centrifugations four or five times at 400 g for 3 min in a wash medium (0.01 molar tris, 0.004 molar potassium phosphate, 0.14 molar sodium chloride, 0.02 molar potassium chloride, pH 7.4) to remove debris and erythrocytes. The cell density was adjusted to give a final concentration of 3 mg of protein/ml. of incubation medium. Thymic lymphocytes were prepared as detailed by Eisen et al.3 from 50-70 g male Wistar rats; cells were diluted to give a final concentration of 0.2 mg of protein/ml. of incubation medium.

Anaerobic incubations were performed by preincubating cells at 37° C with glucose in a 95 per cent nitrogen-5 per cent carbon dioxide atmosphere for 15-30 min before the addition of the inhibitors and isotopically labelled substrates. The incubation medium consisted of 0.04 molar tris, 4 mmolar potassium phosphate, 0.088 molar sodium chloride, 0.02 molar potassium chloride, 2 mmolar magnesium chloride, 2.6 mmolar sodium bicarbonate and 0.66 mmolar glutamine, pH 7.4. The rate of incorporation of ¹⁴C-leucine into cell protein was taken as a measure of protein synthesis. Samples were prepared for counting by adding 0.2-0.3 ml. volumes of the cell suspensions taken at various time intervals to 2-3 ml. of 10 per cent trichloroacetic acid (TCA), filtering on membrane filters with a pore size of 0.45μ, washing three times with 5 per cent TCA, then three times with ether. Isotopic counting was carried out in a Nuclear-Chicago gas-flow counter or a Packard liquid scintillation counter. Rates of incorporation were calculated from the slope of curves of the c.p.m./mg of protein versus time. Protein was determined by the technique of Lowry et al.4 using bovine plasma albumin as standard. ATP was measured fluorometrically as described by Lowry et al.5 on perchloric acid extracts of cell suspensions.

No 14C-leucine was incorporated into protein by either ATC or thymic lymphocytes incubated anaerobically without glucose and there was an increase of about threefold in lactate production in the presence of glucose, thus confirming the completeness of anaerobiosis in this system. In agreement with the original report of Rabinowitze, ATC incubated anaerobically with glucose incorporated ¹⁴C-leucine into protein at 40-60 per cent of the rate in aerobic conditions.

Table 1. Effects of 2,4-dinitrophenol and oligomycin on incorporation of $^{14}\mathrm{C}\text{-LBUCINE}$ into protein and on concentrations of atp in THYMIC LYMPHOCYTES

	incorporation of ¹⁴ C-leucine (c.p.m./ mg of protein × min ⁻¹)	(mμmoles/mg of protein)
Control	6.310	13.32 ± 1.03
2.4-DNP (10-6 molar)	3,170	9.57 ± 3.13
Oligomycin (0.025 µg/mg of protein	3,470	11.50 ± 1.58
2,4-DNP (10-6 molar) + oligomycin (0-025 ug/mg of protein)	1.980	$10 \cdot 10 \pm 2 \cdot 33$

¹⁴C-Leucine was added at 0-667 µc/ml. and had a specific activity of 275 mc./mmole. Five samples were taken in duplicate during 30 min of incubation, and from these rates of incorporation were determined. Experiments were performed anaerobically in the presence of 15 mmolar glucose. Concentrations of ATP were measured in triplicate at 20 min and represent the mean attended deviction. tstandard deviation.

In lymphocytes, 2,4-DNP (10-6 molar) inhibited the anaerobic incorporation of 14C-leucine into protein by about 50 per cent and oligomycin (0.025 μg/mg of protein) impaired protein synthesis by 50 per cent (Table 1). The two agents together caused a multiple effect as if acting on subsequent steps. This would be expected from their actions on ATPase systems and supports the concept that ATP must be changed into some other high energy form before use. The same concentration of oligomycin added to cells incubated aerobically without glucose completely blocked protein synthesis, suggesting interference with mitochondrial ATP production and subsequent inhibition of all extra-mitochondrial energy de-Our studies of isolated fat cells pendent reactions. (unpublished work) confirm these observations on lymphocytes. In contrast to the results noted with thymocytes and fat cells, neither 2,4-DNP (2×10^{-4} molar) nor oligo-

Table 2. Refects of 2,4-dinitrophenol and oligomycin on incorporation of 14 c-leuginh into protein of ehrlich ascites tumour cells

Experiment	; Inhibitor	Incorporation of Control (c.p.m./mg of p	Inhibitor
$\begin{smallmatrix}1\\2\\3\end{smallmatrix}$	2,4-DNP (10^{-4} molar)	1,955	1,922
	2,4-DNP (2×10^{-4} molar)	2,180	1,980
	Oligomyein ($3 \mu g/ml$.)	2,500	2,500

Cells were preincubated in an atmosphere of nitrogen with 15 mmolar glucose as described in the text. The 14 C-leucine was added at 0.3 μ c./ml. and had a specific activity of 4.68 mc./mmole. Five samples were taken during 30 min of incubation, and from these rates of incorporation were calculated.

mycin (3 µg/mg) had any detectable effect on incorporation of 14C-leucine into protein by ATC in anaerobic conditions in the presence of glucose (Table 2).

The data concerning concentrations of ATP in the two cellular systems indicate that the effect of 2,4-DNP and oligomycin on protein synthesis in lymphocytes is not secondary to alterations in concentrations of ATP (Tables 1 and 3). Although oligomycin and 2,4-DNP affect concentrations of ATP to about the same extent in both cellular systems, protein synthesis was affected only in the lymphocytes. The degree of inhibition of this protein synthesis bore no relation to the alterations in the concentration of ATP. Furthermore, there was no additive effect of the two agents on ATP in lymphocytes although a multiple inhibition on protein synthesis was found. Aerobic concentrations of ATP in both the ATC and lymphocytes were in the same range as in anaerobic conditions, showing that glycolysis could maintain normal concentrations of ATP. The ATP data from ATC agree with those of Hempling' on the same cells.

Table 3. EFFECTS OF 2,4-DINITROPHENOL AND OLIGOMYOIN ON CONCENTRATIONS OF ATP IN EHRLICH ASCITES TUMOUR ORLLS

•	mµmoles/mg of prot
Control	34.30 ± 1.07
Oligomycin (3 µg/mg)	$27 \cdot 15 \pm 2 \cdot 11$
2.4-DNP (10-4 molar)	23.20 ± 5.32

Experimental conditions were as in Table 2. ATP was measured at 30 min and represents the mean value of four samples ± standard deviation.

All metabolic processes in mitochondria known to use high energy intermediates of oxidative phosphorylation as energy sources seem to be membrane oriented events; these include reversal of electron transport⁸, ion transport⁹ and protein synthesis10. The conclusion that high energy intermediates of oxidative phosphorylation are involved in these systems is based on the inhibitory effects noted with such agents as 2,4-DNP and oligomycin. Recently, it has been shown that 2,4-DNP and oligomycin sensitive ATPases are not restricted to the mitochondrial membrane but are also present in both plasma¹¹ and microsomal membranes12.

In view of these considerations, it is tempting to interpret the original studies in yeast1 and bacterial cells2 and this study on lymphocytes and fat cells as an indication that some step in protein synthesis in the intact cell is coupled to an ATPase system which is membrane localized and sensitive to DNP and oligomycin. This system functions by converting ATP to another high energy form before use in this step. The lack of effect of these inhibitors on protein synthesis in the ascites tumour cell suggests either a loss or alteration of this step which is dependent on a high energy intermediate in this neoplastic cell. defect is unlikely to reside in an alteration of the membrane localized ATPase system, for Hempling' has shown that sodium and potassium transport in this ascites tumour cell can be inhibited by both DNP and oligomycin in conditions when ATP is supplied exclusively by glycolysis. Rather, it seems that the protein synthetic process itself has been modified so that it can use ATP directly and is no longer dependent on the membrane localized ATPase system. Consistent with this interpretation are the observations of Barbieri¹³ that in normal liver incorporation of amino-acids by membrane bound ribosomes was much greater than that of free ribosomes, whereas in a hepatoma strain the reverse was observed.

One of us (L. J.) is a John and Mary R. Markle Scholar in Medical Sciences.

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Effects of Various Substances on DNA Synthesis in Guinea-pig Skin in vitro

In studies of healing and cell replication in the epidermis. attention has been directed chiefly towards mitosis and the factors controlling it1-9. In the guinea-pig, however, the earliest evidence of an injury response is an increase in the number of cells synthesizing DNA, which can be detected as soon as 4 h after injury¹⁰. Peaks of mitotic activity occur later (24-48 h) and injury seems to impose a temporary synchrony on cell division in the epidermis. so that successive waves of DNA synthesis followed by mitoses take place. Although small peaks of mitotic activity were recorded at about 18 h, Sullivan and Epstein¹¹ showed that the main burst of mitotic activity in humans occurred between 42-60 h.

Bullough⁴⁻⁶ has suggested that the injury response is mediated through a tissue specific antimitotic "chalone" which is neutralized by injury. There is no evidence. however, that this tissue extract is inhibitory to DNA synthesis; and on the other hand, Hell (unpublished observation) has produced evidence of a tissue extract which will stimulate cells to synthesize DNA. In view of the time relations of DNA synthesis and mitosis, it would seem more important to study the former. Accordingly, a range of substances was tested in vitro for their ability to affect the proportion of cells synthesizing DNA. The method used was that of Hell and Cruickshank¹⁰. injured epidermal slices were cut free-hand from the dorsum of the guinea-pig ear and floated on a culture medium (horse serum 40 per cent, Hanks BSS 60 per cent) containing tritiated thymidine. The substance to be tested was added to some cultures and an equal volume of SNS was added to the controls. After incubation for 4 h the skin slices were fixed and sectioned and autoradiographs were prepared according to the method of Pelc12. Two thousand cells were counted in each sample and the percentage of those engaged in DNA synthesis was recorded in the slices with and without additives. The substances were chosen because they were known to be released during injury, or because they were known stimulators or inhibitors of mitosis.

Of the substances tested, no effects were observed with insulin (0.5 mg/ml.), histamine (0.5 mg/ml.) or adrenaline (0.1 mg/ml.). It was found, however, that serotonin (5-hydroxytryptamine) had a distinct stimulating effect

Table 1. SEROTONIN (5-HYDROXYTRYPTAMINE)

Exp. No.	Control Mean % positive	Ser	5 mg/ml. otonin % positive Mean difference	Ser	mg/ml. otonin % positive Mean difference
$\frac{1}{2}$	5·0 2·9	$\frac{7 \cdot 2}{3 \cdot 7}$	$^{+2\cdot2}_{+0\cdot7}$	8.0	+3.0
$\frac{2}{3}$	7·1 3·8	9·4 4·3	+2·3 +0·5	10.9	+3.8
4 5 6	6·9 4·5	10·0 7·7	+3·1 +3·2	9.3	+2-4
7 Averages	5·9 5·1	$8.3 \\ 7.2$	$+2.4$ 2.1 ± 0.38	7∙9 8∙5	$^{+2.0}_{2.8\pm0.39}$

For 0.15 mg/ml. t=5.5, P>0.01 (significant). For 0.3 mg/ml. t=7.2, P>0.01 (significant).

on DNA synthesis (Table 1). The magnitude of this effect after incubation for 4 h was only slightly less than that shown¹⁰ to be induced by injury in vivo.

Thus there is evidence that the inhibition of mitosis by adrenaline reported by Bullough³ is not accompanied by an inhibition of DNA synthesis. Similarly the colcemid effect is reflected in scarcely any interference in DNA synthesis13.

Although serotonin has a marked effect on DNA synthesis in vitro, it would be unwise to conclude that it is a significant factor involved in the increased DNA synthesis after wounding in vitro. It is possible, however, that further work on the control of the initiation of DNA synthesis might be more rewarding than the study of mitosis in the investigation of wound healing.

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Metabolic DNA in Heart and Skeletal Muscle and in the Intestine of Mice

STUDIES of the incorporation of labelled thymidine into the DNA of heart muscle and skeletal muscle in adult animals have shown significant values which indicate regular renewal of some or all of the DNA in these non-dividing organs^{1,2}. Autoradiography has shown labelled muscle nuclei in smooth muscle and in heart muscle and therefore subsidiary cells, such as lymphocytes, cannot be held responsible for the labelling. The existence in plant materials of two separable fractions of DNA with different rates of turnover has been shown by Sampson et al.3, and Sampson and Davies have presented good evidence for the location of a fraction (DNA1) with a high rate of turnover in differentiating cells and of a stable fraction (DNA2) in meristematic cells of Vicia faba. The results of experiments designed to compare the specific activities of the DNA fractions with a molecular weight below 4×10^6 with those above 4×10^6 in heart, skeletal muscle and intestine are reported here.

Adult male mice of strain BALB/c (25-30 g) were injected intravenously with 60 µc. of tritiated thymidine

(specific activity 17 c./mmole, obtained from CEN, Mol). The mice were killed by cervical dislocation 12, 24 and 48 h after injection and the organs—heart, muscle and intestine-were dissected and frozen in dry ice.

The DNA was extracted from the different organs by a method previously described⁵. After homogenization in a saline solution of EDTA in an 'Ultraturax' blender and the addition of sodium lauryl sulphate, the extract was digested with pronase. The nucleic acids were precipitated with alcohol and redissolved in saline EDTA. The solution was treated with RNase and then with pronase. The DNA was precipitated with alcohol and redissolved in saline citrate.

About 500 µg of DNA obtained from different organs was fractionated by chromatography on DEAE cellulose paper⁵. Using discontinuous elution, fractions containing DNA molecules of different molecular weights were recovered. It has been shown that degradation of DNA is not the cause of the appearance of fractions with a low molecular weight. The amount of DNA per fraction was estimated by ultraviolet spectrophotometry, and the radioactivity of the different fractions was estimated on a Packard liquid scintillation counter.

Comparison of the results from the fraction with a molecular weight between 5×10^5 and 4×10^6 (I) with that of a molecular weight above 4×10^6 (II) is shown in Table 1. The specific activity of the molecules of the first group is obviously higher than that of the second group in the three organs. This observation proves again⁶ that the fractions of lower polymers are not the products of degradation of the higher polymer fractions. The (adenine+thymine)/(guanine+cytosine) ratio in mammals is 1.4:1. If the low polymer DNA (I) had an unusually high ratio, say 5:1, the specific activity of fraction I would be only 1.6 times that of fraction II. It can be concluded that differences in the (adenine+ thymine)/(guanine+cytosine) ratios cannot explain our findings, although a correction may be necessary.

Table 1. Specific activity of heart, muscle and intestinal dna after injection of tritiated thymidine

	I	II	I/II
		Adult mice	
Heart	5∙6	0-4	14
Muscle	16-0	1.1	14.6
Intestine	55-7	12.1	4.6
		Young mice	
Heart	6.5	1.2	5.4
Muscle	15.7	2.0	7.8
Intestine	61.5	19.0	3.2

Intestine of 5 19 19 19 3.2 I. Specific activity (d.p.m. $\times 10^{-4}/\mu g$ DNA) of molecular weight of DNA $5\times 10^{4}-4\times 10^{4}$, fractions 4-7. II: Specific activity (d.p.m. $\times 10^{-3}/\mu g$ DNA) of molecular weight of DNA above 4 × 10°, fractions 9-12. I/II: ratio of the specific activities of fractions 4-7 and 9-12. The results for adult mice at various times after injection were pooled; young mice (18-20 days old) were killed 24 h after injection. Taking the amount of DNA in fractions 4-12 as 100 per cent, the metabolic DNA (I) represents 43 per cent in heart, 30 per cent in skeletal muscle and 36 per cent in intestine.

The ratio of the specific activities (I/II) is 14:1 for muscle and 4.6: 1 for intestine. If all labelling of DNA in the intestine were the result of synthesis in preparation for mitosis, a ratio of 1:1 would be expected. The observed ratio of 4.6: I suggests that DNA turns over in some cells in this organ. Disregarding a possible correction for differences in the (adenine + thymine)/(guanine + cytosine) ratios, it would have to be assumed that of the total labelling with tritiated thymidine in the intestine about 25 per cent represents labelling of DNA in cells which are not proceeding to mitosis and 75 per cent is the result of pre-mitotic DNA synthesis. Accurate determinations of the relative number of labelled crypt cells and labelled non-mitotic cells are not available, but preliminary counts do not disagree with this estimate. Up to 6 per cent of the cells are labelled in the lamina propria of the small intestine, where divisions are not seen; in addition labelled nuclei can be found in the smooth muscle surrounding the organ.

The specific activity in the low molecular weight fraction (I in Table 1) is very similar for adult and young mice, while that for fraction II is considerably higher for young animals, which implies some proliferation in the

muscles of young mice.

The high specific activity in the low molecular weight fraction, especially in the non-dividing muscles, can be explained by the assumption that metabolic DNA exists as a separate fraction in mammalian cells and that the average molecular weight of this fraction is relatively Metabolic DNA represents 36-43 per cent of the total DNA scored in our experiments (Table 1). present it is not known whether metabolic DNA is necessarily and permanently in the low polymer form. The evidence for metabolic DNA in mammalian tissues presented here is in accord with that for plant tissues by Sampson et al.3 and by Sampson and Davies4.

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Effect of Cholesterol on the Water Permeability of Thin Lipid Membranes

In order to assess the relative importance of the various membrane constituents in determining the water permeability of thin (<100 Å) lipid membranes^{1,2}, have conducted a series of experiments in which the concentrations of the individual components, particularly cholesterol, in the membrane forming solutions have been varied. The osmotic permeability coefficients, P_f , of membranes formed from the solutions given in Table 1 were determined as described before3.

Table 1. COMPOSITIONS OF MEMBRANE FORMING SOLUTIONS Amounts of lecithin and cholesterol in 1 ml. of solvent with tetradecane* mg of lecithin mg of cholesterol Cholesterol: lecithin #

		(molar ratio
20	0	0:1
20	10	1:1
20	20	2:1
20	40	4:1
20	80	8:1

Amounts of mixed lipids and cholesterol in 1 ml. of solvent with tetradecane* mg of mixed lipids mg of cholesterol Cholesterol: mixed lipidst (molar ratio)

Amounts of mixed lipids and cholesterol in 1 ml. of solvent with tocopherol † Cholesterol: mixed lipidsi (molar ratio) mg of mixed lipids mg of cholesterol

		(money	۰
40	0	0:1	
40	10	1:2	
40	20	1:1	

^{*} The solvent was tetradecane: chloroform: methanol in the volume proportions 18:48:32,
† The solvent was chloroform: methanol in the volume proportions 2:1,
with 200 mg of tocopherol added per ml. of chloroform methanol.
‡ Average molecular weight of lipids was taken as twice that of cholesterol.

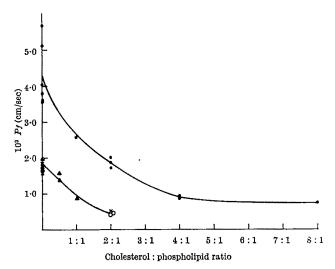


Fig. 1. P_f as a function of molar ratio of cholesterol: phospholipid. All films were formed at $36^{\circ} \pm 0.01^{\circ}$ C by the technique of Mueller et $al.^{1-2}$ in solutions of either unbuffered 100 mmolar sodium chloride or of 100 mmolar sodium chloride + 0.2 mmolar magnesium sulphate + 5 mmolar histidine (pH = 7). After the membrane had become completely black, the concentration of sodium chloride was increased on one side and the rate of water movement was determined. The "mixed lipid" was extracted from ox brain white matter by the method of Mueller et al.' twice split with water to remove protein, and passed through a "Unisil" column to remove cholesterol. • Lecithin membranes formed with tetradecane as the additive; \times , "Mixed lipid" membranes formed with tetradecane as the additive; \wedge , "Mixed lipid" membranes formed with ergosterol as the additive; \wedge , "Mixed lipid" membranes formed with ergosterol instead of cholesterol. (Both tetradecane and dl-atocopherol were present as additives.)

Fig. 1 shows the values of P_f , obtained in independent experiments, as a function of the molar ratios of cholesterol to phospholipid in the membrane forming solutions. The phospholipids were egg lecithin or "mixed lipid" from ox-brain white matter. We found that (a) for both types of membranes the water permeability decreases with relative cholesterol concentration, in lecithin films falling from $4\cdot 2\times 10^{-3}$ cm/sec without cholesterol to $0\cdot 75\times 10^{-3}$ with 8:1 cholesterol: phospholipid, and in "mixed lipid" films, falling from $1\cdot 8\times 10^{-3}$ to $0\cdot 49\times 10^{-3}$ with 2:1 cholesterol: phospholipid; (b) at any given cholesterol concentration, P_f of legithin membranes is consistently larger than P_f of "mixed lipid" membranes (c) P_f is not significantly dependent on the additive (that is tetradecane or dl-atocopherol) used.

We have succeeded in forming several membranes from solutions of the egg lecithin sample after it had been completely hydrogenated. P_f was 1.7×10^{-3} cm/sec, which is approximately 2.5 times less than that of membranes formed from the original partially unsaturated sample.

The knowledge that cholesterol lowers the water permeability of thin lipid membranes is relevant to understanding the mechanism of water transport across them. Hanai and Haydon⁴ have pointed out that the size of P_f is consistent with a simple solubility-diffusion mechanism; that is, at each interphase water partitions (in proportion to its mole fraction in the aqueous phases) into the hydrocarbon region of the membrane, and diffuses through the hydrocarbon region under the concentration gradient thus If this mechanism is operative, then P_f should be sensitive to changes either in the solubility of water or the diffusion coefficient of water in the membrane.

To see whether these factors can reasonably explain the cholesterol effect, we have conducted experiments on bulk hydrocarbons. Assuming that the phospholipids, through their polar groups, and cholesterol, through its hydroxyl group, are anchored at or near the water interface, we have chosen as a model for the hydrocarbon region of the membrane either hexadecane or 1-hexadecene for the phospholipid contribution and cholestane for the cholesterol contribution. The viscosity of a cholestane: 1-hexadecone solution of molar ratio of 1:3 (corresponding in our membranes to a cholesterol: phospholipid ratio of 2:3) was found to be twice that of 1-hexadecene alone. Thus the effect of cholesterol on water permeability can be attributed to an increase in the viscosity of the hydrocarbon region, which produces a proportional decrease in the diffusion coefficient of water within this phase.

It might be possible that chloroform or methanol trapped in the film controls the water permeability, and that cholesterol somehow reduces their concentrations. We have, however, obtained comparable results with membranes formed from mixtures of lecithin and cholesterol dissolved in decane (no chloroform : methanol present), so that this possibility seems to be excluded. It might also be suggested that cholesterol decreases the water permeability by significantly increasing the membrane thickness. Hanai et al. have shown, however, that the electrical capacitance of these membranes increases when cholesterol is added, which suggests a decrease in membrane thickness. In any case, barring tremendous effects of cholesterol on the dielectric constant, this observation indicates no significant increase in thickness.

In addition, we have compared the viscosities of hexadecane and 1-hexadecene, and the solubility of water in these two hydrocarbons. The viscosity of hexadecane is 12 per cent greater than that of hexadecene, while the water solubility is 15 per cent smaller. (Solubilities were determined by the static equilibration method using tritiated water. We checked our method by measuring the solubility of water in benzene and tetradecane; our values agreed with those of Schatzberg'.) Both effects suggest a smaller water permeability of a saturated lipid membrane relative to that of an unsaturated one, and this was observed. More relevant would be comparable data for 8-hexadecene cis, the double bond of which is centrally positioned as are the double bonds in the phospholipids, but this material is unavailable.

It is a priori both hazardous and naive to attempt quantitative comparisons between the properties of bulk hydrocarbon and those of films less than 100 Å thick. The proportion of cholesterol in the films may be quite different from that in the membrane-forming solutions; furthermore, the meaning of "viscosity" in a 100 Å film is not obvious. Nevertheless, the parallel between the properties of the bulk hydrocarbon and the thin membrane is very suggestive. With this solubility diffusion model, the polar ends of the molecules constituting the membrane play no part in determining the value of P_f . The differences we have found between "mixed lipid" films and lecithin films may argue against this, but until comparisons are made between phospholipids with different polar heads but identical hydrocarbon tails, no conclusion can be definitely drawn.

That the water permeability of thin lipid membranes is dependent on cholesterol content may be of physiological relevance. The ubiquity of cholesterol or related sterols (we have obtained a similar decrease in P_f with ergosterol (see Fig. 1)) in plasma membranes of all organisms other than bacteria and its scarcity in some intracellular membranes is well known, but the physiological function of cholesterol in membranes is not understood. It would be interesting to know if there is any correlation between the values of P_f of biological membranes and their cholesterol content; such data are still unavailable. We note, however, that the mucosal membrane of toad bladder epithelial cells is much less permeable to water than the serosal membrane⁸ and that 'Amphotericin B', a polyene antibiotic that seems to interact specifically with sterol containing membranes, affects the mucosal membrane but not the serosal10.

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Proton Conductors in the Respiratory Chain and Artificial Membranes

Many chemical compounds can uncouple electron transfer from energy accumulation in the respiratory chain. Uncouplers stimulate electron transfer in conditions in which the rate of oxidation is limited by coupled phosphorylation. The precise mechanism of uncoupling is obscure.

We have studied the action of uncoupling agents using mitochondria, reconstituted succinate oxidase, artificial phospholipid membranes and solutions of phospholipid in heptane. Rat liver mitochondria were separated in a solution of sucrose 0.25 molar and 0.001 molar EDTA and incubated in a mixture containing 0.25 molar sucrose, 0.02 molar potassium chloride, 0.01 molar potassium phosphate, 0.005 molar magnesium chloride, 0.03 molar tris buffer and 0.002 molar EDTA, pH 7.5. Succinate oxidase was reconstituted from cytochrome c and purified enzyme complexes: succinate-cytochrome c-oxidoreductase and cytochrome c-oxidase. Respiration was measured polarographically, and reduction of NAD and cytochromes by fluorimetry and differential spectrophotometry, respectively.

To prepare artificial bimolecular membranes a solution of brain phospholipid in heptane (10 mg/ml.) was used. The membrane was formed in a hole (diameter of 1-1.5 mm) of a small 'Teflon' vessel with a double wall. The space between the walls was filled with the solution of phospholipid. As a water phase we used the same mixture which was used for the incubation of mitochondria.

Experiments with biological systems gave the following results. (a) All uncouplers, regardless of differences in their chemical structure, have a qualitatively identical effect on mitochondrial respiration in the absence of phosphate acceptor; an increase in the concentration of uncoupler causes the respiration first to be activated and then inhibited. Inhibition was accompanied by the oxidation of NADH and cytochromes^{3,4}. Uncoupling activities are in the following order: p-trifluoromethoxy-carbonyleyanidephenylhydrazone (FCCP) = M-chlorocarbonylcyanidephenylhydrazone (CICCP) ≥ tetrachloro-2-trifluoromethylbenzimidazol (TFB) > dicumarol > 2,4dinitrophenol (DNP) = β -hydroxynaphthoic acid > salicylic acid. (b) The most active uncouplers (CICCP, FCCP, TFB) can stimulate mitochondrial respiration and also inhibit respiration of mitochondria as well as of reconstituted succinate oxidase in amounts equal to or smaller than those of the respiratory enzymes contained in the sample. (c) Inhibition of respiration by uncouplers is competitive with respect to the concentration of oxidation substrate4,5. The affinity of succinate dehydrogenase for the most active uncouplers was found to be many times greater than its affinity for the substrate. Thus the affinity of the reconstructed succinate oxidase system for TFB should be

Table 1. CORRELATION OF THE EFFECTS OF UNCOUPLERS ON MITOCHONDRIAL RESPIRATION AND ON ELECTRIC CONDUCTIVITY OF THE ARTIFICIAL PHOSPHO-LIPID MEMBRANE

Uncouplers	$(C_{1,2})_a$	g at $(C_{1/2})_a$	$(C_{1,2})_i$	g at $(C_{1:1})_i$
DNP	2×10 ⁻⁵ molar	2	5×10^{-4} molar	10
TFB	10 ⁻⁷	2	1.5×10^{-8} ,,	30
FCCP	8×10 ⁻⁸ ,,	3	2.5×10^{-8} ,,	30

 $(C_{1:3})_a$ and $(C_{1:3})_i$ are the concentrations of the uncoupler which cause half maximal activation and half maximal inhibition of mitochondrial respiration, respectively (the oxygen consumption in state 2 was subtracted). Substrate was 0-01 molar succinate. g, Electroconductivity of artificial phospholipid membrane in the presence of the corresponding concentration of the uncoupler; the electroconductivity of the membrane without uncoupler $(3 \times 10^{-3} \, \text{mho} \times \text{cm}^3)$ was taken as a unit.

105-106 times greater than that for succinate. Inhibition by TFB is easily reversible: addition of excess succinate to succinate oxidase after TFB stimulates respiration to the same extent as succinate added before TFB.

These data demonstrate considerable differences between uncouplers and other enzyme inhibitors. The latter: (a) are very specific in relation to their structure; (b) act in quantities not less than those of the enzymes; (c) usually have less affinity for the enzymes than does the substrate, if inhibition is reversible and competitive.

We have investigated possible causes of the unusual action of the uncouplers in model systems. In experiments with artificial bimolecular phospholipid membranes, we confirmed the data of Bielawski, Tompson and Lehninger⁶ regarding the decrease of the electric resistance of the membrane after addition of DNP. Other uncouplers have a similar effect. The effectiveness of the compounds studied in the membrane systems correlates with their effects on mitochondria (Table 1). In natural as well as in artificial systems the strongest uncouplers (FCCP, CICCP, TFB) were four orders of magnitude more active than the weakest one (salicylate); DNP occupies an intermediate position.

The increase in electric conductivity of the membrane caused by uncouplers was a result of the increase in the selective permeability of the membrane to hydrogen ions.

Experiments with solutions of the uncouplers in heptane showed that uncoupling agents (UH) may dissociate by the equation $UH \rightleftharpoons U^- + H^+$ in a hydrophobic system, if phospholipid is added. This is indicated by the characteristic changes in the absorption spectra of uncoupler in heptane after addition of phospholipid (DNP and TFB are studied). The hydrogen ions provided by uncoupler in heptane medium can protonate the nucleophilic groups there. This effect is easily demonstrated by the red staining of the heptane layer after the addition of the uncoupler to the mixture of heptane, phospholipid, methyl red and water. The data of the physical and chemical experiments suggest that the actions of the uncouplers in the biological system are a result of the provision of protons to the hydrophobic part of the enzyme structure.

Considering the uncouplers not as enzyme poisons but as catalysts for hydrogen ion transfer in the hydrophobic medium, the unusual characteristics of the uncouplers can easily be explained. The non-specificity of the chemical structure of the uncouplers is quite understandable if, in the system UH ⇌ U- + H+, the H+ ions are an active part, and U functions chiefly as a mobile proton carrier in the hydrophobic medium.

It can be shown that substitution of the only hydrogen atom in the TFB molecule with a methyl group prevents uncoupling activity (see ref. 7) and decreases the inhibitory action of this substance on respiration. Dinitrobenzene neither uncouples oxidation in mitochondria nor increases electroconductivity in the artificial membrane system.

The ratio [uncoupler] < [enzyme] is also explained by the catalytic character of the action of the uncoupling agents. The stoichiometry [H+]: [enzyme] > 1 is always fulfilled in the experimental conditions because of the buffer capacity provided by the components of the incubation mixture. It is also clear that the unusually high affinity of the enzyme for the uncoupler may only be apparent if the function of the uncoupler is to provide H+. In this case,

it is not necessary for the active centre of the enzyme to contact with UH or U-.

The mechanism of uncouplers as proton donors was first considered by Mitchell's. Mitchell's chemiosmotic concept of coupling predicts a correlation between the respiratory activation effect of the uncoupler and its effect on proton conductivity of the membrane. According to Mitchell's scheme the lipid soluble proton carrier decreases the membrane potential, which is supported by the respiratory chain and is necessary for energy transformation8,9

Another explanation of the uncoupling effect of hydrogen ions10 could be that H+ competes for nucleophilic intermediate $(X^{\delta^{-}})$ in coupling mechanisms with the electrophilic energy carrier $(Y^{\delta^{+}})$. H+ ions might also cause acid hydrolysis of the primary high energy compound $X \sim Y$.

Competitive inhibition of purified dehydrogenases by the uncoupler may be explained by protonation of enzyme groups responsible for attachment to the substrate. In the absence of uncoupler these groups are protected from H+ ions of water by the hydrophobic parts of enzyme or bound phospholipid. Protonation of the dehydrogenase functional groups can be assumed to demand the greater concentration of H+ ions in the hydrophobic medium than protonation of $X^{\delta-}$ (or $X \sim Y$). The uncoupling therefore precedes inhibition of respiration in the course of increasing the concentration of the proton donors. It is interesting that an uncoupler inhibits mitochondrial respiration and reconstituted succinate oxidase respiration as well. The second system differs from the first in lacking the organized membrane structures although it contains phospholipids. Thus the effect of uncouplers as proton conductors is not limited by their action on the membrane.

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Optical Clearing Effect of Dextran on Human Platelet Suspensions

THE optical density of platelet-rich citrated plasma or of platelet suspensions has been seen to decrease irreversibly on the addition of a solution of dextran1. This decrease is biphasic, consisting of an immediate optical clearing effect independent of the molecular weight of the dextran fraction but related to its final concentration, and a delayed effect affected by molecular weight². Because certain dextran fractions induce aggregation of human platelets¹, it was at first assumed that the decrease in optical density of platelet-rich citrated plasma was caused by aggregation¹. Only the delayed changes in optical density, however, seemed to correspond to the degree of platelet aggregation evaluated microscopically after the addition of dextran². The immediate optical clearing effect of dextran has been further examined with a turbidimetric method and re-interpreted in the light of additional work on the optical properties of dextran.

Platelet-rich citrated plasma was prepared as previously described. Platelet suspensions in saline-trishydrochloride were made according to Haslam's method. Platelet aggregation was graded microscopically according to the scheme proposed earlier, and the glassware used was siliconized. The optical density of platelet-rich citrated plasma and platelet suspensions was measured with an EEL titrator using a red (608) filter. Twenty per cent solutions of dextrans of various mean molecular weights (10,000–500,000) were prepared in 0.9 per cent sodium chloride. Refractive indices of solutions of dextran, human albumin and polyvinyl pyrrolidone in 0.9 per cent sodium chloride were measured for a range of concentrations from 0.5 to 20 per cent with an Abbé refractometer.

Solutions of dextran were added to 2 ml. samples of platelet-rich citrated plasma to give a final concentration of 1.8 per cent, and the change in optical density was recorded. Three samples from each cuvette were examined microscopically without delay by an observer unaware of the additive or of the change in optical density, and the degree of aggregation was graded. Despite approximately equal turbidimetric changes with various fractions, the microscopic findings showed pronounced differences. Using similar methods it was found that several antagonists of platelet clumping inhibited aggregation by dextran in relatively low concentrations but had no effect on the immediate reduction in optical density. Thus the optical clearing effect of a material known to aggregate human platelets could not be ascribed entirely to aggregation. Furthermore, human albumin showed a similar optical effect on platelet-rich citrated plasma and platelet suspensions, yet we found no microscopic evidence of aggregation by albumin even in high concentrations.

It is known that changes in optical density of suspensions can also occur from alterations in optical properties at phase boundaries. The quantitative relationship between turbidity and refractive index (the Rayleigh–Gans equation)⁵ as applied to a platelet suspension is as follows:

$$\frac{\tau}{c} = \frac{4\pi\alpha^3}{\lambda} \cdot \frac{d_{12}}{d_2} \left(\frac{m^2-1}{m^2+2}\right)^2 \times 10^{-2}$$

where τ is turbidity; c is "concentration" of platelets; α is $\pi D/\lambda$, a parameter of D, the diameter of the platelets; λ is wavelength of the incident light; d_{12} is the density of the suspension with the additive and d_2 the density of the suspension without the additive; m is μ/μ_0 where μ is the refractive index of the platelets and μ_0 that of the medium. The wavelength of the incident light, λ , was constant. Thus,

$$\frac{\tau}{c} = K' \frac{d_{12}}{d_2} \left(\frac{m^2 - 1}{m^2 + 2} \right)^2$$

and because the ratio d_{12}/d_2 will not change appreciably, then

$$\tau = K'' \left(\frac{m^2 - 1}{m^2 + 2}\right)^2$$

Simplifying further,

$$\frac{\tau}{c} = K'' \left(\frac{\frac{\mu^2}{\mu_0^2} - 1}{\frac{\mu^2}{\mu_0^2} + 2} \right)^2 = K'' \left(\frac{\mu^2 - \mu_0^2}{\mu^2 + 2\mu_0^2} \right)^2$$

Because the change in $(\mu + 2\mu_0^2)$ will be small, then to a first approximation

$$\frac{\tau}{c} = K (\mu^2 - \mu_0^2)^2$$

It should be noted that $\tau=0$ when $\mu=\mu_0$. Thus if the immediate optical clearing effect of dextran is the result of changes in the refractive index of the suspension, K should be constant for all dextran fractions.

The refractive index of platelets in *tris* hydrochloride buffer was found by titrating a platelet suspension with dextran 40. In the titration plot the dextran concentration axis can be interpreted as a refractive index axis and thus the intercept on this axis $(\tau=0)$ gives the value for μ . Extrapolation of the linear part of the curve gave a value of 1.3772. This value is closely similar to the platelet refractive index found with a similar titration using human albumin. The values of K (Table 1) were then calculated from data derived from turbidity changes of a platelet suspension with different concentrations of dextran.

The effect of dextran 10 (molecular weight 10,000) concurred with the Rayleigh-Gans equation at all concentrations. The higher molecular weight fractions (that is, molecular weight 80,000 and more) also accord with it at low concentrations despite microscopic evidence of slight platelet clumping. The optical phenomenon described may therefore prevent detection by optical means of minor degrees of platelet clumping. An increasing departure from the Rayleigh-Gans equation, however, was observed at concentrations of dextran greater than 2 g/100 ml. (Table 1). At such concentrations, even in artificial media free of bivalent cations, pronounced platelet clumping occurred with the higher fractions and was detectable optically.

	Table 1.	VALUES O	F K	
Final dextran concentration (g/100 ml.)	D10†	D40	D80	D 500
1.8	3,105	3,050	3,035	3,020
3.3	3,150	3,007	2,990	2,475*
4.6	3.280	2.980	3,060	1,936*
5.7	3,300	2,925	2,940	1,470*
6-6	3,190	2.685*	2,790*	1,152*
7-5	3.175	2,415*	2,295*	745*
8-2	3,090	2,205*	2,090*	589*

Values were calculated from the experimental data derived from turbidity changes of a human platelet suspension in saline *tris* hydrochloride medium with different final concentrations of dextran. Further details are given in the text

* Calculated values of the constant K which do not concur with the Rayleigh-Gans equation.

† D10, dextran with a mean molecular weight of 10,000; D40, dextran with a mean molecular weight of a0,000, and so on.

Thus the immediate optical changes caused by dextran result from changes in the refractive index of the medium. This reasoning was additionally supported by the finding that the turbidity of platelet suspensions could be reduced almost to zero by raising the refractive index of the medium to that of platelets with other colloids such as polyvinyl pyrolidone or human albumin. Because the refractive indices at any given concentration of a series of polymer fractions are known to be constant this interfacial phenomenon explains the observed independence of the immediate optical clearing effect of the molecular weight of dextran¹.

Thus, although platelet clumping by dextran in vitro is not disputed, our earlier interpretation of its immediate optical clearing effect was in error and our conclusions regarding the relationship between molecular weight and clumping activity were inaccurate. Furthermore,

it is clear that optical properties of the examined materials should be considered when Born's method6 is used to measure platelet aggregation.

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APPLIED SCIENCE

Wind Forces and the Proximity of Cooling Towers to Each Other

A REPLY1 to a letter written by Malik and myself2 on cooling towers and drag forces contains an error to which I wish to draw attention.

Our letter reported some measurements of drag forces on spheres supported in assemblages through which a fluid was constrained to flow. The measurements were represented as the variation of the drag coefficient for a single sphere in our assemblage with the particle Reynolds number, and the well known relationship for the isolated single sphere was included in the graphs as a comparison. Unfortunately, Bearman misread the results as the drag coefficient for unit projected area across the whole assemblage, and quoted a simple application of Torricelli's law to show that our results could be explained by consideration of momentum changes in an ideal fluid. As the assemblage was twenty layers deep, a twenty-fold error was generated, so that Bearman's table for the drag coefficient related to unit projected area for the assemblage should read

	T	s	
a	S	R/eU^{2} (measured)	$\frac{2(1-S)^{s}}{\text{(predicted)}}$
0 0·5 1·0	0·785 0·349 0·197	146 5·0 2·8	8·5 0·41 0·15

...

It would have been surprising if such a theory were to describe the results of the complex fluid-solid interactions found in flow through assemblages of solids, although one could produce a better agreement than that shown in Table 1 by reformulating the assumptions in the light of the experimental results.

We have since published a full account of our experimental results3.

In the latter part of his letter Bearman suggested that our inferences concerning the increase of drag forces on objects caused by proximity did not apply to objects in unconstrained airflow. This is a point which merits some discussion because the proper resolution may be useful in designing experiments to give reliable drag coefficients for groups of bodies.

It is a matter of common experience that in conditions of unconstrained airflow around groupings containing large numbers of bodies, the hydrodynamic resistance of the bodies can so resist penetration of air flow that drag forces on bodies in the centre of the grouping may be very small. For example, conditions inside a forest are still, even in strong winds. Shallow groupings, however, such as the Ferrybridge cooling towers, are much more susceptible to wind penetration, and it is certainly possible

that the difference between drag coefficients measured in constrained and unconstrained conditions of airflow will be small at lateral spacings similar to the cooling towers, although one acknowledges that the difference will increase sharply for very small lateral separations. There is some experimental evidence of increase in drag coefficients of the order of 50 per cent for a sphere in proximity to one other in conditions of unconstrained flow4.

Wind penetration into shallow groupings of bodies will also be affected by the scale of turbulence of the atmospheric flow field (or the distance over which the field is correlated). If the distance over which the flow field is correlated is comparable with, or smaller than, the dimensions of the grouping, one would expect a degree of wind penetration rather greater than found during steady flow.

A further effect of unsteady flow comes to mind from the extensive work of Lunnon⁵, who investigated drag forces on spheres accelerating through fluid. He found that the force acting on an accelerating body was greater than that experienced by the same body in steady conditions at the same velocity, the difference increasing with acceleration.

The report of the committee of inquiry⁵ attributed most of the blame for the failure of the Ferrybridge cooling towers to structural weakness arising from a misinterpretation of the results of wind tunnel tests on isolated towers. Wind tunnel tests on groupings of towers were commissioned, and one may calculate from the table on p. 10 of the report the ratio of drag forces experienced by tower 1A (in the centre of the grouping) to that experienced by an isolated tower as $(84\cdot3/74\cdot6)^2=1\cdot28$, an increased force that is obviously significant particularly when it is borne in mind that but for the diversionary effect of the station buildings on the wind flow the measured increase would probably have been larger.

The results of wind tunnel tests can presumably be relied on to be accurate indications of real conditions only if the conditions measured are related to atmospheric turbulence. In the absence of specific wind tunnel tests on groups, the remarks on wind penetration into groups of bodies suggest that it is not unreasonable to assess the effects of proximity of bodies, in such groups as the Ferrybridge cooling towers, as though the flow were constrained. The results of our experiments show that at interbody spacings similar to that of the Ferrybridge group one would expect an increase in drag forces because of proximity of about 90 per cent-probably something of an overestimation, yet perhaps not very different from the increase that would be experienced by a central tower in a Ferrybridge group without the station buildings.

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Intensive Beef Production from Molasses and Urea

An important problem in many underdeveloped countries is the shortage of protein, particularly animal protein. This is rarely because the animal population is small, but rather because of the poor productivity which results from the dependence on forages and pastures characterized by low energy availability.

In most developed countries extensive methods are being superseded by intensive systems based to an increasing degree on high energy (grain) diets; but underdeveloped countries have neither the economic resources to buy grains nor, at the present time, the necessary technical skills to grow them on a large enough scale. Many underdeveloped countries in tropical areas, however, do have an abundant source of readily available energy in the form of sugar cane. Much of this energy is exported in the form of sugar and molasses, often at low profitability. If means could be found to exploit this energy source as an alternative to grain, more intensive systems of animal production could be developed with obvious economic and social benefits.

Supplementation of pasture with molasses has tended to give disappointing results; for example, in Brazil, Mott et al. giving 2 kg of molasses daily to grazing Zebu steers increased gain by only 0.08 kg/day. In Cuba, addition of urea to the molasses produced better results than molasses alone, but overall performance remained low2. The feeding of fresh Napier grass supplemented with molasses and urea to 247 Zebu bulls in dry lot gave an average daily gain of 0.56 kg with an energetic efficiency of 41.7 Meal of metabolizable energy/kg of gain. This can be compared with a daily gain of 0.97 kg and an energy conversion of 18-8 Mcal/kg for eighty similar bulls given grains and molasses and urea freely (unpublished work of T. R. Preston and M. B. Willis).

Lofgreen³ found that increasing quantities of molasses in a mixed diet were utilized with decreasing efficiency, while others4.5 have demonstrated that large amounts of sugar (as in molasses) depress the digestibility of cellulose. It seemed that the apparent incompatibility of feeding forage and molasses together might best be resolved by reducing the forage to the minimum level commensurate with normal rumen function.

A randomized block experiment was carried out with forty-eight Zebu bulls weighing, on average, 244 kg. They were housed in groups of three on slatted floors. All were given 1.5 kg of green forage maize/100 kg live weight/day and had free access to an aqueous solution of sugar cane molasses (14 per cent soluble solids) containing 0.67 per cent urea. A mineral mixture containing large quantities of phosphorus and sodium was also freely available*. Half the animals were given a protein-mineral-vitamin supplement; at the rate of 200 g/100 kg live weight/day. The other half received a similar supplement, but diluted with an equal part of ground maize and fed at the rate of 400 g/100 kg live weight/day. The feeding period was 168 days and feed allowances were adjusted every 14 days in accordance with live weight. Samples of rumen liquor were taken by stomach tube from all animals half way through the trial. These were analysed for pH, volatile fatty acids and ammonia and counts of bacteria and protozoa were made. The bulls were slaughtered at an average weight of 370 kg and their carcasses dissected according to the procedure outlined by Willis and Preston⁶.

Six animals were removed from the experiment, two because of molasses toxicity, and four because of injuries. The principal results are set out in Table 1.

The most striking features of the work were: (a) an average of 73 per cent of the dry matter and 76 per cent of the total metabolizable energy (calculated) from molasses; (b) an average of 58.6 per cent of the total nitrogen in the form of urea or ammonium sulphate; (c) high daily gains (38 per cent better than with ad libitum forage and molasses-ureas; (d) high killing out percentages (comparable with grain-fed cattle and 13 per cent better than with forage3); (e) an energy conversion only slightly poorer than that with grain and 41 per cent better than with forage3; and (f) conditions in the rumen resembled those of animals being given large amounts of forage and differed markedly from those usually associated with high energy diets (for example, pH 6.8 \pm 0.4; steam volatile fatty acids 93 ± 25 m.equiv/l., ammonia $4.63 \pm$ 3.43 m.equiv/l., and a large and varied protozoan population, particularly Flagellates, Entodinium and Isotrichs).

These findings thus present a new pattern. Gains, feed conversion, killing out percentage and the content of ammonia in the rumen closely resemble those obtained with Zebu cattle feeding on grain, while carcass composition, rumen pH and rumen flora and fauna are similar to those of animals feeding on large quantities of forage3. The concentration of volatile fatty acids in the rumen is remarkably low in view of the high energy intake.

Table 1. MEAN VALUES FOR WEIGHT GAINS, FOOD CONSUMPTION AND CARCASS COMPOSITION FOR BULLS COMPLETING THE TRIAL

	Supplement/100 kg live weight 200 g +			
Item	200 g	200 g maize	S.E.*	
No. of bulls Initial weight (kg) Final weight (kg) Daily gain (kg) Daily gain (kg) Green maize Molasses Supplement Minerals Conversion of metabolizable energy (Meal/kg gain) Killing out percentage Carcass composition (per cent) First quality meat	22 236 355 0·72 1·10 5·28 0·41 0·06 25·2 57·0 29·0	20 252 386 0.83 1.19 5.11 1.06 0.08 24.5 56.2	± 9·0 ± 0·12 	
Total meat Excess fat	73·2 8·7	72·6 9·2	± 0.52 ± 0.62	
Bone	18•1	18.1	± 0.68	

^{*} There were no significant differences between supplements.

Of the nitrogen intake, only 10.6 per cent comes from true protein (fish meal, yeast and grain), while 58.6 per cent is derived from urea and ammonium sulphate, the balance being 19.8 per cent from molasses (the nitrogen of which is predominantly non-protein) and 11.0 per cent from forage. Such a high level of non-protein nitrogen has only been used previously with purified diets without, however, the level of feed utilization and performance recorded here. The usual recommendations are that such synthetic sources of nitrogen should not exceed 25-30 per cent of the total nitrogen8,8.

Three hundred bulls are now involved in experiments designed to develop further this feeding system.

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^{*}The mixture contained 50 per cent CaHPO4; 40 per cent NaCl; 2 per cent ZnCO5; 2-7 per cent FeSO,.5H2O; 2-3 per cent MnSO,.H2O; 1.0 per cent CuSO4.5H2O; 0.01 per cent CoSO4.7H2O; 0.01 per cent KI; 1.98 per cent malze meal.

[†] Supplement contained 25 per cent sunflower seed meal; 25 per cent Torula yeast; 10 per cent fish meal; 7.5 per cent (NH₄)₂SO₄; 9 per cent molasses; 10 per cent CaHPO₄; 4.5 per cent NaCl; 1.5 per cent mineral supplement; 7.5 per cent with a mineral supplement; 7.5 per cent with a and 300,000 IU vitamin D/kg).

BOOK REVIEWS

CHEMISTRY OF BRAIN ACTIVITY

Molecular Basis of Some Aspects of Mental Activity Edited by Otto Walaas. (Proceedings of a NATO Advanced Study Institute held at Drammen, Norway, August 2–14, 1965.) Vol. 1: Pp. xv+476. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 110s.

This is the first of two volumes which contain the proceedings of the NATO meeting. It carries twenty-eight contributions which vary greatly in their quality and in

their relation to mental phenomena.

The mental activities to which its introduction and opening papers are oriented are those of learning and memory, approached first in terms of a particular model system. O. Hechter and C. E. Robinson have sought to apply current concepts of nucleic acid and protein metabolism to develop a theory of memory and recall. This merits serious consideration, although its present support comes largely through analogies to information transfer in genetic systems. The account of directly related experimental work is not convincing; after a subsequent paper "Facilitation of Learning in Rats by Intracisternal Injection of RNA from the Brain of Trained Rats", the recorded discussion displays appropriate scepticism. Other contributions, however, are related in illustrating the importance of protein metabolism to the brain, in giving details of protein synthesis in bacterial and tumour cells, and in describing protein interactions which can condition the growth of neural systems.

Subsequent sections concern inherited metabolic errors, with accounts of phenylketonuria and galactosaemia, the mechanism of hormone action, and biologically active amines. These include valuable accounts of cyclic 3,5-adenosine monophosphate specifically in relation to neural systems; indeed, the mammalian brain is the richest known source of enzymes forming and degrading the cyclic phosphate. Accounts of serotonin and noradrenaline illustrate electrophysiological and pathological approaches to mental phenomena, and an account of the retina and visual pathway contributes an anatomical viewpoint. Some contributions claim no specific relationship to mental activities, such as those describing lactic dehydrogenase isoenzymes and glutaminases, and Cori's excellently factual account of the phosphorylases of liver, muscle and potato. Nevertheless, substrates of the dehydrogenase, glutaminase and phosphorylase, as they operate in the brain in vivo, include compounds which change in concentration with changed cerebral activities.

Discussion sections follow most of the papers and add much to the value of the book; it also carries author and subject indexes.

H. McIlwain

INTRACELLULAR MEMBRANES

Intrace'lular Membraneous Structure

Edited by S. Seno and E. V. Cowdry. (Proceedings of the First International Symposium for Cellular Chemistry at Ohtsu, March 27-31, 1963.) Pp. xix+588. (Okayama, Japan: Japan Society for Cell Biology, 1967.) n.p.

FOR many purposes cells have been conveniently regarded as bags of enzymes surrounded by a selectively permeable membrane. Enzyme studies began by homogenization to obtain a solution or suspension for assay; flux studies assumed that the cell interior behaved as a single undivided compartment. This concept was, of course, a simplification; even before the development of the electron microscope, subdivisions of the cell interior were obvious. Since then a wealth of detailed information has been gained concerning the structure and function of cell organelles. It is a striking fact that many of these organelles consist of membranes arranged in a complex and specific manner; and it is becoming increasingly clear that their functions depend essentially on the character and arrangement of the membranes.

The symposium recorded in this volume brought together electron microscopists, cytochemists, cell physiologists and biochemists, so that there is a full treatment of each topic. The important cellular membranes are dealt with in turn: endoplasmic reticulum, mitochondria, Golgi complex, nuclear membrane and plasma membrane. The standard of the individual papers is very variable, ranging from some masterly reviews of the latest research (such names as Fawcett, Green, Sjöstrand, Novikoff, Robertson and Holter speak for themselves), to some rather disappointing papers, which review older work or are largely speculative. There are also many interesting accounts of more detailed experimental work, mainly by

Japanese authors.
Perhaps the weakest section is the final one on transport processes through membranes. Three of the most interesting papers are not really relevant to the subject: a discussion of the role of moving membranes in active transport relies on morphological evidence, unsupported by the quantitative data which are essential in this field; and a paper on cellular water is best described in the words of the author—"All this, and more, is pure speculation". In this section only the interesting paper of Onoé and Ohno on fat absorption can be regarded as a contribution to knowledge of membrane transport.

Minor defects are frequent misprints and poor English. Some of the reviews also suffer from the delay of four years in publication, which may be connected with the tragic death of two of the Japanese conveners shortly after the conference.

In spite of its defects, the coverage of this symposium is unique and the volume is highly recommended reading for those interested in cell biology. The volume is dedicated to the late Dr Seizo Katsunuma.

D. A. T. DICK

YEAST PROTOPLAST SYMPOSIUM

Symposium über Hefe-Protoplasten/Symposium on Yeast Protoplasts, Jena, 21 bis 24 September 1965 (Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin, Klasse für Medizin, Jahrgang 1966, Nr. 6). Herausgegeben von Rudolf Müller. Pp. vi+ 430. (Berlin: Akademie-Verlag, 1967.) 61.50 D.M.

It often seems that research on yeasts is pursued less actively than on other groups of micro-organisms, for only occasional papers appear in the commonly read microbiological journals. It is therefore a pleasant diversion to find a symposium almost entirely devoted to yeasts. Fifty-one papers are included, of which thirty-three are in English and eighteen in German, with English summaries. Many of the papers have only a tenuous connexion with the subject of yeast protoplasts; eight of the papers are not even concerned with yeasts. The volume offers, however, a very thorough coverage of recent yeast research, especially in the countries of Eastern Europe. The papers are grouped in five sections: microscopic and submicroscopic structure of yeast protoplasts (eight papers), structure and biosynthesis of yeast cell walls (thirteen papers), physiological and biochemical

studies on yeast protoplasts and isolated structures (ten papers), protoplasts of other organisms (seven papers) and studies on intact cells of yeasts and other organisms (thirteen papers). The opening lecture, a survey of trends in yeast research by J. R. Villanueva, is a particularly inspiring review of recent work on the yeast cell wall, including studies on protoplasts and regeneration of cell wall material.

The title is therefore rather misleading; although various aspects of the biochemical activities of yeast protoplasts are discussed, there is no indication of the large proportion of papers concerned mainly with the cell walls of yeasts, with protoplasts achieving only incidental mention. Electron microscopical studies, chemical structure and biosynthesis of the glucan, mannan and peptide or protein components are discussed many times, and the question of chitin-is it or is it not present ?-is revived. Anyone interested in these topics need not be deterred by the specialized title; he will find the book a valuable addition to his library. There is an additional bonus—by collecting contributions from many authors whose papers are normally prepared only in Russian, Czech or in other Eastern European languages, and presenting findings in English or German, a wider range of recent yeast research work is available to those of more limited linguistic ability.

All illustrations and tables are collected as an appendix of 135 pages. The standard of presentation of this section is excellent and the figures and tables referring to any particular paper are easily located. Apart from the index pages and editorial foreword, the text of the book has been produced by offset lithography from typescript. At approximately £5 the book is rather poor value for money in spite of the large number of photographs. Also, why must it have a soft paper cover, and why, in view of the supposedly rapid method of printing, has it taken almost two years since the symposium for the book to appear?

I. CAMPBELL

BIOCHEMISTRY OF PLATELETS

Biochemistry of Blood Platelets

Edited by E. Kowalski and S. Niewiarowski. (Colloquium held on the Occasion of the Third Meeting of the Federation of European Biochemical Societies, Warsaw, April 4-7, 1966.) Pp. vii+191. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc.; Warsaw: PWN-Polish Scientific Publishers, 1967.) 45s.; \$8.

This book contains eleven papers in English and two in French, presented at a colloquium held in Warsaw in April 1966. As so often with the published versions of scientific meetings, one wonders whether the delay of more than 12 months before publication is not too high a price to pay for the eventual elegance of production. A few of the contributions are essentially review articles, but most include accounts of original work; some of this has already been published elsewhere since these papers were delivered, but workers in the field will be glad to have them collected in a compact volume. The value of the book is considerably enhanced by the inclusion of the discussion after each paper, and by a comprehensive subject index; the value of the author index is less

The contributions cover many aspects of platelet biochemistry, including nucleotide metabolism (Holmsen; Spaet and Lejnieks), clotting factors (Deutsch; Niewiarowski), aspects of the "release reaction" (Kowalski; Markwardt), and biochemical abnormalities occurring during storage (Caen) and in congenital platelet disorders (Gross). Mustard discusses the relation of platelet haemostatic function to platelet turnover, Kopeć and her colleagues the effect of fibrinogen degradation products

on platelets, and Davey and Lüscher the protein constituents of platelets. Platelet immunology and ultrastructure are the subjects of papers by Salmon and Libánská respectively. Much active research is continuing on all of these aspects, and this volume is best regarded as a progress report, rather than as a general survey of platelet biochemistry. As such, it will be of interest chiefly to those actively engaged in the field.

R. M. HARDISTY

CHROMOSOME DAMAGE IN MAN

Human Radiation Cytogenetics

Edited by H. J. Evans, W. M. Court Brown and A. S. McLean. Pp. 218. (Proceedings of an International Symposium held in Edinburgh, October 12–15, 1966.) (Amsterdam: North-Holland Publishing Company, 1967.) 70s

This volume is one outcome of a technical advance which has made it possible for chromosomal damage in particular human somatic cells to be studied in considerable detail. In essence, the break-through is the use of phytohaemagglutinin (PHA) to stimulate cultured peripheral blood lymphocytes to go into mitotic division, so that the metaphase chromosomes of large numbers of cells can be scrutinized after appropriate colchicine and hypotonic pretreatments. From the contributed papers and the very short summary of unpublished discussion it is clear that there is now general agreement on methodology. In particular, it has now been realized that the previously preferred sampling time of seventy-two hours after culture initiation gives erroneously low estimates of chromosome damage; fifty hours is now favoured. This agreement is important, because divergent views on optimum fixation times have undoubtedly contributed to the markedly different results obtained in different laboratories. Unfortunately, however, large and puzzling discrepancies still remain.

The first group of papers is concerned with fundamental studies (mainly by H. J. Evans and co-workers) on the mechanics and dynamics of the leucocyte culture system and on the dose-response kinetics of exchange aberrations induced by both X-rays and fast neutrons at different dose rates. Considerable differences were found between these chromosomes and those of higher plants in their radiation response. Altogether, this group of contributions provides a very useful foundation for further work and for the interpretation of in vivo results.

Many subsequent papers describe such in vivo studies on populations exposed in one way or another to excess ionizing radiation. Although some reports are preliminary it is already obvious that this new approach is reaping rich dividends, chiefly in greatly increased knowledge of the nature, extent and persistence of chromosomal damage after both acute and chronic irradiation. Valuable new information has also been gained on the life span and immunological behaviour of the lymphocyte. The possible use of this technique for accurate biological dosimetry and for the detection of other mutagenic agents besides radiation is also discussed.

Full exploitation of this technique for population studies demands the removal of a bottleneck, namely, the time taken to detect and analyse each suitable mitotic cell. It is appropriate, therefore, that two papers should deal with possibilities of automating this process. The problems involved and the solutions advocated are dealt with lucidly and at length, making it clear that the difficulties are not insuperable and that success is only a matter of time.

To the receptive reader this volume should prove as stimulating as PHA to a lymphocyte.

A. G. SEARLE

AFRICAN NECTAR DRINKERS

The Sunbirds of Southern Africa; also the Sugarbirds, the White-Eyes and the Spotted Creeper

By C. J. Skead, assisted by Cecil M. Niven, J. M. Winterbottom and Richard Liversidge. Pp. 351+22 plates+gramophone record. (Cape Town and Amsterdam: A. A. Balkema, 1967. Published for the Trustees of the South African Bird Book Fund.) Rs. 8.50.

This is the second of a series of monographs dealing with certain groups of birds as represented in Southern Africa. It emanates largely from the Percy Fitzpatrick Institute of African Ornithology attached to the University of Cape Town, and its publication is sponsored by the South African Bird Book Fund. It gives a systematic account of the species of four passerine families of the Old World tropics and subtropics, three of them having in common the habit of drinking flower-nectar; two are represented in the area by single genera, puzzling in their taxonomic relationships.

The sunbirds (Nectariniidae) are represented by twenty species and their treatment thus occupies the larger part of the volume. Sunbirds correspond ecologically to the hummingbirds of the New World; and they likewise show brilliantly coloured plumages, well illustrated here by John Perry. Of the white-eyes (Zosteropidae) there are just three representatives, the main concentration lying further to the east. The genus Promerops is peculiar to the area and has two species; it is commonly placed among the honeyeaters (Meliphagidae), otherwise an Australasian family, but in this work judgment is reserved by keeping it in a family of its own. The rather inapt name "sugarbird" probably relates to the remarkably close association between the birds and the Protea bush, suikerbos in Afrikaans. The spotted creeper, of the small genus Salpornis, shows resemblance to the treecreepers (Certhiidae) but is usually regarded as an aberrant nuthatch (Sittidae); it has a slender decurved bill, like the sunbirds and sugarbirds, but is not a nectar drinker.

There is a general review of each family, after which the species are treated separately under such headings as local names, distribution in South Africa and elsewhere, field characters, habits, habitat, food, voice, breeding and taxonomy. In addition to the ten colour plates and twelve plates of photographs, there are line drawings illustrating breeding techniques, courtship attitudes and the like; and there are maps showing distribution in Southern Africa. In a pocket at the back there is a small two-sided gramophone record of sunbird calls and songs, edited by June Stannard. The book is well arranged and attractively produced. In all, a great deal of both old and new information has been usefully put together in convenient form.

LANDSBOROUGH THOMSON

CATALOGUE OF NYCTERIBIIDS

An Illustrated Catalogue of the Rothschild Collection of Nycteribiidae (Diptera) in the British Museum (Natural History)

By Oskar Theodor. Pp. viii + 506 + 5 plates. (Publication No. 655.) (London: British Museum (Natural History), 1967.) 360s.

THE dearth of comprehensive articles on the systematics of Nycteribiids is rectified by this outstanding and essentially monographic opus, which presents a revision of the family as a whole. The number of taxa described as new to science is: one subfamily (of a total of two), one genus (of eight), one subgenus (of nine), thirty-seven species (of 189) and two subspecies (twelve non-nominate).

After discussing and illustrating the complex morphological characters used in the taxonomy of Nycteribiids, the technical terms are defined in a special glossary which

The cites figure references for further clarification. systematics of the various taxa are then presented and the differential characters summarized in table form. names and authors of all species treated are listed. Dichotomous keys to these genera, subgenera, species and subspecies usually employ multiple and alternative diagnostic characters, cite page and figure references, and The taxonomic at times include geographic notes. section resembles in format the laudable system of the companion volumes on Siphonaptera by Hopkins and Thus, complete taxonomic citations are Rothschild. given for each taxon, including designation of synonyms, and succinct diagnoses and descriptions are presented, together with notes on hosts and distribution (supplemented by six maps). The cardinal taxonomic features are illustrated in a set of 898 figures of drawings and nine photomicrographs. Synonyms, misidentifications and major references to valid names can be recognized at a glance in the index.

The figures are clear and illustrate well the characters used in taxonomy. The keys are simple, terse, accurate and sound, and their use is facilitated by the wealth of excellent illustrations. The diagnoses and descriptions are lucid and deal with essential characters, emphasizing why the scheme of systematics employed can confidently be regarded as authoritative throughout.

Despite the complexity of the subject and the innumerable details presented, the book is remarkably free from errors, and in general the volume indicates superior editing and workmanship by the author, by the Hon. Miriam Rothschild and by the staff of the British Museum.

Dr Theodor is to be commended on having prepared a definitive opus which, for the first time, enables the nonspecialist to identify Nycteribiids on a global basis and thereby lays the ground work for much needed research on the habits and bionomics of Nycteribiids and on their possible role as vectors of infection. The volume also contributes to our knowledge of evolution and zoogeography and of the affinities of these parasites and their hosts.

PLANT TERPENES

Terpenoids in Plants

Edited by J. B. Pridham. (Proceedings of the Phytochemical Group Symposium, Aberystwyth, April 1966.) Pp. xi+257. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 70s.; \$12.50.

THE essential oils of plants were among the first naturally occurring materials with which organic chemists concerned themselves. Interest in plant terpenic components and in more complex terpenes from other sources continues unabated and has, indeed, been quickened by the introduction of modern techniques.

The present volume is based on the proceedings of a symposium held under the auspices of the Phytochemical Group in the spring of 1966. The contributions cover a wide field, but it is perhaps a sign of the times that of the twelve chapters seven are concerned in whole or in part with biosynthesis. These include a useful review of methods for the preparation of tritiated and deuterated mevalonate and their application in unravelling the stereochemical course of the biosynthesis of squalene. There are also accounts of the biosynthesis and metabolism of monoterpenes, the in vivo and in vitro skeletal transformations of diterpenes, the biosynthesis of cardenolides, bufadienolides and steroid sapogenins, phytosterol biosynthesis, the biosynthesis of terpenoid quinones and a fascinating review of the variety of sesquiterpenes, mostly derivatives of furan, found in the essential oil of Myoporum deserti A. Cunn. Other chapters are concerned with a discussion of the biological significance of terpenes

in plants, a subject in which interest has been reawakened by the discovery of abscisin and the gibberellins, and accounts of the conformational analysis of terpenes and steroids, structural studies in the carotenoid field (mainly devoted to applications of physical methods), and brief reviews of the chemistry of isoprenoid quinones and of long chain isoprenoid alcohols.

The book will be of greatest value to research chemists and biochemists with a special interest in terpenoid chemistry, for whom it provides authoritative accounts of the position reached in various fields of activity at the beginning of 1966. The level of the contributions is variable, but most are useful and informative. It must be said, however, that the volume remains little more than a disparate collection of short review articles, and one wonders whether publication of books of this type is always really justified. The best articles in this book could just as well, and at less expense, have been published in the ordinary way in review journals, and anyway what is good for a symposium is not necessarily good for a book. A little more discrimination here would have made a better book.

W. Carruthers

ADVANCING STATISTICS

The Advanced Theory of Statistics

By Maurice G. Kendall and Alan Stuart. Vol. 3: Design and Analysis, and Time-Series. Pp. ix + 552. (London: Charles Griffin and Co., Ltd., 1966.) 136s. net.

"This is the final volume of our treatise"; "... we write these final words with a considerable sense of relief". No one can contemplate the 500 pages between these first and last words without profound admiration for the authors and envy of the breadth of their knowledge. Like its two predecessors, this volume is an indispensable work of reference for statisticians whether their primary interests are theoretical or applied. Despite a longer gestation than had been hoped, the work has been kept up to date: the frequency of references dated 1965 or 1966 is high.

The volume divides into three parts. Chapters 35 to 40 are concerned with analysis of variance, Chapters 41 to 44 with the present state of multivariate analysis, and Chapters 45 to 50 with time series. As is inevitable in an encyclopaedic publication, the concise style and concentrated information are not conducive to consecutive reading by a reviewer. A balanced critical discussion of the whole is impracticable; all that can be given here are general comments and discussion of selected details biased towards my special interests.

To a reviewer who believes strongly that statistical theory needs contact with real problems and real observations, the authors' attitude occasionally seems unduly abstract, as in a remark on the avoidance of cross-classifications with one observation per cell. Similarly, a remark about the possibility of covariance analysis when the covariate is affected by treatment surely deserves more critical discussion. On the other hand, a section on the choice of model for analysis of variance is excellent in its insistence on using knowledge of the origin of the observations, and on the relation of design and randomization to the character of inference: "when the observations are not the result of a designed experiment, the validity of the chosen analysis will depend on the insight of the statistician".

In the analysis of variance chapters, the extensive discussion of experimental design and sample survey is valuable, but the distinction is strangely stated: "in surveys we make observations on a sample taken from a finite population of individuals, whereas in experiments we make observations which are in principle generated by a hypothetical infinite population". Sampling perhaps always relates to a finite population, but in some circum-

stances an experiment also may be conducted with reference to a finite population; moreover, the statemen quoted takes no account of the notion of comparison that is inherent in experiments, or of the necessity that the investigator shall decide the allocation of treatments to subjects. A little later, tribute is paid to Fisher's advocacy of randomization: "the most important and the most influential of his many achievements in statistics" the opportunity of reiterating the equally important but slightly different role of randomization in sampling seems to be missed. As the authors well illustrate, the logic of inference from experiments is intimately dependent on the factor structure and the randomization. Tome, however, discussion in terms of "choice of factors to be randomized out" confuses by its seeming implication of deliberate sacrifice of knowledge. In reality, randomization is the protection against ignorance of all the factors in the population that may affect the variate being studied, and the choice important to the experimenter is that of the population of subjects within which he will randomize.

The main categories of experimental design are described briefly, with emphasis on combinatorial structure more than on circumstances of use. A concise matrix presentation of general analysis of variance procedures is of course included. Greater concern for the use of designs might have led to more critical discussion of the consequences of interaction in Latin and higher order orthogonal squares, and of the need for randomization at two levels if inter-block information is to be recovered. The need for fuller study of permutation tests for complicated experiments is strongly argued. This is an issue that statisticians have yet to face fully, instead of accepting the difficulty of the algebra as an excuse for its neglect. Computers may soon make possible the adequate study of distributions underlying the tests of significance, but we must avoid a return to the practice of stressing these to the exclusion of the properties of estimates of para-The sections on lattice and factorial designs meters. scarcely suffice to show their connexions. Moreover, too little attention is paid to the exploitation of confounding and fractional replication in concentrating the information from an experiment on the issues of greatest interest; again the concern seems to be more with combinatorial niceties than with the refining of a research instrument.

As might be expected, the authors are more at home with sample survey, although relative to the major texts on sampling these seventy pages can only outline the theory with little mention of the practical complexities. Thus the reader finds here excellent accounts of procedures for removing or minimizing the purely probabilistic bias of various estimators, but he must go elsewhere for guidance on biases arising from questionnaire design or non-response. Somewhat surprisingly, the special problems of compromise in planning a survey when many variates must be studied simultaneously (as in most real surveys) are scarcely mentioned.

The hundred pages on multivariate statistics summarize well the theory of a subject in which practice has lagged behind theory until the coming of computers. A chapter on the main distributions is followed by an excellent account of types of hypotheses and how they are tested. Three branches of canonical analysis are considered under the headings of component analysis, canonical correlations, and factor analysis; admirable though the text is, the impression remains that much yet remains to be learned of the relations between the three and the parts that each should play in the analysis of data. The final chapter of this part is concerned with discrimination and classification, a field in which objectives are more clearly defined. Though brief, this survey of methods is especially welcome in view of the great current interest in applications as diverse as medical diagnosis and the principles of taxonomy. The systematic presentation of types of classification problems is particularly valuable.

I am not competent to discuss the chapters on time series. An important addition (relative to Dr Kendall's previous volumes) is an account of spectrum theory. A final chapter, recognizing the special difficulties in applying the theory of time series to "real" data, briefly reviews special topics connected with estimation of parameters, testing the adequacy of a fitted model and multivariate series.

In reviewing a book of this magnitude, to pick out particular errors or lapses in clarity is much easier than to evaluate fairly the features that are good but less specific. To say that the three volumes of Kendall and Stuart have weaknesses is in no way a denial of the great achievement they represent. For at least the next ten years, they will occupy a central place in statistical literature; for many years longer, they will be a standard reference. Will these authors, or any others, ever have the courage and skills to produce a new work on even grander scale in 1977?

D. J. Finney

ABSTRACT ALGEBRA

Abstract Algebra

By Andrew O. Lindstrum, jun. (Holden-Day Series in Mathematics.) Pp. xii+211. (San Francisco and London: Holden-Day, Inc., 1967.) \$10.

Most of the topics treated in this textbook of abstract algebra would find a place in courses for honours degrees in pure mathematics in this country at the present time. The author may have attempted to cover too many topics in the space available to him, for the treatment is sometimes rather compressed, particularly in group theory and linear algebra. Much care has been taken, however, in the preparation of the material and many points of detail have received thoughtful treatment.

While the substance of the book is good in many respects and I have a feeling that the lectures on which the book is based were very successful, it does not in its present form seem to be an attractive exposition for an undergraduate reader. He would not read it with the pleasure he should get from such a course, and his interest might not be maintained. He might get used to the abstract and formal style and should make an effort to do so, but the practice of requiring the reader to supply his own proofs of various theorems is overdone. In moderation, the practice can stimulate and encourage a student, but here it might well irritate and depress. This defect of presentation is regrettable because the book has some interesting and commendable features.

The bibliography would have been more useful if it had been related in a more specific way with the various chapters of the book, and if some of the books mentioned were recommended for collateral reading and others for a study of further developments.

H. O. FOULKES

CLASSICAL ANALYSIS

A Second Course in Complex Analysis

By William A. Veech. Pp. ix+246. (New York and Amsterdam: W. A. Benjamin, Inc., 1967.) \$8.75.

VEECH builds on an assumed standard first course in complex variable, dealing with Cauchy's theorem, residues and simple mappings. He then pursues a number of related topics, all of which are now important parts of classical analysis. The style is midway between that of an elementary text and a research memoir, and should prepare the honours student for the reading of periodical literature.

The first four chapters hang very closely together, with a strong geometrical interest. A chapter on analytic

continuation and one on conformal mappings, using the Schwarz lemma and reflexion principle, prepare for the chief theme, the relation between analytical and topological equivalence expressed most simply in the Riemann mapping theorem, here obtained as a corollary to the more general Koebe mapping theorem. In this domain, the language of covering surfaces and covering maps is conveniently employed. Following on this are the results springing from the Picard theorem that an integral function has at most one exceptional value, the Landau and Schottky theorems, Montel's normal families, the Koebe-Faber distortion theorem, Bloch's constant. In this section, modular functions are used but the requisite properties are obtained in the text.

The remaining two chapters are a little detached from the chief theme. One deals with integral functions, deriving the Weierstrass product and the more difficult Hadamard product for functions of finite order. The last chapter discusses the Riemann zeta function and gives Ikehara's proof of the prime number theorem. In mentioning the way in which Landau, Wiener and Ikehara successively minimized the amount of zeta function theory required, the impetus given by the Tauberian theorems of Hardy and Littlewood is not noted.

T. A. A. BROADBENT

ELECTRIC MACHINES

Generalized Electric Machines

By A. J. Ellison. Pp. 146. (London: George G. Harrap and Co., Ltd., 1967.) 20s. net.

Electrical Machines

(Electrical Engineering Series.) By A. Draper. Pp. xx+384. (London: Longmans, Green and Co., Ltd., 1967.) 63s. net.

DURING the past few years a large number of books have appeared dealing with electrical machines. Most of these have been concerned to try to present a "generalized" approach to the subject, beginning with a description of electro-mechanical transducers in either circuit or energy terms and often going some way towards considering the systems in which the transducers are employed.

Of the two books reviewed here, Ellison comes closer to the current fashion. It is a companion volume to the same author's book Electromechanical Energy Conversion. published in 1965; the present book, however, is unique in being wholly concerned with the structure and details of a laboratory course directed at complementing typical modern lecture courses employing a generalized approach. The title, which is thus seen to be somewhat misleading as a description of the subject matter, presumably refers to the special purpose laboratory machines now widely available as vehicles for courses such as those described. The author has contributed much to the development of modern laboratory courses and, in this country, pioneered the first generalized laboratory machine in conjunction with AEI. Material sufficient for a course of up to twelve three hour periods is described, comprising for each experiment an introduction, a description of apparatus and general arrangements, a procedure, an outline of the relevant theory and calculations and a discussion and set of questions for the student. The course described is a good one, as would now, we believe, be generally agreed. But the fact that this agreement largely exists already, raises the question of exactly what need this text fulfils. Experienced teachers will have their own views on courseplanning, and will prepare laboratory sheets which will supersede this book as far as their students are concerned; but it could certainly be of value to younger and less experienced teachers and to those needing persuasion to make a change from a traditional to a generalized laboratory.

The book by Draper is closer to the traditional approach to machine exposition than most of those written recently. This is not to be critical of it, but to indicate that it will meet a need for those who feel that the more fashionable approach is too far removed from practical devices and leaves the student with problems of visualization and appreciation. It is the second edition of the successful book which first appeared in 1956. This new edition follows the same pattern as the earlier one and is improved chiefly by the addition of a chapter dealing briefly with the principles and application of semi-conductor devices; also the treatment of the induction motor has been extended somewhat, and one or two ideas on closed-loop control are included. The treatment of single-phase machines remains brief, and matrix methods, although referred to in the preface, are not seriously exploited; however, both these topics are treated in the author's companion text Electrical Circuits (including Machines), and cross-references are given. The present book is remarkable for the breadth of its coverage—all practically important machines, together with mercury-arc rectifiers, are included, even if briefly. That this should be possible without the treatment becoming unacceptably superficial or scrappy is due to the author's commendably simple and clear style. Because of its coverage and its readability, this book will attract many students.

P. J. LAWRENSON G. W. CARTER

TEXTILE CHEMISTRY

Textile Chemistry
By R. H. Peters. Vol. 2: Impurities in Fibres; Purification of Fibres. Pp. xiii+374. (Amsterdam, London and New York; Elsevier Publishing Company, 1967.)

In the main this book comprises an excellent and detailed description of the industrial chemistry associated with the processes of scouring and bleaching of cotton and other cellulosic fibres. There are chapters on the chemistry, scouring and bleaching of wool, and particularly on the fundamental work on the reactions of wool in aqueous media. There is some reference to man made and regenerated cellulose and its esters, and also to the aqueous reactions of silk fibroin.

The first chapter gives an account of the industrial uses and chemical content and contamination of water from various sources and forms a fitting introduction to the aqueous fibre chemistry which follows. There follows a detailed physico-chemical study of sequestering agents and co-ordination compounds. The bearing of this subject on dyestuff absorption is obvious, and in the next chapter ion-exchange phenomena are discussed with a strong mathematical accent on sorption and swelling properties of fibres. The fourth chapter is a rather short description of some of the natural fibres; possibly this could have been expanded with some added value to the book. A description is then given of the chemistry of sizes, gums and waxes and their technical applications are outlined.

There follows a series of chapters on the chemical technology of cotton and other cellulosic fibres. In this field, the book is sound and comprehensive and makes an excellent reference work in this field. The thirteenth chapter on linen and man made fibres is a useful contribution but in no way as comprehensive as the work on cotton. A similar comment can be made about the three chapters on wool chemistry and also the seventeenth chapter on the scouring and bleaching of wool. They do, however, give a general survey of the subject and the references are a useful guide to any reader who wishes for a more detailed study of the chemistry of wool and

related products. The final chapter gives a comprehensive survey of the technical chemistry of the mercerization of cotton.

The book is the second of this series, and when the third volume is available the series should make a valuable contribution in the field of the applied chemistry of fibres. F. HAPPEY

OBITUARIES

Dr Jan Kruszynski

DR JAN KRUSZYNSKI, senior lecturer in histology in the University of Liverpool, died on October 14 aged 64.

Kruszynski studied medicine at the Stefan Batory University of Wilno. In his second year, his beautiful histological drawings came to the attention of Professor J. S. Alexandrowicz and soon led to a junior appointment to the teaching staff of the Department of Histology. Kruszynski graduated MD in 1930 and attained the degree of Docent in 1936.

He remained at the Department of Histology at Wilno until his capture by the Russians in 1939. He was a prisoner of war until 1941; he did not care to discuss his terrible experiences during that period. He joined the Polish Army in Russia in 1941, being appointed pathologist to a military hospital, with which he served as it travelled across the Caspian Sea to Italy, via Egypt, Palestine, Iraq and Persia. He reached Britain in 1947. He was appointed assistant lecturer in histology at Liverpool in 1948, lecturer in 1950 and senior lecturer in 1963.

Jan Kruszynski was a gentle, self effacing scholar who gave service of inestimable value to the department and to the medical school as a whole. He had a profound knowledge of classical histology, its literature, its methods and results. His artistic gifts made him a superb illustrator of histological appearances; he went to untold trouble on the students' behalf in preparing demonstrations and lectures illustrated by the clearest of diagrams and drawings. He was always ready to share his wide experience of histological appearances and was consulted by colleagues from many different departments about their problems. His own special research interest was in inorganic histochemistry; from an early stage in his career he was interested in microincineration and became recognized internationally, through lectures and published papers, as the chief authority in that field.

His hobbies reflected his artistic interests: art galleries, photography, engraving and country walking. He will be sorely missed, not only for his professional accomplishments, but for his cheerful, modest personality and the complete integrity of his character. N. M. HANCOX

Dr W. J. Rees

It is a great shock to marine biologists in many countries to learn of Dr W. J. Rees' sudden death on October 12 at the age of 54. He was born on July 2, 1913, and graduated from the University of Wales at Aberystwyth in 1933 and was awarded the DSc in 1942. He is known internationally for his research on three distinct groups of marine animals.

His first research as a postgraduate student at Aberystwyth, was on the helminth parasites of molluscs. He gave this group up somewhat reluctantly in 1936 to become research assistant at the Plymouth Marine Laboratory where he started his researches on the Hydrozoa which were to prove his primary interest for the rest of his life. From 1940 to 1946 he served with the Royal Air Force Volunteer Reserve, first with Coastal Command and later

with the Admiralty where he became a leading authority on the recognition of fishing craft, writing a series of books of reference for the services on fishing craft, first on those of western Europe and then on those of far eastern countries.

In 1946 Rees was appointed to the scientific staff of the British Museum (Natural History) and for the next eight years was in charge of the mollusc section. During this time he published important papers, chiefly on the Cephalopoda, being specially interested in giant squids, and he made very interesting investigations on the larval stages of the octopus in the English Channel. He also did sound work on heteropods and terrestrial gastropods including a report for the Colonial Office on the giant snail, Achatina. He reviewed the breathing devices of terrestrial pulmonates and wrote on the aerial dispersion of Mollusca. He did not, however, conceal his eagerness to return to work on the coelenterates and when the opportunity arose in 1954 he was transferred to the coelenterate section as a principal scientific officer and was able to resume his researches on the Hydromedusae. He did not, however, abandon his interest in the mollusca and was president of the Malacological Society from 1963 to 1966.

Rees pursued his studies in many marine laboratories in Europe and latterly in the United States, and in addition to discovering a number of new species of hydroid was often successful in linking for the first time a known medusa to its hydroid, which was often known by a different name. His interest in the classification of the Hydrozoa culminated in an international symposium on "Cnidaria and their Evolution", which was effectively organized and edited by him for the Zoological Society of London.

J. P. Harding

Dr P. D. F. Murray

DR P. D. F. MURRAY died suddenly on May 17 on board ship while on his way from Australia to Cambridge. He was one of Australia's most distinguished zoologists.

Murray was born on June 18, 1900, at Dorchester. He went to Australia at an early age and was educated at Riverview College and the University of Sydney, from which he graduated with first class honours and the university medal in zoology. He was awarded the Macleay Fellowship of the Linnean Society of New South Wales and began research work for which he was awarded the DSc. After staying for a few years as lecturer, he left Sydney and spent some time at the Universities of Freiburg and Oxford before taking up a Royal Society Smithson Research Fellowship at the Strangeways Laboratory at Cambridge. In 1936 he was appointed university demonstrator at Bedford College and three years later reader in biology and comparative anatomy at the Medical College of St. Bartholomew's Hospital.

In 1949 the Challis chair in zoology in the University of Sydney became vacant and Murray returned to take up this appointment. Ill health, however, forced him to relinquish it and in 1960 he went to the University of New England at Armidale in New South Wales, where he had been appointed reader in zoology. This position enabled him to pursue his research without the heavy load of administration that had undermined his health in Sydney. He retired from this post last year and the university honoured him by appointing him an honorary research fellow.

Besides holding the degrees of the University of Sydney, Murray also held a BSc from the University of Oxford and an honorary MA from Cambridge. When the Australian Academy of Science was established he became one of the Foundation Fellows.

Murray's research interests were in the field of experimental embryology and morphogenesis. While still at the University of Sydney early in his career he carried out work on the development of single somites, of unsegmented mesoderm and of the skeleton in chick embryos.

He used the technique of chorio-allantoic grafts, which, together with tissue cultures, became his major techniques in his later work.

After he left Sydney, Murray continued his research with studies on the development of the heart and the blood vessels and the effects of various cations on the heartbeat and the fibrillation in the developing chick heart. Later he concentrated on experimental studies of the skeletal tissues and during the past few years worked on the histology and the experimental induction of adventitious cartilage.

His research interests, however, do not tell the full story of Murray's role in academic life. He was a man of great modesty but of firm purpose. His clear mind and wide interests in many facets of biology, his integrity and disinterestedness, were generally acknowledged and caused his advice to be sought on many occasions. And because his taking up the position of professor of zoology came at the beginning of a rapid expansion of university teaching with the establishment of new institutions the calls on his advice, his time and his energy were many.

When he took up the readership in the University of New England he was freed from many of the more arduous tasks of administration. He could again concentrate on his research work and the supervision and stimulation of work of younger colleagues. On his retirement he looked forward with keen anticipation to a renewal of old friendships at the Strangeways Laboratory, where he intended to spend the next two years. His sudden death, depriving the world of an outstanding scientist, is therefore the more poignant and tragic.

ALEX STOCK

Professor Carl Kling

PROFESSOR CARL KLING died in July at the age of 88. He became interested in microbiology as a medical student, and after graduation joined the staff of the Swedish National Bacteriological Laboratory. He was appointed director in 1924 and held this post until his retirement in 1945.

In 1911 Sweden experienced what was to develop into one of the most devastating poliomyelitis epidemics ever recorded. Klir g dedicated himself to a study of the epidemiology of the disease and developed an almost obsessive interest in this subject which stayed with him for the rest of his life.

His most significant contribution came in 1929 when, after observations on several European epidemics and a re-evaluation of earlier laboratory findings, he concluded that poliomyelitis must indeed be an intestinal infection. This represented a radical departure from the generally accepted concept of poliovirus as an exclusively neurotropic agent. It took almost ten years and a great deal of confirmatory evidence to convince the medical world that Kling's ideas were essentially correct.

His hypothesis when first presented had rested partly on his intuition and power of scientific imagination. In the following years, in experimental studies of the pathogenesis and epidemiology of the disease, he added much of the substantial evidence that finally revolutionized the concepts of the natural history of poliomyelitis.

In later years he engaged himself in a less fruitless search for extrahuman virus reservoirs. He remained active long after his retirement; only in his last two years did age begin to dampen his spirits.

Under Kling's directorship the National Bacteriological Laboratory g ew to acquire both national and international recognition and long served as Sweden's central breeding place for the microbiological sciences. This development did not take place entirely without controversy. With his refreshingly nonconformist attitude to both life and science, Kling made a few enemies as well as many devoted friends. With him Swedish medical science has lost one of its most colourful personalities.

SVEN GARD

University News:

Bradford

MR D. P. Howson, formerly senior lecturer in the Department of Electronic and Electrical Engineering in the University of Birmingham, has been appointed to the additional chair of electrical engineering, and Dr G. Brown, a reader in physics in the university, has been appointed to a personal chair in nuclear physics. Professor D. C. Johnson, a visiting professor in the university, has been appointed to the chair of industrial technology.

Reading

THE personal title of professor has been conferred on the following: Dr R. N. Curnow, at present reader in applied statistics and head of the Department of Applied Statistics; Dr G. W. Maynard, at present reader in economics; Mr E. J. Rolfe, principal of the National College of Food Technology; Dr B. G. F. Weitz, director of the National Institute for Research in Dairying.

Salford

DR G. O. PHILLIPS, formerly senior lecturer in chemistry at the University College of South Wales, has been appointed professor of chemistry.

Appointments

MR A. E. BAILEY, formerly head of the Strategic Systems Division at the Signals Research and Development Establishment, has been appointed superintendent of a new Division of Electrical Science at the National Physical Laboratory.

MR F. H. Felberg has been appointed assistant laboratory director for plans and programmes in the Jet Propulsion Laboratory, California Institute of Technology; Mr J. N. James has been appointed to Mr Felberg's former position of assistant laboratory director for Technical Divisions.

Announcements

MR F. L. WARING, deputy chairman and managing director of Coalite and Chemical Products Ltd, has been elected president of Chemical Industries Association, in succession to Sir Peter Allen, deputy chairman of Imperial Chemical Industries Ltd.

AT the annual statutory meeting of the Royal Society of Edinburgh on October 23, 1967, the following were elected officers and council for the session 1967-68: President, Professor N. Feather of the University of Edinburgh; Vice-presidents, Professor C. H. Waddington of the Institute of Animal Genetics, Edinburgh; Dr H. E. Butler of the Royal Observatory, Edinburgh; Professor I. N. Sneddon of the University of Edinburgh; Dr J. R. Peddie of Edinburgh; Professor T. C. Phemister of the University of Aberdeen; Professor J. D. Robertson of the University of Glasgow; General Secretary, Professor A. E. Ritchie of the University of St. Andrews; Secretaries to Ordinary Meetings, Sir William Weipers of the University of Glasgow Veterinary Hospital; Professor Neil Campbell of the University of Edinburgh; Treasurer, Lord Balerno of Currie; Curator, Dr R. Schlapp of the University of Edinburgh; Ordinary Members of Council, Professor P. W. Brian of the University of Glasgow; Sir David Lowe; Dr D. G. Sopwith; Professor E. M. Patterson of the University of Aberdeen; Professor P. D. Ritchie of the University of Strathclyde; Professor R. S. Silver of the University of Glasgow; Professor H. W. Wilson of the Scottish Universities' Reactor Centre; Professor W. N. Everitt of the University of Dundee; Professor N. Kemmer of the University of Edinburgh; Dr D. Traill of Edinburgh; Professor G. R. Tristram of the University of St. Andrews; and Dr C. D. Waterson of the Royal Scottish Museum, Edinburgh.

Meetings

PERMEABILITY Problems, July 2-9, 1968, Jerusalem (The Secretariat, Symposium on Permeability Problems, Polymer Department, Weizmann Institute of Science, Rehovoth, Israel).

CORRIGENDUM. In the article "Theoretical Astronomy at Cambridge", by F. L. Westwater (*Nature*, 216, 432; 1967) the SRC grant was incorrectly given as £25,000. This should have been £250,000.

CORRIGENDUM. In the article "Bacterial Inhibitors in Milk and other Biological Fluids" by B. Reiter and J. D. Oram (Nature, 216, 328; 1967) the last sentence should read "... and less than 1 µg of purified lipopolysaccharide (E. coli 005: B5, Difco) produces leucocytosis in 4-5 h".

ERRATUM. In the obituary of Sir John Cockcroft (*Nature*, 216, 621; 1967), m.amp should read μamp. The site for the heavy water reactor on the Ottawa River was selected in 1945, not 1946.

ERRATUM. In the article "Formation of Zinc Ferrite at Low Temperatures" by J. Beretka and M. J. Ridge (Nature, 216, 473; 1967), in the second line of the fourth paragraph organic oxides should read R₂O₂, and in the ninth line of the fourth paragraph the word chlorine should read chloride.

CORRESPONDENCE

Assessing the AGR

Sir,—To put your recent article on "Assessing the AGR" (Nature, 216, 213; 1967) in its proper perspective, we ought to emphasize a few points and add some comments to what was written.

Although the AGR came out unfavourably compared with the other systems in our assessment, the differences in terms of generating costs are marginal. Taking into account the uncertainties in the basic cost data used, we would agree with AEA that our results are by no means conclusive and we would further agree that only through actual tendering will it be possible to establish the real costs.

The fact that we for a certain period concentrated on a more extensive comparison between light and heavy water boiling reactors should not be given more importance than we in Norway have attached to it—a mere continuation of our paper exercise.

The cost picture for the different reactor types is changing rapidly and, as AEA points out, there has, since our assessment was carried out, been a general increase in the cost of light water reactors while there has been a definite decrease in the cost for the advanced gas cooled reactor. It is also generally acknowledged that the AGR has more to gain from a further development than have the light water reactors.

Since the results referred to in your article were published, we have obtained additional cost figures for both the AGR and the American light water reactors (PWR and BWR) indicating that, according to our ground rules, the generating costs for these reactors now work out roughly equal.

With due regard to the above development, we decided earlier this year to include an AGR in our reference design study of a 500 MWe nuclear power plant under Norwegian conditions. We have recently initiated a collaboration with UKAEA Reactor Group, Risley, to carry this study through.

Yours faithfully,

HENRIK AGER-HANSSEN Assistant Director, Project Manager.

Institutt for Atomenergi. Kjeller.

FORTHCOMING EVENTS

1986

(Meetings marked with an asterisk are open to the public.)

Monday, November 20

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (at the Science Museum, Exhibition Road, London, SW7), at 5.30 p.m.—Mr F. I. G. Rawlins: "Physical Chemistry or Chemical Physics—a History of Two Movements".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr W. D. Humpage and Dr T. N. Saha: "Digital Computer Methods in Dynamic Response Analysis Turbogenerator Units".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5.30 p.m.—Dr Arthur Engel: "Perspectives in Health Planning. III. Mass Screening for Asymptomatic Disease as a Public Health Measure". (Third of four Heath Clark Lectures.)*

Society for Analytical Chemistry, Radiochemical Methods Group (at the Pharmaceutical Society, 17 Bloomsbury Square, London, WCI). at 6 p.m.—Annual General Meeting, followed by Dr P. G. Jeffrey: "The Application of Isotope Neutron Sources in Analytical Chemistry"; Dr K. Ansell: "Isotope Radiation Sources for X-ray Analysis"; Mr J. Johnston: "Bremsstrahlung Methods in Industry".

Tuesday, November 21

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 1.20 p.m.—Mr A. N. Maxwell: "How are Sensations Related to Brain Processes?".*

UNIVERSITY OF LONDON (in the Chemistry Department, Imperial College of Science and Technology, London, SW7), at 5 p.m.—Professor J. H. de Boey (Technological University of Delit): "Solved and Unsolved Problems after 40 Years of Adsorption Research".*

SOCIETY OF INSTRUMENT TECHNOLOGY (joint meeting with the Plastics Institute, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 5,30 p.m.—Mr D. Grant and Mr P. Wilkinson: "Temperature Control Extrusion"; "The Requirements for Temperature Control D. E. A. Brooks and Mr P. R. Lever; "Strategies for the Temperature Control of Extruders".

University of Aston in Birmingham (at Gosta Green, Birmingham, 4), at 5.30 p.m.—Professor E. Braun: "Commonsense and Physical Law" (Inangural Lecture).

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr F. H. Doyle: "Radiological Measurements in Endocrine and Metabolic Diseases". (Eleventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5.30 p.m.—Dr Arthur Engel: "Perspectives in Health Planning. IV. The Swedish Regionalized Hospital and Health System". (Last of four Heath Clark Lectures.)*

UNIVERSITY OF LOXDON (in the Botany Theatre, University College London, Gower Street, London, WC1), at 5.30 p.m.—Dr D. H. Notthcote: "Growth and Differentiation of Plant Cells" (further lecture on November 22).*

UNIVERSITY OF SURREY (in the Great Hall of the University, Battersea Park Road, London, SW11), at 5.30 p.m.—Professor J. Waterlow: "The Body's Need for Protein—a Challenge for Research".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), ten Design" opened by Mr N. L. Sigournay, and a speaker from the Decca Navigator Co.

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Art, Science and Technology in Engineering Design".

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, SW1), at 6 p.m.—Mr G. W. Hemy: "The Russian Chemical Industry".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Eccleston Hotel, Victoria, London, SW1), at 7 p.m.—Mr. H. G. Parker: "Health Hazards in the Rubber Industry".

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W1), at 7 p.m.—Twenty-third Annual General Meeting, followed by Dr G. F. Reynolds: "Advances in Electrophoresis".

Tuesday, November 21-Thursday, November 23

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2)—Conference on "Servocomponents".

Wednesday, November 22

Geological Society (at Burlington House, Piccadilly, London, W1), at 5 p.m.—Dr George W. White: "Pleistocene Deposits of the North-West Allegheny Plateau"; Dr A. C. Dunham: "The Felsites, Granophyre, Explosion Breccias and Tuffisites of the North-Eastern Margin of the Tertiary Igneous Complex of Rhum, Inverness-shire".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr J. Evans: "Solid State Electronics".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.,—Mr I. I. Artobolewskii: "Some Aspects of the Development of the Theory of Automatic Machines by Soviet Scientists".

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, WCI). at 6 p.m.—Dr A. M. Jelliffe and Dr P. T. Lascelles: "The Use and Misuse of Ionizing Radiation in Neurology—Clinical and Biochemical Aspects".*

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (joint meeting with the Plastics and Polymer Group, at 14 Belgrave Square, London, SW1), at 6.15 p.m.—Dr W. A. P. Black: "The Use of Aigal Polysaccharides in the Food Industry".

Thursday, November 23

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.ms.—Professor George Porter, F.R.S.: "Thermodynamics and Chemical Change" (Civil Service Lecture).

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Cleveland Street, London, W1), at 5 p.m.—Professor A. Kekwick: "Food and Energy".*

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr H. K. Weinbren: "Control of Growth and Regeneration in the Liver". (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "The Place of Liberal Studies in Engineering Courses".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, WC2), at 6.30 p.m.—Professor J. G. Davies: "Radio Astronomy".

Friday, November 24

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Dr J. R. Froines: "Studies of Chlorophyll Monolayers".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 2.30 p.m.—Colloquium on "Integrated Circuits and Logical Design with Integrated Circuits. (b) Partitioning with Integrated Circuits".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Lt.-Col. R. H. Smith, R.E.M.E. (retd.), T.D.: "Developments Since 1897 of Technical Equipment Used by the London Electrical Engineers".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 9 p.m.—Professor Pearce Williams: "Michael Faraday and the Ether—a Study in Heresy".

Friday, November 24-Sunday, November 26

INSTITUTION OF MECHANICAL ENGINEERS, THE OPERATIONAL RESPASSOR SOCIETY, and the BRITISH COMPETER SOCIETY (at The Grand Hotel, Eastbourne)—Joint Conference on "Problems of Project Management Using Network Analysis".

Saturday, November 25

BOTANICAL SOCIETY OF THE BRITISH ISLES (in the Botany Department, British Museum (Natural History), Cromwell Road, London, SW7), from 2 p.m. to 5.30 p.m.—Annual Exhibition Meeting.

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3.30 p.m.—Dr J. M. Cullen: "Colour and the Eye—Experiments on the Role of Colour in Animal Behaviour".*

Monday, November 27

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2)-Colloquium on "Energy from Natural Gas".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 1.15 p.m.—Professor L. Pearce Williams: "Physics and Philosophy in the Early Nineteenth Century".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr C. S. E. Phillips: "Computers and Adaptive Radar".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at 1 Birdage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Systematic Approach to Design", opened by Mr R. L. Latham and Mr G. J. Terry.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the

dates mentioned:
TEMPORARY ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, The University, Sheffield (November 27).
TEMPORARY LECTURER/SENIOR LECTURER IN MATHEMATICS AT Britannia Royal Naval College, Dartmouth, Devon—Ministry of Defence, Navy Department SEI(N) 368, Old War Office Building, London, SWI (November 672)

CHAIR OF PHYSIOLOGY at St. Mary's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, WC1 (December 1).

(December 1).

DEMONSTRATOR (male or female, medically qualified) in the DEPARTMENT
OF ANATOMY—The Registrar and Secretary, The University, Bristol
(December 4).

LECTURERS (2) IN CIVIL ENGINEERING at the University of Queensland,
Australia—The Association of Commonwealth Universities (Branch Office),
Marlborough House, Pall Mall. London, SW1 (London and Brisbane, December 2).

Mariborough House, Pail Mall, London, SWI (London and Brisdare, December 8).

Research Associate/Senior Research Associate (graduate with experience of electronic circuits and preferably some knowledge of acoustics) in the Department of Electronic and December 18, to work on a challenging project concerned with the exploitation of non-linear acoustice phenomena in a new type of sonar system—The Assistant Registrar (8). P.O. Box 363, The University, Birmingham, 15 (December 9).

PROFESSOR OF MATHEMATICS at the University of Ife. Nigeria—The Inter-University Council, 33 Bedford Place, London, WC1 (December 12).

LECTUERR OF ASSISTANT LECTURER in the DEPARTMENT OF MATHEMATICS—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (December 15).

READER OF SENIOR LECTURER IN PHYSICAL CHEMISTRY; and a SECON LECTURER of LECTURER (preferably with interests in organometable chemistry) in Organic Chemistry—The Registrar, The New University of Ulster, Coleraine, Northern Ireland (December 15).

LECTURERS/ASSISTANT LECTURERS (with at least a good honours degree or equivalent, plus suitable teaching and research experience, and preferably specialized in one of the following fields: inorganic chemistry, analytical chemistry, physical chemistry or chemical technology) in Chemistry at the University of Malaya—The Association or Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Kuala Lumpur and London, December 17).

SENIOR LECTURER, LECTURER or ASSISTANT LECTURER IN SCIENCE EDUCATION, with special reference to the teaching of mathematics at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, WC1 (December 17).

TION, with special reference to the teaching of mathematics at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, WC1 (December 17).

LECTURER/SENIOR LECTURER (preferably with interests in one or more of the following fields: economic geography, urban geography, biogeography, South-East Asia) in the DEPARTYENT OF GEOGRAPHY. Monash University—The Academic Registrar, Monash University, Clayton, Victoria, Australia; or The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (December 22).

LECTURER IN ENTRACTIVE METALLURGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (London and Brisbane, December 31).

PROFESSOR OF APPLIED PSYCHOLOGY at the University of New South Wales—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, December 31).

SENIOR LECTURER OF LECTURER IN PSYCHOLOGY at Flinders University of South Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, December 31).

SENIOR LECTURER OF LECTURER IN PSYCHOLOGY at Flinders University of South Australia. Bedford Park, South Australia (December 31).

CHAIR OF ELECTRONICS at Chelsea College of Science and Technology—The Academic Registrar (A), University of London, Senate House, London, WC1 (January 3).

CHAIR OF ENTROPOLOGY at the London School of Economics—The Academic Registrar (A), University of London, Senate House, London, WC1 (March 1).

(March 1).

HEADSHIP OF THE DEPARTMENT AND CHAIR OF PHYSICS—The Academic Registrar, The City University, St. John Street, London, EC1, quoting ref. P/T.

LECTURER IN ANTHROPOLOGY; and a LECTURER IN SOCIOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1.

SENIOR LECTURER (man or woman with good academic qualifications) in MATHEMATICS; and a LECTURER (man or woman with good academic qualifications) in MATHEMATICS—The Secretary to the Principal, Homerton College, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Universities Federation for Animal Welfare. Report and Accounts, 1st April 1966—31st March 1967. Pp. 38. (London: Universities Federation for Animal Welfare, 1967.)

Forestry Commission. Booklet No. 17: Thinning Control in British Woodlands. By R. T. Bradley. Pp. 31. 10s. 6d. net. Booklet No. 19: Timber Extraction by Light Agricultural Tractor. By J. W. Barraclough. Pp. vi + 41 + 12 plates. 5s. net. Leaflet No. 5: Fomes annosus: a Fungus Causing Butt Rot, Root Rot and Death of Conifers. Pp. 11. 2s. net. Leaflet No. 43: Keithla Disease of Western Red Cedar, Thuja plicata. Pp. 7. Forest Record No. 52: Home Grown Roundwood: a Survey of Estimated Industrial

Requirements 1965—80 Compared with Potential Production. By B. W. Holtam. Pp. 34. 4s. 6d. net. Forest Record No. 60: Procedures Used for Progeny-Testing in Britain with Special Reference to Forest Nursery Practice. By R. Faulkner. Pp. 22 (5 plates). 3s. net. (London: H.M. Stationery Office, 1967.)

Society for Psychical Research. Report of the Annual General Meeting held on Saturday, April 22, 1967. Pp. 6. (London: Society for Psychical Research, 1967.)

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Other Countries

U.S. Department of Commerce: National Bureau of Standards. NBS Monograph No. 102: Effects of Finite Lattice Heat Capacity on Spin-Lattice Relaxation: Theory and Numerical Analysis. Ry Robert L. Peterson. Pp. iii +21. (Washington, D.C.; Government Printing Office, 1967). [298]
Records of the Australian Museum. Vol. 27. No. 6 (14th July, 1967). Skull and Tooth Variation in the Genus Perameles. Part 1: Anatomical Features. By L. Freedman. Pp. 147-166. 90c. Vol. 27. No. 7 (14th July, 1967). 8401. [298]
1967): A New Species of Attenuatella (Brachiopoda) from Permian Beds Near Drake, New South Wales. By J. B. Waterhouse. Pp. 167-173 + plate 24. 30c. Vol. 27. No. 9 (21st July, 1967): Skull and Tooth Variation in the Genus Perameles. Part 2: Metrical Features of P. nassuta. By L. Freedman and A. D. Joffe. Pp. 183-195 + plates 28-31. 60c. Vol. 27. No. 10 (21st July, 1967): Skull and Tooth Variation in the Genus Perameles. Part 3: Metrical Features of P. nassuta. By L. Freedman and A. D. Joffe. Pp. 197-212 + plates 32-34. 70c. (Sydney: The Australian Museum, 1967.)

World Health Organization. Technical Report Series, No. 369: Arboviruses and Human Disease—Report of a WHO Scientific Group. Pp. 84. (Geneva: World Health Organization; London: H.M. Stationery Office, 1967.) 4 Sw. francs; 6s. 8d.; \$1.25.

[298]
Ministère de l'Agriculture et de la Colonisation du Québec: Le Conseil des Recherches Agricoles. Recherches Agrinomiques—Sommaire des Résultats, 1965/1966. Pp. 79. (Québec: Ministère de l'Agriculture et de la Colon sation, 1966.)

Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series, No. 19: Lands of the Isaac—Comet Area, Queensland. Comprising papers by R. Story, R. W. Galloway, R. H. Gunn and E. A. Fitzpatrick. Pp. 151. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1967.)

Report of the King Institute of Preventive Medicine, Guindy, for the period from 1st April 1964 to 31st March 1965. By Dr S. Govindarajan. Pp. 11. (Guindy, Madras King Institute o

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Mr Wedgwood Benn's Epistle to Caithness

MR WEDGWOOD BENN obviously fancies himself as a letter writer but, on the evidence of his letter to the fast reactor men at Dounreay, he has some way to go before he ranks with St Paul and Jonathan Swift or even Sir Harold Nicolson. To be sure, when he warned the people at Dounreay against the blandishments of the Westinghouse Company, he did not know for certain that there was devaluation just around the corner. That might have made him choose more subtle phrases or even silence. In the event, however, no great harm seems to have been done. Those already talking to the company have not been maddened by the unction of Mr Wedgwood Benn's declaration to clinch the deal. Those already determined to stay because they like their work or their life have seemed not to have been persuaded to reconsider their decisions now that they have been told that they should stay in Britain out of a sense of guilt, not necessarily from a sense of purpose. Everybody will be glad of

At the same time, it would be a great misfortune if the content of Mr Wedgwood Benn's letter were to be forgotten simply because it has done as little harm as good, particularly in a week when the international value of the British currency has had to be reduced. The sad truth is that Mr Wedgwood Benn has fallen into a great many of the pitfalls in the relationship between corporate employers and technical employees which the Jones Committee on the brain drain was complaining about only a few weeks ago.

The problem which must now be faced quite squarely by employers of technical manpower in Britain and other countries similarly placed is that for all practical purposes there is now an international market in technically skilled manpower. This, in a nutshell, is the message of the Jones Report. This is the reason why that committee spent so much of its time urging that industrial employers should now be cajoled into paying better salaries to scientists and engineers. This is why it asked that means should be found of promoting more scientists and engineers to positions of responsibility. And what applies to industrial employers should also apply to the British Government. In short, even if Mr Wedgwood Benn is right in his assertion that Westinghouse is making a deliberate attempt to tempt away skilled people with special knowledge of the fast reactor now being designed in Britain, it cuts very little ice for him to say in reply that "no Westinghouse establishment has got the facilities, scope or resources that Britain has made available to you through the Atomic Energy Auth-

ority". A part of the trouble is that continuing uncertainty about the future of the Atomic Energy Authority does not fully reassure those who fear that the splendid work so far accomplished as part of the fast reactor programme will be fully and energetically exploited. But it is also entirely proper that scientists and engineers should be concerned with the kind of life which they and their families are able to enjoy. There is—or there should be—no shame in making cold-blooded calculations that life could be more attractive elsewhere.

But how to strike a balance between emigration and staying at home? Skilled people throughout Europe are continually having to make this kind of calculation. This is not merely a recurring necessity but an important intellectual exercise in its own right, and it deserves to be brought more explicitly into the open. In particular, it is wrong to hide from the plain fact that the economic standard of life, and quite often its quality, is demonstrably better in North America than anywhere in Europe. And who is to say that a British scientist or engineer behaves scandalously by going off to the United States to work if he does so because of a cold-blooded calculation that his children would then have a better chance of going to a university? Who can complain if somebody chooses to leave for the United States because he and his family, for any one of a host of reasons, will then be happier? Everybody has his own calculation to make. On the face of things, at least, the benefits of crossing the Atlantic towards the west are bound to be immense for a great many European families. The wonder is not that there is such a flood of emigrants, but that so many people stay behind in places like Dounreay.

This is the point at which the sharpest rejoinder to Mr Wedgwood Benn's epistle suggests itself. true, of course, that special groups of people such as the designers of the fast reactor now being built in Scotland are likely to be particularly valuable to the Atomic Energy Authority in the years ahead. So why not pay them more? Money may not be everything, but it is important—even in remote places such as Dounreay. And although salaries in the Atomic Energy Authority compare favourably with those in the Government service proper, and even with those in industry, they are by no means the kinds of salaries which give those who receive them a sense of being well paid. Much younger men with a flair for knowing about computers can often earn much more, for example. But, worse still, there is a great rigidity about the pattern of salaries in the public corporations.

The hierarchical pattern cannot be too much distorted without causing trouble. And for all the technical excitement of the work they do, the people at Dounreay must be well aware that there is very little prospect of the kind of salary which, in Britain, is to be counted as more than merely modest. So much can be told from the way in which Sir Richard Clarke was boasting modestly, earlier this week, of the way in which the Ministry of Technology now employs more than seventy people at salaries of more than £5,000 a year. The trouble, of course, is that a good many quite small companies could probably make a prouder boast. And if somebody from Dounreay decides to try his luck in industry, is there any reason to expect that he will first bestow his favours on some British company? The Jones Committee made several useful suggestions for improving the attractiveness of British industry, but nothing much has been done about them yet.

It is more difficult to make a list of the reasons why people stay in Europe, although this is just as important. But it would be wrong to think that mere inertia has led the continued presence of people with saleable skills in Western Europe—even as far north For one thing, Europe remains an as Caithness. interesting place in which to live. People are varied and enterprising. The place is intellectually alive. The rewards are not always cancelled out by the frustra-Yet there is one quality of life in Western Europe and particularly in Britain which is increasingly a thorn in the flesh of those who have not yet emigrated. In one way and another, the pattern of life has become too rigid and inflexible. People may be fully agreed on some course of action but apparently powerless to follow it through.

The events of the past few months in Britain contain many splendid illustrations of this sad truth. Every-

body has agreed, for twenty years, that there will have to be some kind of national reference library for science and technology, but it will be the best part of a decade yet before the real need is met. The educational system is ripe for change, particularly at the interface between the schools and the universities yet nothing much is happening (but see page 740). The Economic Planning Council for the South-East of England has produced a plan the most obvious virtue. of which is that nobody is likely to take it seriously. Closer to Mr Wedgwood Benn's parish, the Atomic Energy Authority remains uncertain of its future after what amounts to between two and four years of continuous uncertainty. Occasionally it seems as if any change, more or less in any direction, would be better than the accidie, which seems from time to time to infest some parts of Western Europe.

There is no doubting Mr Wedgwood Benn's views and his ministry's objectives on this general issue. Almost by definition, they are on the side of change. Yet there remains a curious air of stillness. Things stay much the same, and those who are most hamstrung by circumstances are also prevented by circumstances from battling on their own behalf. The fast reactor men are good examples. In this sense the paternalism of Mr Wedgwood Benn's assurance that he will regard fast reactors as his favourite children from now on will be less valuable than would have been some greater sense that the men involved in the development of fast reactors could also have felt more able to influence the uses that will be made of these machines in the years ahead. This is the direction in which Mr Wedgwood Benn should push. It is also a point which he should keep in mind when reading the report of the Select Committee of the House of Commons on the nuclear power industry.

Relations between Universities and Schools

THE meeting of the academic advisory committee called by the Committee of Vice-Chancellors (see page 740) has been a great success, but it would be wrong to think that there are now no problems left in regulating the transition between schools and universities. For several months there have been signs that the universities have been coming round to the belief that a more liberal curriculum in the sixth forms would be a great advantage. A host of university lecturers in economics, now forced to spend time teaching mathematics to undergraduates, are among the most recent recruits to this point of view. Yet there cannot at this stage be any certainty that the enthusiasm for change among the delegates to the vice-chancellors' meeting will be fully echoed in the universities.

Even if there should be general agreement that reform has somehow become essential, the universities are likely to be hard pressed in putting forward some constructive alternative to the proposals of the Schools Council. Should there be four subjects or five in the school-leaving curriculum? How great should be the element of compulsion? It would be a splendid

thing if the universities and schools could agree that mathematics should be an essential part of every university entrant's studies, but this is asking a great deal. Another complex of problems yet to be tackled is the extent to which the universities will accept one of the most radical implications of the course upon which they have embarked—the likelihood that many students arriving at universities will have had no specific preparation in the particular specialisms of of which they wish to make a special study. This will be a sharp break with the British tradition, but it has also become a necessity. In the circumstances, nobody will be surprised if many universities decide that most undergraduate courses will have to last four years and not three.

There are also formidable problems to be tackled in the relationship between the universities and the schools. In the past few years, the pressure of the competition for places at the universities has persuaded many schools that the most urgent need is somehow to reduce the burden of external examinations. This is the origin of the Schools Council's plan for a sixth

form curriculum in which students seeking entrance to a university would follow only two externally examined courses. The university representatives at the vicechancellors' meeting were right to argue that these proposals would narrow and not broaden the education of students reaching the universities, but this seems not to be the present interest of the schools. Obviously it will be hard for the universities now to push them in the direction of extra examinations. The fact that *the examinations may be less exacting will not entirely comfort those schools in which teachers value jealously their right to mimic the universities in the sixth forms. Evidently the universities will need not merely a reasonable and coherent case to put to the Schools Council in the spring, but tact as well. If they are wise, they will pay close attention to Professor Butler's argument for deliberate experiment, and there is, of course, no reason why the universities should not elect, without further discussion, to embark on some of the controlled experiments which the introduction of a more liberal curriculum in the schools will make essential. But if this is to be the method, the universities will have to hurry. Britain has waited too long already for a more sensible pattern of education in the schools. If there are to be experiments, there is no reason why they should not begin in September 1968.

Hung Jury

ALTHOUGH it is too soon properly to assess the first report of the Select Committee on Science and Technology appointed by the House of Commons, one thing is already clear—the committee has done its reputation a great disservice by coming out with a report which is crudely divided on party grounds. The committee's credibility has already suffered from its lack of the professional assistance that would have enabled it to ask more penetrating questions. Its failure to agree on proposals for the reorganization of the civil nuclear power industry is a greater setback. The committee might have done better to contain its ambitions within its capabilities, on this first exercise at least.

"In present circumstances, the best interests of the country would be served by the combination in a single organization or company of the skill and resources of those now separately engaged in the design and construction of nuclear boilers." With this sentence the Select Committee on Science and Technology finally resolved discussion of its examination of the nuclear power industry in Britain. The report of the committee, published on November 22, comes down. by a majority of 7 to 5, in favour of what has become known as a "central design authority". sortium system, it says, should be phased out, and the generating boards should be free to place contracts with individual companies for nuclear stations in the same way as they do for conventional stations. The commercial research and development of the AEA should go to the single nuclear boiler organization or company. The AEA should, however, retain its responsibility for pure research, and the Government should carry out a full review to make sure that it is concentrating on pure research, and that any functions not "inextricably linked" to the primary task are

passed to more appropriate organizations. This clearly is a reference to the diversification into fields such as desalination which the AEA has been engaged on.

In the production of fuel, the AEA monopoly should be broken by the establishment of a new British fuel supply and manufacturing company, which should include the AEA and others-Rolls-Royce is one possibility. In the export market, the British Nuclear Export Executive should be wound up, but the committee proposes no new organization to take its place. Instead, there should be a survey by the Board of Trade of export markets-hardly an inspiring rallying cry. The committee seems enthusiastic about high temperature reactors—partly on the evidence of the sub-committee which visited Europe—and says that high temperature reactor research should be supported. and that the Dragon reactor should be kept going, if necessary by Britain alone. Water reactors, the committee says, and particularly the steam generating heavy water reactor, should be speeded up. Finally, marine nuclear power is given support, if only in the recommendation that a departmental committee should be set up to examine the possibilities. As for fusion, the committee says that the Ministry of Technology should review the whole field of fusion research to ensure that Britain takes advantage of any technological breakthrough.

The committee seems keen to chip away at the power of the AEA. The AEA holds, it says, "a position of considerable advantage". In another place, it is described as "virtually unassailable". To counteract this, a technical assessment unit should be set up to advise the Government on the merits of AEA projects. The committee would also like to see the emergence in Britain of a body like the United States Joint Congressional Committee, with an expert staff.

The questions which the report leaves unanswered give plenty of room for further argument. If the AEA is to be divided, where will the division fall? Will the change in responsibility of the AEA need parliamentary legislation? And the greatest ambiguity of all—will the central design authority be an organization like the AEA is now, or will it be more like a nationalized industry? Throughout the report, it is referred to as a "single organization or company", no doubt in part to give way to Conservative critics. The saddest thing about the report is that it is not unanimous; the division along party lines may well have the effect of reducing the argument to party terms.

Counting Heads

In Britain, more graduates are going on to further study or training than are entering industry. situation, first documented by the Swann Committee report, has been substantiated by a report just issued by the University Grants Committee. But there has been a narrowing of the gap between the two careers —the number going on the postgraduate training or research rose from 12,084 in 1964-65 to 13,417 in 1965-66, while the number going into industry went up from 10,904 to 12,851. If the trend continues, industry will this year be taking almost exactly as many graduates from British universities as choose to remain in academic life or teaching. The balance, almost 20 per cent, includes overseas students going home. British students going abroad, married women and the unemployed. Happily, the last category is comparatively small; at the end of December 1966, 3 per cent of arts and social science graduates were still looking for work, while only 1-6 per cent of scientists were still looking. While the trend is an encouraging one, not too much should be made of it, as for the first time the colleges of advanced technology have been included in the list—and they send 67 per cent of their graduates into industry.

The report shows that the total number of graduates in 1965-66 was 32,166, while 5,821 qualified for higher The statistics show how many of these graduates went overseas, either permanently or temporarily. Among first degree graduates, only a small and relatively constant proportion admitted to joining the brain drain; in pure science, for example, 1.2 per cent of men went overseas for further training or research while 1.3 per cent took permanent employment overseas and 1.5 per cent temporary employment overseas. The figures are higher for applied science— 3.4 per cent of men graduating in applied science gained permanent employment overseas. Among higher degree graduates, the proportions are much higher; 7 per cent took up permanent employment overseas, and 6 per cent took up temporary employment. The figures for higher graduates in pure science show an interesting trend; in 1965, 13.9 per cent took up permanent employment overseas, while only 4.7 per cent took short-term employment. By 1966, however, possibly as a result of the adverse publicity of the brain drain, only 6.8 per cent admitted to taking permanent employment overseas, while the number who said that their overseas employment was short term had shot up to 11.8 per cent. The total, 18.6 per cent, is exactly the same in each year, so it looks as if employers abroad, or graduates going abroad, are responding to criticism of the brain drain by claiming that the appointments are short term. Applied scientists have no such scruples—only 1.8 per cent said that their jobs overseas were short term, while 8.5 per cent said that they were permanent.

Strings with the Money

The University Grants Committee has now sent to Vice-Chancellors a memorandum which sets out the pattern for universities over the next five years. The financial allocation was made public several weeks ago, and the UGC has now followed this up with some indication of its philosophy. The memorandum does not attempt to establish hard and fast rules for university development, but tries to put the financial provisions into context. "... It did seem at this point of time there would be certain background factors or general lines of development to which it would be proper for the committee to call attention."

The most startling recommendation in the memorandum is made in an almost throwaway style. After saying that undergraduate numbers are "a genuine priority" because of pressure from the schools, the report says that "the major increase must be in the number of arts-based, rather than science-based, students". This recommendation is made in the light of A-level trends, which show the now familiar swing away from science. As for postgraduates, the UGC says that the expansion it has allowed for is in many cases smaller than the universities wished, and gives a

number of reasons for this; as well as the pressure on undergraduate places, there is the fact that the proportion of undergraduates going on to higher degrees already exceeds the Robbins estimate, and that too many are staying in the universities. Finally, the slowing of the expansion in the universities means that the demand for recruits to university staffs will slacken off.

There is also talk in the memorandum of the unit costs of universities. In a passage which will no doubt alarm some Vice-Chancellors, the UGC says that the unit costs of universities are one of the considerations which should be taken into account in the distribution of grants. The UGC, the memorandum says, has a considerable amount of information on the subject, and it has "made some adjustments where there seemed to them to be under—or over—financing of individual universities in relation to their responsibilities".

The memorandum also makes encouraging noises about collaboration between universities and between universities and industry. Collaboration with institutions in the "non-autonomous" sector should also be encouraged, and the committee welcomes the investigations into the use of plant and equipment which the Committee of Vice-Chancellors and Principals has set in motion. In letters to universities, the UGC has indicated certain areas which it believes merit greater support, and for which more money has been supplied (though not on an earmarked basis). These include libraries, for which expenditure should increase by 20 per cent, administration, audio-visual aids, computer maintenance, and other developments such as dentistry, management studies and town and country planning.

Other areas, on the other hand, get a cooler appraisal. The committee has recommended that agriculture departments at three universities should close, and that some concentration of resources in agricultural economics is also desirable. Although there should be more student architects, there should not be more schools of architecture; equally, there should be no more schools of biological sciences, although the committee has not yet recommended that any be closed down. Social studies should see a big expansion, but mainly at universities already entrenched in the subject. Particular importance is attached to the development of economics, law and statistics.

New Director-General

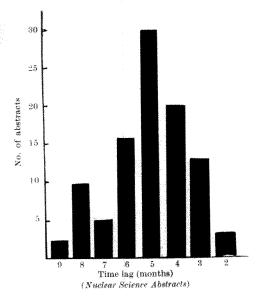
On November 20, Dr A. H. Boerma was elected Director-General of the United Nations Food and Agriculture Organization. He succeeds Dr B. R. Sen of India. Of Dutch origin, Dr Boerma is no stranger to FAO: for the past five years he has been Executive-Director of the World Food Programme. Before that he represented the Netherlands on the FAO council before becoming the organization's regional representative for Europe from 1948 to 1951. Dr Boerma has not so far committed himself publicly to a policy for FAO in the years ahead, but the annual conference now being held in Rome will be an opportunity for him to take soundings among the member governments. It will be interesting to see whether a man from the developed world will be entirely acceptable to underdeveloped countries. At the FAO as elsewhere, however, change at the top can be enlivening by itself.

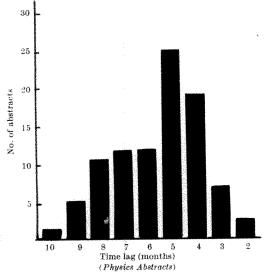
Slow Abstracts

How useful are abstracts? Because of the expansion of scientific literature, many of the functions once performed by scientific journals are now taken over by the abstract journals. By scanning more primary publications than any individual is able to, the abstracts provide a running record of what has been published

and keep their readers up to date.

But examination of two abstract journals in physics Physics Abstracts, published by the Institution of Electrical Engineers, and Nuclear Science Abstracts, published by the United States Atomic Energy Commission—shows that their readers are by no means as up to date as they might be. The distribution curves show the time lags calculated for October editions of both abstract journals. The delay times are those between the publication of the original paper and the appearance of an abstract of it. The curves were calculated from the dates of original publication given in the abstracts. The figures thus take no account of the abstracts for which no date is given. The figures are based on two counts, each of 100 abstracts, taken at random from corresponding editions of Physics Abstracts and Nuclear Science Abstracts. The curves





show that Nuclear Science Abstracts is slightly the quicker of the two-the median date of publication for abstracts listed in the issue of October 15 was June 4, 1967. For Physics Abstracts, published nominally on October 1, the median date of original publication was May 18, 1967. Physicists thus had to wait nearly five months in order for abstracts to appear. In some cases, of course—and particularly in Nuclear Science Abstracts-they had to wait even longer; a cursory reading of the journal brought to light a British patent-"Improved Process for the Production of Graphite" granted as long ago as January 11, 1961, and with a priority date in France of February 29, 1956. Unfortunately, it is not only patents which take a long time to seep through; the same issue of Nuclear Science Abstracts contains a Russian paper published in English translation in January 1961, and an article from New Scientist ("Medical and Surgical Uses for the Laser", by Leon Goldman) which appeared in January 1964. Really out of date papers are much less common in Physics Abstracts, possibly because it does not include patents.

The IEE is conscious of the delay, and is hoping to accelerate the process and reduce the backlog. The recent move of the publications department to Stevenage has caused transitory difficulties, but should ultimately help to speed the process. But, despite this, there will still be an "unavoidable delay" of at least two months. Computer techniques are unlikely

to help, at least in the short term.

Foot and Mouth Vaccine

A NEW factory has been built at Grossburgwedel, outside Hanover, for the production of foot and mouth disease vaccine. The factory, which was planned, built and ready for operation within one year, is the seventh link in a chain of production units established by the Wellcome Foundation Ltd to meet demands from those countries where control measures are based on vaccination. It was opened on November 17 by Dr Fred Wrigley, deputy chairman of the foundation.

Vaccine will be produced at Grossburgwedel by a suspended cell culture technique developed by Wellcome which permits bulk production. Vaccine against those types of foot and mouth disease virus known to occur in Germany will be produced, but facilities are also available for switching production to other virus

types at short notice.

The new unit, which cost about £0.5 million, covers an area of some 40,000 sq. ft. and comprises three sections. Vaccine is produced in the main laboratory building and filled and packed under a rigid control system. Vaccine is tested under the control of a government veterinary officer in the testing station, which is the second section; the third section is a quarantine stable which provides accommodation for cattle and pigs. The research team at Gross-burgwedel will be led by Dr Rolf Bandau.

Foot and mouth disease vaccine is also produced in Wellcome factories in Spain, Kenya, Uruguay, Argentina and Brazil. In Britain and Eire, unlike other European countries, vaccination against foot and mouth disease is not used. It will be interesting to see whether the British Government will be compelled to change this policy by the current epidemic, still spread-

ing.

Relaying Telegrams

THE General Post Office recently opened in London a new telegraphy relay centre, designed to speed up the transmission of overseas telegrams. As well as sending out cables which originate in the United Kingdom, the centre, at Cardinal House in Farringdon Street, will also handle the large quantities of telegrams which simply pass through London on their way from, say, Montreal to Sydney.

The new system is almost entirely automatic, but because automatic routing requires an extra line in the message—equal in length to 10 per cent of the average cable provision is made for manual routing for telegraph administrations unwilling to pay extra. Messages arriving at Cardinal House are stored temporarily in ferrite stores and, if there is a suitable line available, are retransmitted immediately. If there is no line available, the messages are stored on magnetic tape to wait for a suitable line. The ferrite stores are small—2,000 characters—but the tape stores have a capacity of 40,000 characters each, and can if necessary accumulate messages over a period of hours if any outgoing line is out of action. Messages stored in this way are retransmitted in order of priority. system replaces a much cruder arrangement at Electra House in which cables were punched out on tape, torn off manually and retransmitted on another machine.

The GPO is now looking forward to the ideal system—still ten years away—which would be automated from start to finish and controlled by computers. As well as routing the cables, the computer could also be used to work out the bills.

More Fire-raising

Fire Research 1966, published on October 17, is the annual report of the Joint Fire Research Organization of the Ministry of Technology and the Fire Offices' Committee. This partnership between government and insurance came into being for the purpose of carrying out research into all aspects of fire and the danger it represents to both life and property.

According to the report, the direct fire losses are of the order of £75 million a year, with a further £75 million in indirect losses. In addition to this, the fire brigade service costs about another £75 million. The size of fires has on an average decreased, but there are now considerably more of them.

The Fire Research Station, which costs £300,000 a year to run, carries out research and also undertakes a great deal of testing for industry, government and insurance. A study of the statistics of incendiarism suggests that more fires may be caused by arson than are reported, and, moreover, the cost of these fires is likely to be greater than for fires started by other means. Of the ten most recent fires estimated to have cost £1 million each in direct fire loss, three have been attributed to incendiarism, six to unknown causes and one to smokers' requisites, as they are called.

The ratio of fire load to ventilation is an important factor in the extent of damage to structures and it is possible that the spread of fire in warehouses could be reduced or even prevented by providing water curtains. Smoke detectors have been found to give a much more rapid response than heat detectors, and there is evidence that a substantial reduction in fire losses could

be made by the provision of efficient early warning systems.

A new method of protection has been developed in the chemistry and chemical engineering section for protecting industrial equipment which contains sparking components or flames. The method involves incorporation of flame arresters in the casing of equipment so that, if an explosion should occur in the casing, the pressure is relieved through vents but flame does not spread to the outside; this, it is hoped, may result in a saving of £250,000 per annum.

Parliament in Britain

Students

THE Secretary of State for Education and Science, Mr P. Gordon Walker, gave the provisional number of full-time registered students in the universities of the United Kingdom on October 31, 1967, as 199,372, of whom 37,614 were new entrants to undergraduate and lower level courses. These figures compare with 184,510 and 54,058 respectively in the previous year. In addition, in Northern Ireland there were 5,230 students, including 1,489 new entrants, compared with 5,025 and 1,399 respectively in the previous year. (Written answer, November 16.)

Machine Tools

MR A. WEDGWOOD BENN, the Minister of Technology, stated that the National Research Development Corporation was managing on behalf of the ministry a scheme intended to encourage the wider use of numerically controlled machine tools. The scheme enables suppliers to offer machine tools to customers on a sale or return basis. Twelve manufacturers were participating in the scheme and three machine tools had been sold and delivered, with further sales in prospect. The scheme was capable of supporting sales to the value of £3.5-£4 million, but the response from users had so far been very limited. Mr Benn also stated that the Ministry of Technology was helping to establish low-cost automation centres at universities and technical colleges throughout the UK. (Written answer, November 10.)

Equipment Grants

BARONESS PHILLIPS said that the total amount of grant authorized for university expenditure on furniture and equipment in the current financial year was £25.5 million, and by July 1967 the universities had drawn about £8.5 million. Total drawings to date were about £14 million. (Answer, November 16.)

Research

In 1966-67 the Ministry of Technology spent £238 million on research and development in industry and within its own establishments. Dr Jeremy Bray, who gave the figures, said that the Government's policy was to place as many research and development contracts as possible in development areas, including Scotland. Total expenditure on research and development by the Ministry of Technology/Ministry of Aviation in 1966-67 was £289 million, of which £53 million was spent in the combined departments' establishments. Expenditure by industrial research associations in the financial year ending in 1966 was £13·2 million, and Government grants to the associations totalled £3·2 million. (Answer, November 14.)



Large meeting hall and library at Carlton House Terrace.

Moving House

The Royal Society has now completed its move to new premises at Carlton House Terrace, and the rooms were opened on Tuesday by the Queen, who is Patron of the Society. During the three centuries of its history the society has occupied six different sets of rooms, the most recent being at Burlington House, where it has been since 1857. The new rooms, built between 1829 and 1832 to the designs of John Nash, are part of a fine terrace with an imposing array of Corinthian pillars stretching the full length. The terrace faces St James's Park and the Mall, and the society will be occupying the four houses at the eastward end of the terrace, ending at the Duke of York's steps.

The striking appearance of the houses from the outside has been preserved, but inside there has been a radical rebuilding, including, in the case of number 9, the replacement of most of the load bearing walls below ground level. By removing walls, a lecture theatre capable of seating 250 people has been constructed in number 9, and the society's library will take up all the deep basements and all the first floor at number 6. All four houses have been knocked together to form one unit, modern heating and air conditioning have been installed and lifts and remodelled staircases have been built. Although the whole task could have been accomplished more cheaply by knocking down the old terrace and building anew, this would not have been

Much of the internal decoration, in a variety of styles, has been retained. The history of the houses is varied. Number 6 was once occupied by a Buenos Aires millionaire, Mr C. H. Sandford, who installed interior decoration of the most elaborate sort-mother of pearl ceilings, and Spanish and Florentine carvings and inlays-at the end of the nineteenth century. More recently, numbers 8 and 9 had housed the German Embassy in the era of Von Ribbentrop, and still bore traces of the taste of the Third Reich. Lord Holford, the architect for the conversion, barely conceals his feelings. In an article written for the society's own publicity, he describes the decoration: "Although it was not in a style (or mixture of styles) that would have been preferred by the Royal Society, or by any other learned society in the middle of the twentieth



century, much of the existing decoration had to be retained". The basements, he says, were dungeon-like and the construction of the buildings indifferent. Only unusual advantages of situation and environment

justified the conversion.

The society first showed an interest in moving to Carlton House Terrace in 1961. Originally it was thought that only Nos. 7, 8 and 9, with the basement of number 6, would be needed. In 1963, the Chancellor of the Exchequer gave his approval, announcing that the Government was prepared to pay the rent and annual maintenance for the 99 year lease of the four houses. The cost of conversion, then estimated at £500,000, was to be paid by the society. The floor space, 72,000 sq. ft., is more than two and a half times what was available at Burlington House. Furthermore, the President, Executive Secretary, and caretaker will all have flats in the new premises. (The present President, Professor P. M. S. Blackett, whose home is in London, will not be moving into his.) But the costs of the move were soon seen to have been underestimated, and they rose to £1 million. By making a variety of economies, the bill was reduced to £662,411, but further increases since then have increased it again, to some £850,000. Of this money the Nuffield Foundation gave £250,000, the Government £125,000 and the Wellcome Trust £103,000. Despite a number of smaller benefactions, the full amount has yet to be collected.

The Royal Society's move has brought comfort to others than its own fellows. The premises which it has vacated in Burlington House are now being divided between a number of societies. The Chemical Society of London has inherited the lion's share of the old apartments (and has launched an appeal to equip them properly). The Royal Astronomical Society, much cramped in recent years, has acquired some breathing space. Yet there remains among the societies a somewhat depressed nostalgia for the old dream of one building for all the learned societies in London.

Universities for Reform

The first private meeting on November 17 of the Academic Advisory Committee organized by the Committee of Vice-Chancellors seems to have been not merely a success but a landmark in the development of relations between British universities and British schools. One of the participants said afterwards that he had been astonished. "I had not expected anything like this." The outstanding feature of the proceedings seems to have been the unanimous agreement that the basis for selection into universities must be changed. In the process, the proposals put forward by the Schools Council for changing the present system seem not to have found many supporters.

Those present at the meeting in the Senate House of the University of London included most of the vice-chancellors of the British universities as well as academics nominated by the university senates. The chairman was Dr D. G. Christopherson, chairman of the Committee of Vice-Chancellors, and the audience ran to about 100. The proceedings were opened by Dr Geoffrey Templeman, Vice-Chancellor of the University of Kent and a member of the Schools Council, who described the origins of the Schools Council's proposal for a reform of the sixth form curriculum. A document embodying these proposals has already been circulated to the universities and will be published in the near

examined studies of a sixth form pupil should be restricted to two Advanced level subjects, and that there should be internally moderated courses (called "electives") as well

The nub of the scheme is that the externally

"electives") as well.

The surprise at Friday's meeting seems to have been the way in which the principal speakers, like those who followed them from the floor, argued in favour of a broader curriculum and against what seemed to them to be the narrowing character of the proposals of the Schools Council. The ball was set rolling, from the platform, by Professor J. T. Allanson of the University of Birmingham, who argued that the preparation at school for a scientific education should be more liberal; he would see the present pattern of three A-levels broadened to include at least four courses in the sixth form. Professor J. G. Wilson followed next, with the argument that the pattern of entrance to universities determined by performance in three A-level courses is one of the reasons for the drift from science to the arts subjects; in his view, too, the Schools Council's scheme would hinder and not help. The same theme seems to have been echoed at the conference by Mr J. Morrison, Principal of University College, Cambridge, who argued instead in favour of a pattern of five courses in the sixth form, spanning the arts and the sciences. By all accounts, this was very much like the scheme which Mr Morrison described at the Cambridge conference reported in Nature on September 23, 1967.

One experienced and philosophical observer of discussions among university representatives on academic policy said after the meeting that it was a great surprise that "not one voice was raised against the general character of the objections against the Schools Councils' proposals". The general opinion seems to have been one of sympathy with the objectives of the Schools Council, but scepticism about its methods. Dr F. S.

British universities seem prepared for change in entrance requirements.

Dainton, Vice-Chancellor of the University of Leicester, is known to have supported Professor Wilson's argument. Vice-Chancellors Aitken, Swann and Edwards (of Birmingham, Edinburgh and Broadford) are known to have intervened in favour of a broadened curriculum. Lord Annan (né Noel Annan) seems to have come out on the side of the angels in a characteristically impish way.

Professor Bryan Thwaites argued strongly at the conference for a course in mathematics designed for all students in British sixth forms. By all accounts, Professor Thwaites's proposals have already been put to the Schools Council, but the appropriate committees seem so far to have been unable to resolve the differences between the pure and the not-so-pure mathematicians, with the result that the council has not yet embarked on the development of the appropriate curriculum. The view at the meeting seems to have been that if compulsion of any kind is to be imposed on students leaving British schools, courses in mathematics and English should be obligatory.

What will this mean for the universities? One view, championed by Professor L. R. B. Elton (University of Surrey), was that the universities should somehow be able to accommodate more liberally educated entrants within their present three-year pattern of undergraduate courses. There seemed, however, to be more support for the notion that a more liberal sixth form would bring with it the need for four year courses. On this occasion, the universities seem to have been less readily disposed than on previous occasions to assume that such a development is unthinkable on financial

grounds.

The day's discussion seems, by all accounts, to have been splendidly capped by a stirring speech from Professor C. C. Butler (Imperial College), who urged the need for reform and the need for an evolutionary approach to it. He did not think that the British system could be made to change overnight, and he urged that the universities should collaborate with the schools in a number of experiments designed to test the feasibility and the advantages of different patterns of entrance qualifications. What he has in mind, by all accounts, is a series of experiments in which selected groups of schools would be encouraged to embark on alternative patterns of sixth form work in return for an undertaking by the universities that pupils so involved would not be handicapped in seeking entrance to universities.

But a heady day plotting reform does not mean that everything will now be different. For one thing, there is no certainty that the opinions of the university representatives last week will be confirmed by university senates, which are being asked by the Vice-Chancellors' Committee to submit their first comments on the proposals of the Schools Council by the end of this year. Early in the new year there is likely to be a discussion within the Standing Conference on Entrance to Universities from which, with luck, there may emerge a co-ordinated university view to put to the Schools Council by the spring of 1968. On the present showing, it seems very much as if this reply will take the form of an alternative proposal.



AND VIEWS

about the Cosmic Microwave Background

vidently something of a boom in studies ural phenomenon known as the cosmic background. This issue of *Nature*, for ntains no fewer than four articles on various

aspects of the subject. Elsewhere, the first results are beginning to appear of the refined methods of measurements designed two years ago when it was first recognized how tangible and how potentially important the microwave background may be. The group at Princeton University, for example, has within the past few weeks reported measurements of the background intensity at wavelengths between 8 mm and 10 cm which imply more certainly than previous measurements that the electromagnetic spectrum does accurately correspond to that of a blackbody. that the temperature obtained by this group—2.7° K is somewhat lower than that reported from other laboratories, usually with longer wavelengths, so that there is still room for argument on the central issue of whether the microwave radiation which seems to pervade the universe does indeed correspond to blackbody radiation, but this is unlikely to be an important The uncertainties of measurement are still great enough to make the comparison of measurements made by different instruments a somewhat hazardous undertaking.

That said, there is obviously a great deal to be learned from the studies of the microwave background which are already under way. The detailed study of the isotropic character of the radiation is obviously of the greatest interest. The point here is that the motion of the Solar System through the microwave background should be reflected in an anisotropy of the measured background. In other words, the apparent temperature of the radiation should vary from one direction to another. Hitherto it has been supposed that this phenomenon would be determined largely by the speed at which the Solar System is moving as a consequence of the rotation of the galaxy—a speed of 300 kilometres a second or thereabouts. The interest of the argument by Drs Stewart and Sciama (see page 748) is that it shows how the same kinds of measurements may throw light on the structure of the universe on a somewhat larger scale. In particular, sufficiently refined measurements of the microwave radiation may be able to suggest whether the local cluster of galaxies is rotating, and at what speed. The authors of this argument may have underestimated some of the difficulties of making precise measurements of the background—the problems are concerned not so much with the design of instruments as with the elimination

of external sources of interference. But the prospect which they offer is exciting.

This, however, is only one of the possibilities now to be followed up. The possibility of an interaction between fast electrons in the cosmic rays and photons of the microwave background is intriguing and potentially important. For sufficiently fast electrons, for example, even a sea of radiation corresponding to a temperature of 3° K or thereabouts would be practically opaque, which has led to the suggestion that there should be a limit to the energy of the cosmic rays, which can reach the Earth. But with the cosmic rays, as with the radiation itself, the search for anisotropy may be profitable. Evidently the boom in the microwave background is only just beginning.

Measurements and Standards

from a Correspondent

A CONFERENCE on radio-frequency measurements and standards was held in the Glazebrook Hall at the National Physical Laboratory last week. The Institution of Electronic and Radio Engineers, in collaboration with the Institution of Electrical Engineers, organized the conference to coincide with the inauguration of the British Calibration Service. Following the opening by Sir Leonard Atkinson, president elect of the IERE, eighteen scientific papers were read by authors from government, industrial and university backgrounds. The British contribution to the science of r.f. measurement in the range 100 KHz–1GHz was particularly stressed.

Development of techniques, comparable with the best available internationally, was the keynote of two contributions from the Electrical Inspection Directorate of the Ministry of Technology. The papers covered the design of a precision bridge and outlined methods for carrying out both attenuation and power measurement to a high degree of accuracy. A paper entitled "Noise Source Calibration in the Decimetre Band" described work carried out in the Services Valve Test Laboratory which, when coupled with a complementary paper from Ferranti Ltd. Edinburgh, on a noise source primary standard, set up new and improved standards for noise measurement.

Novel solutions to a number of problems appeared in the contributions from industry. Marconi Instruments put forward a design of co-axial thermocouple and associated r.f. load using thin film techniques which can be used to measure r.f. power to an accuracy of 5 per cent from below about 1 GHz, as the basis for a new range of commercial absorption power meters. In the measurement of lumped immitance, Wayne Kerr out-

lined the so-called crevasse entry technique and showed its corresponding impact on design of r.f. bridges.

In describing a technique for measurement of impedance within a micro-circuit module, authors from the University of Southampton showed how they had been able to achieve a satisfactory connexion between circuit and measuring bridge by use of a micro-strip line. Certain authors chose to support their papers by practical demonstrations of relevant equipment. It was interesting, after a paper on attenuation measurement, to see Russian and American standard attenuation measuring equipment compared side by side.

Whilst the conference succeeded in its prime aim of highlighting British work on r.f. measurements, it must be regretted that no corresponding European contribution was forthcoming.

New Way of Looking at Plants

The scanning electron microscope, with its wide depth of field giving almost three-dimensional pictures, is clearly going to be very useful to botanists. At the meeting of the Linnean Society on November 16, Dr P. Echlin described some of the techniques being developed for use with this microscope, and Professor V. H. Heywood showed how these techniques can be particularly valuable to the taxonomist.

The advantage of the machine is that whole specimens can be viewed rather than the sections which are necessary with the transmission electron microscope. Dr Echlin and his colleagues at the University of Cambridge are looking for the best ways of fixing the specimens, which he had classified as robust dry, robust wet and labile. Robust dry specimens, such as pollen grains, seeds and fruits, can be glued directly on to a clean surface. Dust, which will show up in the pictures. is a considerable problem, and at Cambridge a vacuum pump is used as a mini-vacuum cleaner to keep specimens as clean as possible. The robust wet specimens. which include diatoms and some algae and microfossils, can be placed without glue on to a surface and dried in a dust free atmosphere. Labile specimens present most problems, and although they can be dried by more conventional methods, such as passage through a series of alcohols, freeze drying has proved the most successful. Dr Echlin has developed a technique which involves dipping specimens into isopentane or 'Freon 22' cooled in liquid nitrogen. Using this technique, Chlorella can be fixed without collapsing. as it does when dried by more conventional methods. Blue-green algae and bacteria are also successfully fixed in this way.

Although the scanning electron microscope can give little information that cannot be obtained from serial sections in the transmission version, the use of whole specimens makes investigation very much easier. In one hour, perhaps five or six pollen grains or fruits can be examined and photographed. This is valuable for people such as Professor Heywood and his team at the University of Liverpool. They are conducting a taxonomic study of the tribe Caucalidae of the Umbelliferae, and are collecting characters from as many parts of the plant as possible. When examining the fruits for distinguishing characters, and having found more than forty with little effort, the team began to use the scanning electron microscope. This is revealing many



The apex of a main spine on the fruit of Torilis leptophylla (x 1,250).

features of the ridges, hairs and spines of fruits which were previously not seen, or were seen but not understood. These are features such as the apex of the spine on the fruit of *Torilis leptophylla* which is shown in the photograph. *Turgenia latifolia*, for example, has revealed surface projections on the fruit which are unique to the species.

Now that taxonomists are finding more characters than ever before, they have the problem of handling all this information. To begin with, a new terminology is needed to describe the surface features of the fruits, seeds or pollen grains. Methods of scoring and processing the data which are being so effectively recorded have also to be developed before the full value of the new techniques can be appreciated.

Elementary Genetic Control

from a Correspondent in Cell Biology

Factors which control gene activity of necessity control development. They are elusive, but there are indications that the control of some chromosomal genes is the product of some structural feature of the chromosome itself. There is one well known example—in female mammalian cells genes on one of the two X chromosomes are inactivated because one of the chromosomes becomes condensed or heterochromatic. Whether the paternal or maternal X chromosome is inactivated in this way is a matter of chance.

This process of inactivation has been explored by Cattenach and Isaacson (Genetics, 57, 331; 1967). In 1965 Cattenach showed that when part of the autosome carrying some of the genes controlling coat colour is inserted into one of the X chromosomes (X^T), these genes may be inactivated, but to a variable degree. One result is that two lines of mice with distinctive coat colour patterns can be selected and the stability of the inactivation studied directly by breeding experiments. In practice the inactivation of X^T is found to change on passage through several generations. Cattenach and Isaacson account for this change in the state of gene activity by proposing that there is a "controlling element" on the X chromosome which

influences the inactivation of the genes on the inserted segment, most probably by influencing the degree of heterochromatinization of the inserted segment. The same experiments demonstrate that the potency of the

controlling element can vary.

Similar changes of the state of genes have been followed in maize. McClintock (Carnegie Inst. Yearbook, 65, 568; 1966) has shown that pigmentation in certain tissues of maize is not regularly inherited, which suggests a modification of gene control in the germ line—as Cattenach has suggested for the mouse breeding experiments—or in early embryonic growth. There are also parallels with certain models for the regulation of genes in bacteria.

In maize as well as mice, heterochromatin is implicated in the mechanisms for gene control. In the fruit fly it is possible to influence the degree to which gene loci, seen on the bands of salivary gland chromosomes, can be made heterochromatic by varying the temperature at which the flies develop. Hartmann-Goldstein (Genet. Res., 10, 143; 1967) finds correlation between cold-induced heterochromatinization of bands locating genes for two morphological characters and the reduced expression of these genes. Her work shows how gene activity can be manipulated experimentally by altering chromosome structure. But if the controlling elements are heterochromatin, then it may be possible to influence their activity by environmental change. With this in mind, Mikula (Genetics, 56, 733; 1967) showed that day length can cause a heritable change at the R locus in maize. Such findings may seem heretical, but if genetics is to be of full value in developmental studies then phenomena like these must be fully understood.

Single Cell Proteins

from Dr A. C. Frazer

MORE than 400 people from 22 different countries took part in a three day discussion on single cell proteins at the Massachusetts Institute of Technology from Opening the conference, Dr Nevin October 9-11. Scrimshaw emphasized the urgent need for more protein to feed the ever-increasing population of the world. The problem, he said, could be met in several ways, such as reduction of current wastage, increase of local production, or the development of new sources of protein, either from plant sources or from fish, or by synthesis in single cell systems. No one method should be regarded as a panacea. Although the need was now recognized by responsible people, the urgency of the situation was not yet fully appre-

The background was presented by leading authorities in the fields of global food production and distribution, protein needs, and the agricultural and economic aspects of low cost protein production. The conference reviewed the various forms of single cell systems that might be used for protein production—yeasts, algae, fungi and bacteria. The biological, engineering and other problems relating to protein production in these various systems were presented. The basic raw materials to be used in these systems were also considered; these included agricultural and industrial waste materials and petroleum products. The safety evaluation of the protein produced by single cell systems was discussed. Although a considerable amount of work seems to have been done, there was some hesitation in putting the available facts fully before the meeting.

The next phase of the meeting was concerned with the practical experiences of people who had used some of these products for feeding studies in human subjects and in animals. The results appear to be promising. Several speakers stressed the importance of studying the acceptability of the various protein products, and there is little doubt that there is an urgent need for an adequate educational programme which will enable people to have a better understanding of the value of protein foods and also train those concerned in the

proper use of additional protein supplies.

In these discussions, it was apparent that there are two main approaches. One is conservative and aims to supply more low cost protein in animal feeds, so that the contribution to human nutrition is indirect. The other aims to increase the human protein intake directly. This could be done by supplementing existing foods with protein material or even by converting a new source of protein into analogues of conventional protein foods. Whatever may be the outcome of present research, there is little doubt that any new protein product will have to compete successfully with others which become available and with those already on the market. Although it is true that the economic factors concerned in producing a new substance change as development proceeds, it is the market-place which eventually decides the future of any new product. Many times during the conference it became clear that local factors might well be decisive in determining which type of protein supplementation was possible and—if a single cell system were used—which raw material would be economically feasible. In general, transport costs seemed likely to be a limiting factor.

A great many interesting and important issues were raised during this conference and the proceedings will be published in the near future. Perhaps the most encouraging feature was the fact that there is a worldwide interest in these problems and that many developing countries are beginning to play an increasingly active part in solving the practical difficulties. This kind of participation by developing countries in improving protein production and distribution is necessary for the future welfare of mankind.

Assembly of Quaternary Structures

from our Molecular Biology Correspondent

Most proteins contain several polypeptide chains. Most commonly, there are two types of these sub-units, which are under separate genetic control. The manner in which the rates of production of the sub-units are geared to each other, and the mechanism by which the finished protein is assembled, are matters which have until quite recently been altogether obscure.

In the archetypal case—haemoglobin—which is made up of four sub-units, two α and two β , so that its structure may be written $a_2\beta_2$, it was reported that completed a-chains are found attached to polysomes. This observation and its implications for the regulation of haemoglobin synthesis have been followed up by Baglioni and Campana (Europ. J. Biochem., 4, 480; 1967). They have found, by pulse-labelling experiments, that there are two polypeptide intermediates in rabbit reticulocytes; these have been identified by chromatographic properties as completed a-chains and free globin. By treatment with detergent the a-chains are released from the ribosomes, and are found to be free of tRNA. It therefore appears that they must have been synthesized at an earlier stage, and it is convincingly surmised that these same ribosomes are in fact producing β -chains. The a-chains evidently combine with the β -chains while these are yet incomplete; the $a\beta$ complexes—that is to say, free globin in its usual dimeric state—are then released, combine with haem and finally associate to form the $a_2\beta_2$ tetramer. Labelling experiments indicate that the latter processes may proceed by way of intermediates containing less than their full complement of haem.

This last observation is in good accord with findings on the mechanism of the haem-globin reaction in vitro. Winterhalter and Deranleau (Biochemistry, 6, 3136; 1967) have found all three haem-deficient haemoglobin species in mixtures of haem with excess globin. The form in which two of the chains carry haem groups is the most prominent, and was isolated chromatographically. The molecule was shown indeed to be tetrameric, but with an increased tendency to dissociate to dimers at low concentrations. By addition of labelled haemin, and separation of the chains, it was established that the a-sub-units were the ones that had already contained haem groups.

The concept of a stable species containing only two haems between four chains receives independent support from the work of Heidrich and Schroeder (Biochim. Biophys. Acta, 147, 389; 1967), who find that when separated haem and globin are recombined, no less than 25 per cent of such a component is formed; it will combine with haemin only when incubated with the cyanide derivative (which is also the one used throughout by Winterhalter and Deranleau).

As it has long been known that globin synthesis is stimulated by added haem, the differential reactivity of the a- and β -chains may lead to an overall regulatory effect, whereby, for example, the synthesis of a-chains is regulated by the haem supply; the availability of the a-chains then makes possible release of the β -chains from the ribosome, with the formation of complete haemoglobin, so that effective synchrony is achieved. It is interesting to note, as Baglioni and Campana also point out, that the H-chains of immunoglobulins have been shown similarly to be released from the ribosome only after association with completed L-chains. This sequence may therefore constitute a general feature of the biosynthesis of sub-unit proteins.

Flagella Morphogenesis

from our Cell Biology Correspondent

CILIA and flagella from protozoan to mammalian cells have the same basic structural pattern. Two central microtubules surrounded by a ring of nine outer paired tubules run the length of the organelle, forming the basic 9+2 pattern of the axoneme. Where the axoneme enters the cell body, the two central tubules terminate in a basal plate, but the nine outer doublets are continuous with two of the three tubules in the nine triplets of the basal body.

What specifies this very exact structure? Electron microscopy has shown that the basal bodies somehow

act as organizers for the axonemes, but that, of course. leaves unanswered the question of the specification of the basal body structure. In several protozoans the pattern of ciliation on the cell surface appears to be controlled by extranuclear genes, and there are several reports—the latest from Smith-Sonneborn and Plaut $(\hat{J}. Cell Sci., 2, 225; 1967)$ —that basal bodies, like mitochondria and chloroplasts, contain DNA. This immediately raises the possibility that basal bodies are self-replicating organelles in the sense that the DNA could act, no matter how many steps of RNA and protein synthesis intervene, as an organizer for the sub-unit protein of microtubules and perhaps even specify the microtubular protein as well. But it is far from certain that all basal bodies contain DNA. In the current Journal of Cell Biology (35, 323; 1967), Outka and Kluss suggest that in the protozoan Tetramitus rostratus the basal bodies are not self replicating and that in this species nuclear DNA, not basal body DNA, acts as an organizer for the assembly of flagella.

Tetramitus has, like the more familiar Naegleria gruberi, the very remarkable capacity of existing either as an amoeboid cell or as a flagellate and of transforming from one form to the other. Within two hours, amoeboid Tetramitus devoid of microtubules can change into a tetraflagellate cell with cytoplasmic microtubules and a "mouth" supported by a microtubular system. This flagellate form is stable; it both feeds and divides. Thus the transformation process provides a useful system for studying flagella morphogenesis.

The amoeboid Tetramitus is devoid of basal bodies and their formation is the first requirement of transformation. The problem, of course, is to recognize the very earliest stage of basal body formation. What Outka and Kluss have seen in the electron microscope is the association of recognizable developing basal bodies with amorphous material, which on morphological grounds they tentatively identify as unassembled microtubular protein. They have also seen striking and apparently transitory projections from the nucleus to the basal body. They reasonably interpret this as meaning that a nuclear gene product somehow seeds the assembly of microtubular protein into the ordered basal body structure and once this nucleation has occurred the flagella can grow, perhaps by self assembly.

Once the basal body has formed, a pocket of the cell balloons out from the cell surface above it, and, at the same time, axonemal microtubules develop from the basal body into the outgrowth. The central pair of the axoneme always appears before the nine outer doublets, which at early stages are randomly arranged before they become sequentially positioned in the outer ring. As the growing axonemal microtubules often terminate in fine filaments, and as similar filaments are scattered throughout the outgrowth, it seems likely that microtubular development involves first the production of filaments by linear aggregation of subunit protein and then their assembly into the wall of the microtubule.

In the absence of correlated biochemical or genetic studies, any interpretation of a dynamic cell process from a series of electron micrographs must be only tentative. Nonetheless, the structure and timing of the association between developing basal bodies and nuclear outgrowths clearly suggest that the nucleus may play a very direct part in flagella morphogenesis in some species.

Organization of Ministry of Technology

The following extracts were taken from the prepared text of a speech by Sir Richard Clarke, Permanent Secretary, Ministry of Technology, in Birmingham, November 21.

THE present Ministry of Technology really dates from February 15, 1967, with the merger between the industrial part of the seven year old Ministry of Aviation (the civil aviation side having already gone to the Board of Trade) and the two year old Ministry of Technology (started in October 1964). The Ministry of Aviation has not swallowed up the old Ministry of Technology: nor has the Ministry of Technology swallowed up the Ministry of Aviation. We have a new entity which is swallowing up both.

The department controls seven [government research establishments] from the old Ministry of Aviation (plus two factories) and nine from the old DSIR—a total staff of 23,000. If we include AEA establishments, the total R and D manpower is about 9,000 qualified (graduate equivalent) engineers and scientists, compared with 55,000 enguged nation-wide on R and D. In addition, the department supports 45 industrial research associations with about 1,800 qualified men (formerly supported by DSIR). It is responsible for the National Research Development Corporation, which had been with the Board of Trade from the outset in 1948 to end-1964, and is currently handling 184 development projects with an investment of nearly £22 million.

Change in Whitehall

Since the early 1950s, most of the six blocks of public business [controlled by the department] have been regrouped with others and restyled at least three times. All large organizations need built-in arrangements for change and adaptation; and most will benefit from one major restructuring every ten years or so. But three or four major reshuffles in fifteen years is a different matter.

The essence of this industrial work is to establish effective communication and mutual confidence between the department and the industry. These are not easy relationships to create and maintain, and repeated changes in the machinery of government make them much more difficult. The detailed work tends to continue to be done by the same people. But the movement of staffs from one department to another presents every time major problems of organization and human adjustments and career planning, and of building and rebuilding the personal relationships and loyalties which are the heart of organization. The benefits from change and regrouping, like those from all mergers, take time to secure and are slow to accrue. Too often there is not enough time to get the benefits from one major change of structure before the next change is made. Let me repeat that changes in the administrative structure of government may be very necessary, in order to reflect a change in the long-term content and direction of the government's relationship to industry. But there is a real price to be paid in terms of the hard efficiency of the work of the governmental machine and thus of the effectiveness with which governments can carry out their industrial policy.

I have been speaking so far of changes in departmental structure and organization, and thus of changes in the duties and responsibilities of ministers and senior officials. These are in addition to the changes of ministers and senior officials which take place in the normal flow of governmental reconstructions and civil service appointments. The two together have had quite striking results in the

years November 1, 1957, to October 31, 1967. In this period, there have been 4 ministers and 4 permanent secretaries in charge of the government's relationship with merchant shipbuilding; 8 ministers and 6 permanent secretaries for aircraft; 6 ministers and 4 permanent secretaries in charge of the government's relationship with the engineering industry. These ten years cover both Labour and Conservative administrations, and what I say applies equally to both. One cannot say whether this dilution of the personal responsibilities for the government's contact with these sectors of industry is a contributory cause or a consequence of the difficulty which this country has been experiencing in finding an effective relationship between government and industry since the early 1950s. But it must be important.

Technology/Aviation Merger

[By November 1966 the department had responsibility for computer, electronic, telecommunications, machine tool, engineering and shipbuilding industries.] Powerful arguments were being made also for bringing some of the responsibilities of the Ministry of Aviation into the new department. It made little sense to talk about strengthening the technology of industry without regard for one of the most advanced technologies of all and the one receiving most government support—the aircraft industry. biggest research establishments, with most technological "fall-out", were those of the Ministry of Aviation. It was ineffective for the Ministry of Technology to be trying to strengthen the electronic industry's structure, when the main government relationship with the industry was the Ministry of Aviation's massive procurement. All this added up to a decisive argument for the ministry to take over much of the responsibility for the Ministry of Aviation. This was announced by the Prime Minister in June 1966. The old Ministry of Supply saw its primary duty in defence, with the civil industrial side a poor second. The new department has a large and important defence supply function, but its centre of gravity is definitely on the industrial side and will remain so. The old idea has been abandoned that "science" should be kept at arm's length from the main government machine in order to preserve the integrity of research: the task is now seen as one of bringing industry and the government research establishments together in order to get the best possible cross-fertilization and market orientation: we now regard science and technology as being in industrial policy right up to the neck. Thus, we now see an articulated chain of government activity, linking together scientific and technological research and innovation, the problems of industrial structure, and the role of public procurement.

The New Department

I would like to say a little about the human beings who have this task to carry out. The civil service tends to be rather restrained in its references to itself, so that quite absurd caricatures tend to become accepted as revealed truth. We are a very large department, total over 36,000, almost all from the Ministry of Aviation, the Board of Trade and DSIR. The total is perhaps a little misleading, for our research establishments and factories account for

Table 1. NON-INDUSTRIAL STAFFS

	Total (rounded)	Head- quarters	establish- ments and factories	Inspec- torates
Administrative	150	150		
Executive	2,300	1.870	360	60
Clerical and typing	5,150	3,000	1,750	420
Professional I (1)	3,200	900	2,000	300
Professional II (2)	8,300	1,160	5,700	1,420
Ancillary technical (3)	1,750	400	1,200	150
Miscellaneous	1,050	520	520	10
Total	21,900	8,000	11,530	2,360

Scientists, engineers, chemists, accountants, etc.
 Scientific assistants, technical grades, experimental officers, draughtsinen, etc.
 Drawing office assistants, photographers, stores grades, telephonists, etc.

about 23,000 of the 36,000, and the inspectorates for about 4,000, with something over 8,000 at headquarters.

Included in the 36,000 are nearly every kind of skill. Over 13,000 are industrial workers; 1,000 are Service personnel; the 22,000 non-industrials are divided:

This is a great weight of experience and expertise; and we devote quite large resources to the job of managament. On this I must limit myself to two short points. First, career planning. This has always been well done for the administrative grade. The Ministry of Aviation was developing it effectively for scientists and engineers. The department now has 1,900 in the scientific grades, approaching double the old Ministry of Aviation. We see here a widening of the field of opportunity for our scientists, and a formidable but repaying task of extending career planning. Second, training. Here again we have a large operation from the old Ministry of Aviation, with central training in department-wide organizational, management and specialist courses; with group training for staffs in particular branches. It is difficult to show the scale by statistics. However, this year we trained over 1,000 of our staff in central management and specialist courses.

The Senior Staff

In terms of senior and highly qualified staff, the department is very large. We have about 70 earning £5,000 or more, which is about twice as many, for example, as the Treasury or the Board of Trade. These are about equally divided between (i) administrators, (ii) headquarters engineers and scientists and other specialists, and (iii) those in the research establishments. About one-quarter are under 50; and about one-quarter of the whole 70 reached this level at the age of 42 or less. These age averages are virtually the same for administrators, engincers and scientists alike; so the chances of an able man to get into the top group relatively young are probably about the same on all ladders.

In this top group of 70 there are 21 men and 1 woman of the administrative grade (under-secretaries and above). Half of them were at Oxford or Cambridge, but only 9 entered the administrative grade by the direct route from the university; 5 were promoted from lower grades, and 8 came into the civil service after experience outside. Nevertheless, they are highly professional: three-quarters of them have been working in or near the field of the present department ever since the War.

Lastly, I should mention the 11 top men in the department. These consist of 5 administrators—the permanent and second permanent secretaries and three deputy secretaries: one came up the ladder from the old Ministry of Supply/Aviation, one from the Board of Trade, two from the Defence Department and one from the Treasury. There are four controllers—Aircraft, Research, Guided Weapons and Electronics, Industrial Technology. One is an air marshal, a scientist who came into the RAF during the War: one is an electrical engineer, formerly head of the Royal Radar Establishment at Malvern: one is an aeronautical engineer, now president of the Royal Aeronautical Society: the fourth an electronic engineer from Aldermaston, who was made an FRS

this year. At the same level are the directors of the two senior research establishments, the Royal Aircraft Establishment at Farnborough and the National Physical Laboratory. These 11 are a mixed group, all in their fifties; two Scots and two Welsh and seven English; three from private boarding schools; six Oxbridge; eight graduates in scientific subjects and three in arts. Between them, they share a wide variety of background and experience, inside and outside the civil service, which is not easy to reconcile with the picture which sometimes gets painted.

Organization of the Department

Each of the three groups-Aviation, Research and Engineering—has a fairly clearly defined set of tasks to carry out. Some of these are self-contained: others impinge upon other groups and upon the links between the groups.

Research Group

[Having discussed the Aviation group, Sir Richard went on: I said earlier that the department's research establishments plus those of the Atomic Energy Authority, employ about 9,000 qualified engineers and scientists. This is the biggest force under one central direction in Western Europe; and our task is to deploy these resources to the best possible advantage of the national economy.

I have been talking here in terms of numbers—the of the government-financed establishments, and whether it should be increased or reduced and by how much. But it isn't just "How many?" It is also "How good?" It is important whether there are 8,500 or 9,000 or 9,500 qualified scientists or engineers in our research establishments. But it is much more important that they should be doing the right kind of work; and even more important again that the fruits of that work should go to where they can be used effectively for the benefit of industry.

Engineering Group

[Having spoken of the role of the five industrial divisions -electronics; computers; machine tools and manufacturing machinery; vehicles and mechanical engineering; shipbuilding, electrical and process plant-in fostering economic and technological development and competitive power, Sir Richard said:] This work brings us immediately to problems of industrial structure. In many parts of the engineering industry, we have become impressed by the disadvantages of smallness. Where R and D expenditure, and the much larger expenditures involved in bringing R and D into successful production, is important, the size of turnover over which the expenditure can be spread is highly relevant. IBM's R and D expenditure, though huge, is only about 5 per cent of its turnover. should not be so silly as to favour bigness for its own sake. A big organization may be just as sleepy as a small one. Mergers are valuable only if they are used to cut out dead wood. We have formed the opinion, however, that especially in the industries with a big component of advanced technology, larger units have their advantage. This is where the department's work fits in with the Industrial Reorganization Corporation, whose job it is to seek opportunities to stimulate constructive industrial re-organizations. Throughout the engineering industry, events are on the move. In shipbuilding, the mergers postulated in the Geddes Report are under way. In electrical engineering, we are clearly in a period of rogrouping. In machine tools, a number of large firms have emerged. We are getting, for better or for worsewould say for better-very large changes in industrial structure.

Conclusion

To sum up, we see our task as being to sharpon public awareness of the decisive position of industrial technology and productive efficiency in the attainment of all our national objectives.

Some Sociological Concomitants of Sixth Form Subject Specialization

by RONALD KING University of Exeter The choice between science and the arts at sixth form level may be conditioned by the social background of the pupil and his involvement in the school.

THE studies of Hudson indicate that there may be significant psychological differences between science and arts specialist students both at sixth form and university level. The sociology of subject specialization has received less attention, except at a purely speculative level, by those interested in the "short-fall" in science students or the "swing to the arts". A few empirical studies have intimated some of the social factors that may condition subject choice, as, for example, the recent study of Box and Ford which explores the role of the working class chemistry student in terms of the concept of marginality².

The empirical study reported here sheds a little light on the influence that a pupil's social background and the formal organization of the school may have on subject choice. The evidence is drawn from a larger piece of research involving pupils of all ages in a London boys' grammar school. In terms of its size, staffing ratio, proportion in the sixth form, examination success, qualifications of staff and social composition, the school was quite typical of boys' grammar schools as described in the Department of Education and Science statistics.

The case study was one of the social values and involvement of pupils in the school. A survey of the teachers indicated which values they purported to transmit to the pupils. This included their approval or disapproval of interests and activities in terms of their suitability for the pupils. A questionnaire administered to the pupils included items and scales designed to measure these attitudes, values and interests. Although the responses to each item were scored and analysed, it was also convenient to assign each pupil a score on a short four point scale (0-3) for the holding of values approved by the school. Involvement was measured by the attitudes of the pupils to different aspects of school, and their actual behaviour in terms of the frequency with which they joined school clubs. Each pupil was thus assigned a position on a four point scale (0-3) for involvement in the school.

The principal independent variables in the analysis were pupil age, social background and stream status. The most significant social background factor was found to be the pupils' parents' own experience of grammar school education. Thus pupils were classified as first or second generation grammar school pupils. The streaming in the school took the form of "expressing", in which the thirty most successful pupils at the end of the first year of school were given an accelerated course to take O-level in their fourth year.

The sample of sixth formers (n=99) represented virtually all the sixth formers in the school. Apart from analyses using the expressed/non-expressed, first generation/second generation dichotomies, analysis was also made in terms of subject choice—arts or science. Four social factors were found to be associated with this subject choice: (1) the educational experience of parents; (2) the incidence of expressing; (3) the extent to which a pupil held school-approved values; (4) the degree of the pupil's involvement in the school.

Table 1. ARTS AND SCIENCE SPECIALISTS BY EDUCATION OF PARENTS AND EXPRESSING

		Arts			Science		
	n	f	%	f	%		
First generation expressed Second generation expressed First generation non-expressed Second generation non-expressed	29 14 35 21 99	9 8 21 11 49	31.0 57.1 60.0 52.4 49.5	20 6 14 10 50	69·0 42·9 40·0 47·6 50·5		

The choice of science was particularly associated with first generation expressed pupils (P=0.02 by χ^2 , Table 1).

Wilkinson has shown that success in a subject at O-level often leads to further A-level study³. The results for science examinations at O-level for expressed pupils were very good in the subject school—thus the disposition for expressed pupils to continue science studies. Chown has shown that working class pupils in grammar schools rely mainly on their teachers for educational and vocational guidance4. These first generation, mainly working class, expressed pupils may have responded to the formal and informal guidance given by their science teachers because science subjects can lead to occupations of which the working class pupils can have some fairly specific image. Box and Ford see the choice of chemistry on the part of working class university students as a way of resolving their "crisis of identity". The bright working class student, estranged from his family by the changes his education has brought about in him, may embrace the role of professional scientist because this is seen as being marginal in its social class connotations, and so he can escape the middle class life-style which he perceives as his alternative ultimate destiny.

An analysis was made of the mean scores for values and involvement. Slight, but not significant, trends emerged. When a similar analysis was made of those fifth formers who eventually became sixth form subject specialists, similar, but still not significant, trends were found. By combining the results of the existing subject specialists with the eventual specialists, significant patterns did emerge (Tables 2 and 3). Specialization in science was associated with: (1) first generation expressed pupils

Table 2. MEAN SCORES FOR VALUES—ACTUAL AND EVENTUAL SUBJECT SPECIALISTS

	n	Arts Mean	S.D.	n	Science Mean	S.D.
First generation expressed	18	1·22	0·55	30	1·93	0·49
Second generation expressed	11	2·27	0·76	11	1·36	0·71
First generation non-expressed	30	2·10	0·67	24	1·26	1·20
Second generation non-expressed	16	1·81	0·58	18	2·11	0·45

Table 3. MEAN SCORES FOR INVOLVEMENT—ACTUAL AND EVENTUAL SUBJECT SPECIALISTS

	n	Arts Mean	S.D.	n	Mean	
First generation expressed	18	1·72	1·19	30	2·20	1·11
Second generation expressed	11	2·27	1·34	11	1·54	0·79
First generation non-expressed	30	1·84	1·01	24	1·17	0·74
Second generation non-expressed	16	1·56	1·23	18	1·67	1·06

highly involved in the school (P = 0.01 by t test); (2) second generation expressed pupils with low involvement in the school (P = 0.01); (3) first generation nonexpressed pupils of low involvement (P = 0.05); and poor school-approved values (P = 0.01).

These results clearly indicate that any differences associated with subject specialism may in turn be related to the social origins, school experience and value-set of the specialists concerned. Further results of this study do, however, reveal features of sixth formers which seem to relate directly to their chosen subject. (These differences have been shown not to be associated with the different social compositions of the arts and science groups.)

Science students were found to be less sceptical of textbooks, subject teachers and scientific laws as authoritative sources of information. Science at the intermediate level deals with certainties rather than speculations. The trust of textbooks and teachers reflects the emphasis on facts and exact procedures in sixth form science. In their assessment of the qualifications of teachers they "got on well with", science students stressed clear exposition on the part of the teacher rather than any special personal pupil/teacher relationship. Their acceptance of scientific laws forms the basis of much intermediate experimental work and calculation problems.

Science students read less than arts students. They joined fewer school clubs and this certainly contributed to their being made prefects less often. Like the scepticism differences, these too may be associated with the nature of science at the sixth form level, as well as the general pressure of school work felt by these young science specialists, most of whom (74.0 per cent) hoped to go to university (compared with 53.0 per cent of arts specialists).

It is possible that a student's experience of a subject will be conditioned by his expectations of that subject, and these will probably be related to the kind of social factors already discussed. It is possible that pupils with socially determined need-dispositions, like the establishment of a social identity, select subjects which seem to fulfil these needs. Finally, it is clear that any logistical analysis of the supply of scientific manpower will need to take into account the kind of social factors outlined and discussed here.

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Peculiar Velocity of the Sun and its Relation to the Cosmic Microwave Background

by

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If the microwave blackbody radiation is both cosmological and isotropic, it will only be isotropic to an observer who is at rest in the rest frame of distant matter which last scattered the radiation. In this article an estimate is made of the velocity of the Sun relative to distant matter, from which a prediction can be made of the anisotropy to be expected in the microwave radiation. It will soon be possible to compare this prediction with experimental results.

Until comparatively recently, the science of cosmology was based on one experimental fact, the recession of extragalactic objects. The theories used to explain the expansion of the universe usually assume that the universe is homogeneous and isotropic, and number counts of galaxies, radio galaxies, etc., in various regions of space have been made to check these assumptions to varying degrees of accuracy, and also to refute some theories which were in conflict with the observations. There has been, however, an important recent observational development which is the discovery of the excess microwave background¹, assuming of course that it is cosmological in origin². While there are still doubts as to its exact effective temperature, it is certainly highly isotropic3. Thorne has suggested4 that the isotropy of the microwave background gives a very strong restriction on the possible anisotropy in the early stages of the universe, while Misner⁵ has pointed out that neutrino viscosity could make the present isotropy (or lack of it) independent of still earlier conditions.

In this article we shall discuss that component of the angular distribution of the microwave radiation which has a 24 h period in any plane. Throughout the rest of this article, anisotropy refers only to this component. Then the radiation defines a rest frame at our location in space, namely, that frame in which the radiation is isotropic. If the Sun is not at rest in this frame, its peculiar motion can be measured from the resulting anisotropy of the radiation arising from the Doppler effect. The best measurements to date show that the component of the Sun's peculiar velocity close to the celestial equator $(\delta = -8^{\circ})$ is less than 300 km/sec. Measurements in other directions and to greater precision should be available soon.

If the radiation is cosmological in origin, then the rest frame it defines is the rest frame defined by the matter which last scattered it. (We regard it as unlikely that any anisotropy of primordial origin should have a 24 h period.) This matter is extremely distant, because on the most conservative assumptions it cannot have a red-shift less than about 7, and the actual red-shift may be much greater. According to Mach's principle7 this rest frame of distant matter defines an inertial reference frame at the Sun. If we could estimate the motion of the Sun relative to this inertial frame, then we should be able to predict the resulting anisotropy in the microwave radiation. Partridge and Wilkinson³ pointed out that, relative to an inertial frame, the Sun rotates around the centre of the Galaxy with a velocity of about 250 km/sec, and they suggested that a relatively small increase in the accuracy of their measurements should reveal this rota-Any inertial frame chosen locally, however, could well have a uniform rectilinear velocity with respect to the rest frame of distant matter (although according to Mach's principle it could not have an angular velocity with respect to distant matter) and this would affect the

anisotropy prediction. If we choose as a reference frame a frame which is inertial and at rest relative to some suitably defined average of all nearby matter, then the larger the amount of matter considered, the more nearly our inertial frame should correspond to the rest frame of distant matter.

We consider here an inertial frame based on the local supercluster of galaxies. One of us made a rough estimate of the Sun's peculiar velocity relative to this frame, and we now want to give a more detailed discussion of this question. In particular, we wish to predict a range of directions in which the peculiar velocity of the Sun relative to this local inertial frame should lie. It will then be possible to compare these theoretical results with the observations, when sufficiently sensitive measurements of the anisotropy of the microwave radiation in all directions become available.

Motion of the Galaxy in the Local Supercluster

A study of the distribution of bright galaxies (see, for example, ref. 9) strongly suggests that the Local Group belongs to a supercluster of galaxies (Fig. 1). De Vaucouleurs has estimated that this supercluster is roughly a spheroid, with an axial ratio of 1:5, centred on the Virgo cluster of galaxies. The Local Group would lie near the central plane and at about two-thirds of the distance from the centre to the edge. The flattening of the supercluster suggests that it might be rotating about its axis through the Virgo cluster, and this possibility has been investigated by many authors, especially Rubin¹¹, Ogorodnikov¹² and de Vaucouleurs¹⁰.

away from Virgo, making it less than that predicted by the Hubble law (as determined from distant galaxies). A crude calculation shows that the difference is at most 100 to 200 km/sec. Because our tangential velocity is thus likely to be the major component of our total peculiar velocity relative to Virgo, it is most important to decide its sense, and if possible to obtain a better estimate of its We have therefore attempted to determine the differential velocity field in the vicinity of the Local Group by an Oort-type analysis of the radial velocities of nearby galaxies. If the Virgo cluster were not stable, however, then a substantial fraction of the mass of the supercluster could be distributed fairly uniformly in its plane, and we might have to contend with a rigid body rotation superimposed on the differential velocity field. A more sophisticated analysis would then be needed to predict the magnitude of such a rotation.

The analysis is similar in principle to that used in the Oort theory of the rotation of the Galaxy¹⁶, except that no proper motions are available, and allowance must be made for the substantial rate of expansion of the supercluster. There are unfortunately serious practical difficulties in analysing the motion of the supercluster: (a) the measured radial velocities are not particularly accurate, and it is well known that groups of galaxies have velocity

measured radial velocities are not particularly deceased, and it is well known that groups of galaxies have velocity dispersions which may be large enough to disrupt the groups; (b) we are concerned with distances in the range 1-8 Mparsec and the secondary distance indicators (HII regions, angular diameters, etc.) which have to be used in this range have significant random and systematic errors; (c) if we select only those galaxies which are close

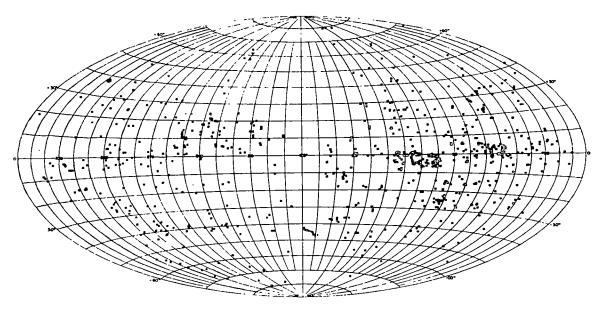


Fig. 1. Distribution of 2590 bright galaxies in supergalactic co-ordinates. Note the concentration to the supergalactic plane especially in the northern galactic hemisphere. (Source: ref. 9.)

Although Ogorodnikov's study¹² is based on data which are now obsolete, we shall adopt his qualitative result that differential velocities in the plane of the supercluster are an order of magnitude greater than those perpendicular to the plane. Thus we are essentially dealing with plane motion. If the Virgo cluster is stable and so has the mass (about 10⁴8 g) implied by the virial theorem¹³, the circular velocity at the position of the Local Group would be ~770 km/sec (if its distance from Virgo is 11⋅5 Mparsec¹⁴). The gravitational effect of the Virgo cluster could also affect our radial velocity

enough for the usual first-order theory to apply, then there are too few galaxies to obtain reliable results. If we include more distant galaxies, a higher order theory must be used, and there will be too many undetermined parameters in comparison with the amount of data available. Because the distances to the galaxies are so poorly determined, it does not seem worthwhile to use more than the simplest first-order analysis. We have made such an analysis, using the results of de Vaucouleur's recent study¹⁴ of the grouping of galaxies within the supercluster.

We introduce cylindrical polar co-ordinates (ρ, θ, z) fixed with respect to the supercluster, and with the axis along the supercluster axis through Virgo. Let the components of the velocity of the local standard of rest be (π, θ, Ez) , where E is the local Hubble constant for velocities perpendicular to the supergalactic plane. In defining the local standard of rest we have made allowance both for the rotation of the Galaxy and for its peculiar motion relative to the Local Group. We have also used the assumption mentioned earlier, that there is no peculiar velocity in the z-direction.

and D is not much greater than $(A^2+C^2)^{\frac{1}{2}}$, which was certainly found to be the case by Ogorodnikov¹², then the term proportional to D can be neglected. Also because E is presumably not significantly greater than K, this restriction on B means that the term involving E can be neglected.

Following de Vaucouleurs¹⁴, we have classified into groups all the galaxies with $|B| \lesssim 20^{\circ}$, and which lie outside the Local Group, but within 8 Mparsec (on the distance scale given by $H=100 \text{ km sec}^{-1}$ Mparsec⁻¹ for very distant galaxies); the details are shown in Fig. 2 and Table 1.

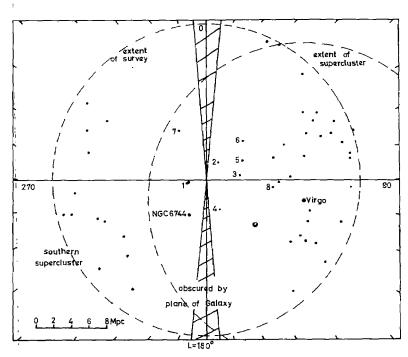


Fig. 2. Space distribution of nearby groups projected on to the supergalactic plane. The Galaxy is at the centre of the diagram. The numbers refer to the groups the members of which were used in the analysis (see Table 1). Data were taken from ref. 14.

Now if v is the radial velocity of a galaxy at a distance r from the Local Group with supergalactic longitude and latitude L and B, and if r is small compared with the dimensions of the supercluster, an Oort-type analysis gives to first order 15,16

$$\begin{split} v &= r \text{cos}^2 B(K + A \text{sin} 2(L - L_0) + C \text{cos} 2(L - L_0)) + \\ &r D \text{sin} 2B + r E \text{sin}^2 B + \delta v, \\ &= r \text{cos}^2 B(K + (A^2 + C^2)^{\frac{1}{2}} \text{sin} 2(L - L_0 + \varepsilon)) + \\ &r D \text{sin} 2B + r E \text{sin}^2 B + \delta v, \end{split}$$

where

$$K = \frac{1}{2} \left(\frac{\partial \pi}{\partial \rho} + \frac{\pi}{\rho} + \frac{1}{\rho} \frac{\partial \Theta}{\partial \overline{\Theta}} \right),$$

$$A = \frac{1}{2} \left(\frac{\partial \Theta}{\partial \rho} - \frac{\Theta}{\rho} + \frac{1}{\rho} \frac{\partial \pi}{\partial \overline{\Theta}} \right),$$

$$C = \frac{1}{2} \left(\frac{\partial \pi}{\partial \rho} - \frac{\pi}{\rho} - \frac{1}{\rho} \frac{\partial \Theta}{\partial \overline{\Theta}} \right),$$

 $D=\frac{1}{2}\frac{\partial \pi}{\partial z}$, $2\varepsilon=\tan^{-1}\left(C/A\right)$, L_0 is the longitude of the axis of rotation, δv is the residual velocity of the galaxy, and all derivatives are evaluated at the local standard of rest. Provided we restrict the data to low latitudes $(|B| \lesssim 20^{\circ})$,

For each group we have estimated a distance by considering the distance determinations for individual group members cited in Table 1. These were reduced to the distance scale for which $H\!=\!100~{\rm km~sec^{-1}~Mparsec^{-1}}$ for very distant galaxies, and a weighted average was used to give the distance of each group. Then for each galaxy we evaluated the effective Hubble constant

$$\frac{v\sec^2 B}{r} = K + A\sin 2(L - L_0) + C\cos 2(L - L_0) + \frac{\delta v\sec^2 B}{r}$$

A double sine curve was fitted to the data by means of a least squares analysis, and confidence limits were deduced for K, A and C by consideration of the residuals. There are two plausible ways of analysing the data; the simplest is to give equal weights to the brightest galaxies in each group. If, however, the groups are stable it is preferable to weight the data according to the masses of the individual galaxies, or according to their luminosities because all the bright nearby galaxies are spirals with roughly the same mass-luminosity ratio. (We also assume that the first brightest galaxies in the groups have roughly the same absolute magnitude.) Apart from the obvious dynamical reasons, this weighting is advantageous because it decreases the number of galaxies to be considered and lessens the error due to a non-member galaxy being included in a group. (Such an interloper would usually lie behind the group, and so would tend to be fainter than

the group members.) If, however, the groups are unstable this method will not remove the residuals effectively, because great weight is given to the brightest galaxies in each group. The data and the two fitted curves are shown in Fig. 3. From these we have deduced the following parameters.

	Equal weighting	Mass weighting
K (km sec ⁻¹ Mparsec ⁻¹)	91 (±6)	93 (±6)
A (km sec-1 Mparsec-1)	$-24 (\pm 8)$	$-22 (\pm 8)$
C (km sec-1 Mparsec-1)	$-8(\pm 9)$	$-9 (\pm 10)$
ε (in degrees)	$+9(\pm 11)$	$+11 (\pm 13)$

These values will be inaccurate because: (a) the radial velocities are poorly determined and include residuals: (b) the distances are probably very inaccurate (but apart from a scale factor the errors are probably random); (c) the linearized model is not a completely adequate description of the local kinematics. Provided that these errors are random and do not include any systematic effects, then the probability that K, A and C lie outside the limits given is <10 per cent. We might expect the real errors to be rather less than the quoted values for the following reason. The mass weighting is equivalent to choosing (for special dynamical reasons) a sub-set of our set of galaxies, without regard to their values of L and their Hubble constant, and giving the members of this sub-set equal weight. If our fitted curves contain large errors because of the scatter in the diagram, we could not expect the agreement between them to be as good as has been found. Because the two sets of values are roughly the same, we shall henceforth use the mass weighting values.

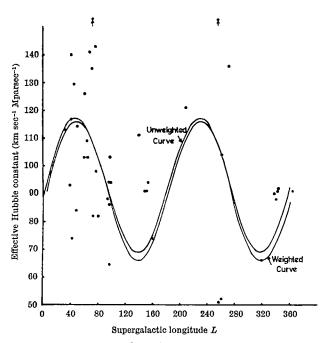


Fig. 3. The effective Hubble constant for nearby galaxies plotted as a function of supergalactic longitude L. The double sine curves were fitted by a least squares analysis. For details of the weighting, see text.

The next step is to derive the net tangential velocity of the local standard of rest from the differential velocity field we have just determined. This cannot, however, be done without further assumptions. We shall assume in the first place that most of the mass in the supercluster is concentrated towards its centre so that there is no rigid rotation superposed on the differential rotation. Second,

we note that the local value of A for circular orbits under the gravitational action of a mass of 1048 g located at Virgo would be about 50 km sec⁻¹ Mparsec⁻¹. of the same general order, though twice as large, as the value of A we have found from our kinematical analysis. We shall therefore assume that our actual tangential velocity, Θ , is related to our derived value of A in the same way as for circular orbits, that is

$$\Theta \sim \frac{4}{3} A_{\rm P}$$

$$= -340(\pm 120) \text{ km/sec}$$

As regards the peculiar radial velocity of the local standard of rest we cannot argue in an analogous way, because our value for C has the wrong sign for the differential expansion to be explained in terms of the retarding

Table 1. THE DATA USED TO ANALYSE THE RADIAL VELOCITIES

Group (1)	NGC No. (2)	L (3)	B (4)	Appt. Mag. (5)	v_0 (km/sec) (6)	r (Mpar- sec) (7)
1	0253 0055 0300 0247 7793 0045	272 257 261 277 262 272	-5 -2 -10 -4 3	8·1 8·2 9·0 9·9 10·3 11·2	277a 97 95 - 129 197 489	1.01
2	3031 2403 3034 4236 2976	41 32 41 48 42	$ \begin{array}{r} 1 \\ -9 \\ 1 \\ 11 \\ -1 \end{array} $	7.88 9.07 9.57 10.40 11.09	88 255 322 186 <i>c</i> 169	1.96
3	4736 4258 4826 4449 4214	77 69 96 73 80	10 6 6 6 2	8·91 9·19 9·60 10·08 10·38	362 530 352 269 311	2.3d
4	5236 5128 4945 5102 5068 5258	149 160 166 154 139 151	$ \begin{array}{r} 1 \\ -5 \\ -10 \\ -4 \\ 0 \\ 1 \end{array} $	8·22 8·38 9·48 10·55 11·05 11·14	$335 \\ 271 \\ f \\ 348 \\ 410 \\ 229$	3·8e
5	5457 5194 5055 5195 5585 5474 5204	64 72 77 72 61 65	23 17 14 17 25 23 18	8.58 9.03 9.52 10.94 11.66 11.54 12.03	415 444h 600 444h 467 895 416	3·7g
6	2841 2681 2541 2500 2552 2537	50 46 41 39 42 42	-16 -18 -23 -22 -22 -26	10·27 11·34 12·14 12·39 12·54 12·55	671 751 634m 513 f 422	4·5j
7	1023 0925 1058 1003	342 336 341 344	$ \begin{array}{r} -9 \\ -10l \\ -10 \\ -8 \end{array} $	10.65 10.96 12.26 12.45	$729 \\ 712l \\ 665m \\ 741$	
8	3627 3628 3623 3489 3593	97 97 97 94 97	-18 -18 -19 -23 -20	9·89 10·43 10·51 11·24 11·91	591 730 640 570 429	7-8n
Field galaxy	6744	209	10		544	7·6p 4·6q

All data are taken from Reference Catalogue of Bright Galaxies³, except: a Radial velocity from ref. 22.
b Most recent distance determination for a member of this group²³.
c Lick velocity for a bright emission patch²⁰.
d Distance is an average of Sersic²⁴ for group, Sersic²⁵ for 3031, Sandage¹⁷, odge²⁵ and van den Bergh²⁷ for 2403 and Lynds and Sandage²⁵ for 3034.
here is a large scatter. d Distance is an average of Sersic¹⁴ for group, Sersic²⁵ for 9031, Sandage¹⁷, Hodge²⁵ and van den Bergh²⁷ for 2403 and Lynds and Sandage¹⁹ for 3034. There is a large scatter.

e Average of Sersic²⁴ for group, Hodge¹⁵, Sandage¹⁷ for 4449, Sandage¹⁷ for 4258, van den Bergh¹² and Rubin et al. ²⁵ for 4826.

f No radial velocity known.

g Average of Sersic²⁶ for 5128, de Vaucouleurs²¹ for 4945, Hodge²⁶ for 5068.

h Radial velocity from ref. 32.

f Average of Sersic²⁶ for group, Sersic²⁵ for 5457.

k Distance from ref 27.

l Correction to Reference Catalogue²⁵.

m Velocity of de Vaucouleurs²⁶.

n Average of Sersic¹⁵, Sandage¹⁷ and Hodge²⁶.

p Distance of de Vaucouleurs²⁶.

action of Virgo. Presumably the supercluster is not completely relaxed. Nevertheless we can infer that the peculiar radial velocity is small, from the fact, stressed especially by Sandage (ref. 17, p. 378), that Virgo fits well onto the red-shift-apparent magnitude relation for the brightest members of distant clusters of galaxies. If we assume that the absolute magnitudes of the brightest galaxies in Virgo do not differ by more than ± 0.3 m from the corresponding means for all rich clusters, then we can estimate that our peculiar radial velocity is unlikely to exceed ± 200 km/sec.

We may note in passing that the expansion rate of the supercluster relative to the local standard of rest in the tangential direction is

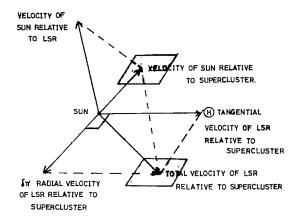
$$K-C=102(\pm 12) \text{ km sec}^{-1} \text{ Mparsec}^{-1}$$

If the tangential velocity Θ is independent of azimuth θ (as it would be if the supercluster has circular symmetry about Virgo), then the tangential expansion rate should be equal to our actual expansion rate away from Virgo. This is indeed the case because, as we have just seen, this latter expansion rate is close to the expansion rate for very distant galaxies of 100 km sec⁻¹ Mparsec⁻¹.

Peculiar Velocity of the Sun

When our results come to be compared with future observations, it will be convenient to work in terms of the peculiar velocity of the local standard of rest as defined here. In the supercluster reference frame, this peculiar velocity is the sum of the rotational velocity of the local standard of rest around the Virgo cluster. some 220 to 460 km/sec towards $l_{\rm H}=319^{\circ}$, $b_{\rm H}=-14^{\circ}$, and the peculiar velocity of the local standard of rest towards Virgo, some -200 to +200 km/sec towards $l_{\rm II} = 287^{\circ}$, $b_{\rm II} = 72^{\circ}$. Although the magnitudes of these velocities are not well determined, their directions are, and so the net peculiar velocity lies in a well defined plane. The limits on the velocities restrict the direction of the net peculiar velocity to a rectangle in this plane which is shown in Fig. 4. Referred to new galactic co-ordinates. the direction of the peculiar velocity of the local standard of rest relative to the supercluster reference frame should lie on the minor arc of the great circle between $l_{\rm II}=312^{\circ}$.

 $b_{\rm H}=27^{\circ}$, and $l_{\rm H}=331^{\circ}$, $b_{\rm H}=-56^{\circ}$. If Mach's principle, were true, it would be expected that this "local" inertial frame of the supercluster would be non-rotating with respect to distant matter. It will be possible to check this when more information is available about the possible anisotropy of the microwave



•VIRGO

Fig. 4. The components of the peculiar velocity of the Sun relative to the supercluster. The shaded areas represent the range of directions within which the total peculiar velocities should lie.

background. Knowledge of the amplitude and phase of the anisotropy component with a 24 h period would determine the peculiar velocity of the Sun with respect to the very distant matter which last scattered the radia tion. To make the comparison we must subtract from this velocity the solar motion with respect to the local standard of rest, which is caused by the rotation of the Galaxy, and its peculiar motion relative to the Local Group^{18,19}. Then we obtain the peculiar velocity of the local standard of rest with respect to distant matter, which can be compared with our estimate of the peculiar velocity with respect to the supercluster. If our estimate is correct the difference between the two should give the velocity of the supercluster with respect to distant matter (see Fig. 4). In view of the measurements of Partridge and Wilkinson³, the component of this velocity along $\delta = -8^{\circ}$ cannot exceed a few hundred km/sec. If in fact future observations show that the peculiar velocity of the local standard of rest is in reasonable accord with our estimate, then this would tend to confirm Mach's principle, that local inertial frames are non-rotating with respect to distant matter, to an accuracy of 10 3 sec of are/century, a 500-fold increase over the present precision of the comparison*.

Finally, we may note that if this picture of the super cluster turns out to be in accord with the observations. it would help to explain the curious result noted by Sandage²⁰ and de Vaucouleurs²¹ that the Hubble constant derived from galaxies in the northern galactic hemisphere is about 25 per cent less than that observed in the south. using the same distance scale. The galaxies in the north lie in our supercluster and partake in its peculiar contraction, so that the measured Hubble constant will be less than its true value. In the south, nearly all the galaxies used lie in the nearby supercluster, the edge of which can be seen in Fig. 2. They are spread roughly symmetrically about the line joining the Galaxy to Virgo. and so on average the rotation of the nearby supercluster will have no net effect on the radial velocities of these galaxies. Because these galaxies partake in the peculiar contraction of their own supercluster, the measured Hubble constant for them will be larger than the true value. A rough calculation shows that this effect is of the right general order of magnitude.

Conclusions

Future observations of the 24 h anisotropy of the cosmic microwave background are expected to reveal our peculiar velocity with respect to distant matter. Although part of this velocity is caused by our rotation in the Galaxy. our discussion shows that a significant contribution could arise from our motion in the local supercluster. Numerical estimates of the magnitude of this motion are rather uncertain at present, partly because of the scatter in the data and partly because there are so few observed radial velocities and distances of galaxies in the southern hemisphere. The direction of this motion, however, is hemisphere. better determined, and when it can be compared with the direction of our observed peculiar velocity with respect to distant matter we should be able to derive important information concerning the nature of the local supercluster and the validity of Mach's principle.
We thank Professor G. de Vaucouleurs for a helpful

We thank Professor G. de Vaucouleurs for a helpful discussion on the analysis, and Dr M. J. Rees for his many comments and criticisms of the theory.

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- *In a previous paper* by one of us (D. W. S.) the increase in preci ion was mistakenly given as 5,000-fold. We are grateful to Protessor Schiff for drawing this error to our attention. (See also his discussion of the precision with which Mach's principle is presently confirmed.)
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Spectrum of the 3° K Cosmic Microwave Radiation

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Measurements of the 3° K cosmic blackbody radiation at frequencies below about I GHz have a limited accuracy because radiation from the Galaxy becomes a dominant component. Measurements at 408 MHz and 610 MHz nevertheless show that there is a radiation component at these frequencies which is consistent with a blackbody spectrum of about 3° K.

MEASUREMENTS of the intensity of the cosmic microwave radiation have already been made over the range of frequency 1.407 GHz to 36.6 GHz (refs. 1-7) and all are consistent with this radiation having a blackbody spectrum corresponding to a temperature between 2.5° K and 3.0° K. The work of Scheuers and Weymanns, however, has shown that at lower frequencies deviations from this blackbody spectrum may perhaps be expected because of the presence of free-free radiation from intergalactic gas, the precise nature of the deviations depending on the density and thermal history of the gas. In view of the importance of further information on these matters, an attempt has been made to determine the spectrum at the frequencies of 408 MHz and 610 MHz, even though the accuracy attainable at frequencies below 1 GHz is limited by the fact that radiation from the Galaxy is the dominant component.

The experimental method has been described previously3. It relies on a measurement of the difference of noise output from an antenna and from a reference termination immersed in liquid helium. The antennas used were optimal horns with E-plane slant heights of 6 \(\lambda \), which provided beam-widths of 15°. The scaled dimensions lead to identical reception patterns, thus simplifying the comparison of the antenna temperatures at each frequency. The horns were oriented with their E-planes along the meridian.

Table 1 gives a summary of the measured antenna temperatures for the celestial North Pole and the corrections necessary to derive the effective sky temperatures. The passage of the galactic plane through the side-lobes of

	Table 1	
	408 MHz	610 MHz
Excess temperature, antenna	21·0 ± 0·7° K	7·85 ± 0·5° K
over load Load temperature	5·6±0·2° K 1·3±0·1° K	5·7 ±0·2° K 1·95 ± 0·1° K
Atmospheric emission Ground contribution Contribution from loss in horn	0·6±0·4° K 0·4±0·2° K	0·8 ± 0·4° K 0·4 ± 0·2° K
Corrected pole temperature	$24.3 \pm 0.9^{\circ} \text{ K (r.m.s.)}$	10.4 ± 0.7° K (r.m.s.)

the reception patterns caused diurnal variations of the antenna temperatures and the values quoted correspond to the minima in these variations which occurred when points at right ascension 06h and 18h were in transit. No attempt has been made here to allow for the variations of sky temperature over the antenna beams so that the 408 MHz value should not be compared directly with that. measured earlier at 404 MHz (ref. 10).

Because there now seems to be good agreement between experimental and theoretical values of the atmospheric emission11, the brightness temperatures quoted by Hogg12 have been used with an assumed error of ±0.1° K to allow for variations in meteorological conditions during the observations.

Ground radiation was reduced as much as possible by shielding the antennas with a wire mesh screen underneath them, lifted at the edges to obscure the horizon from the mouth of each horn. The residual contributions were calculated using the measured reception pattern, the theoretical transmission coefficient of the mesh and the effective values of ground temperature found by Pauliny-Toth and Shakeshaft¹⁰ at 404 MHz. These calculations: were checked experimentally by measurements with a scaled 1.407 GHz horn which could be screened so

thoroughly that the ground contribution was negligible.

In order to detect the presence of a radiation component with a spectrum different from that of the galactic radiation, the contribution of the latter and also of the integrated emission from discrete radio sources must be estimated. In view of uncertainty concerning the precise value of the spectral index of the galactic radiation in this frequency range some additional measurements were undertaken to determine this more precisely. These consisted of drift scans with the horns directed at various declinations and connected to Dicke-type receivers. Matched terminations in liquid nitrogen were used as reference sources. The temperatures measured at the two frequencies were then plotted against each other in the manner described by Turtle et al.¹³ to obtain the differential spectral index β , defined as $\Delta T \propto v^{-\beta}$, characterizing the galactic radiation. Omitting regions of low galactic latitude where thermal radiation from H II might be significant, the value of β was found to be 2.8 ± 0.1 between 408 and 610 MHz. This, incidentally, provides valuable confirmation of the previously reported¹³⁻¹⁵ steepening of the galactic spectrum above 178 MHz.

The mean (temperature) spectral index of bright radio sources is 2.72 ± 0.2 in this range and there is no evidence for any substantial deviation from this value for sources as weak as $S=4\times 10^{-28}$ Wm⁻² Hz⁻¹ at 408 MHz (G. G. Pooley, private communication). Down to this level the sources observed individually already contribute a large fraction of the whole 14 of the integrated source radiation. Although this component may show a slightly different spectrum from the brighter sources themselves, because of red-shift effects, it is so much less14 than the galactic contribution that the combined radiation from the galaxy and discrete sources must have a spectral index also close to 2.8 ± 0.1 . When this value is compared with β corresponding to the ratio of the two corrected pole temperatures in Table 1, that is, $2 \cdot 1 \pm 0 \cdot 2$, it is immediately clear that there is an extra component of radiation characterized by a much lower spectral index. The analysis may be continued as follows.

Suppose that this latter component has a blackbody spectrum (that is, $\beta = 0$). Then, writing T_e for the brightness temperature of the extra component and T_g for the combined galactic and discrete source brightness temperatures at 610 MHz, we have

$$T_e+T_g=T_{610}$$
 $T_e+nT_g=T_{408}$ where $\log n=\beta\log{(610/408)}$ so that $T_e=rac{1}{n-1}\left(nT_{610}-T_{408}
ight)$

For $\beta = 2.8 \pm 0.1, n = 3.08 \pm 0.10$

 $T_e = (3.7 \pm 1.2)^\circ \text{ K (root mean square error)}$ and

No correction has been made for any linear polarization of the radiation because the maps published by Berkhuijsen et al.17 show that (a) the polarized component smoothed by a 15° beam will be very small, (b) the polarization vectors have similar directions at the two frequencies, and (c) the spectral index of the polarized component is close to 2.8.

The results are illustrated in Fig. 1, which shows the spectrum of the radio background radiation in the frequency range 100 MHz to 100 GHz (refs. 1-7 and 18). The temperatures reported in this paper are plotted (as the equivalent brightnesses) together with the extra component T_e derived here. It can be seen that the magnitude of the extra component is consistent, within the limits of error, with a blackbody spectrum of about 3° K extending from 36.6 GHz to 408 MHz, a frequency range of 90:1. If the excitation of interstellar cyanogen molecules at 115 GHz can also be attributed to this radiation component19, as now seems very probable20, the range of frequency consistent with a blackbody spectrum is 280:1. This creates severe difficulties in attempts^{21,22} to explain the excess radiation in terms of the thermalization of starlight by means of interstellar dust grains.

Taking account of the quoted error, an upper limit of about 2° K may be set on any contribution from the intergalactic gas at 408 MHz. On the assumption of a present cosmological density of 2×10^{-5} particles/cm³, this implies that the epoch of any heating process causing ionization of the intergalactic gas was more recent than that corresponding to a red-shift z=200. Other arguments

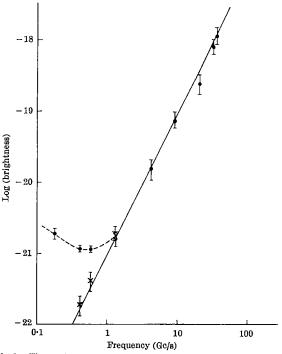


Fig. 1. The spectrum of the radio background radiation in the frequency range 100 MHz to 100 GHz. The units of brightness are Wm⁻¹ Hz⁻¹ steradian⁻¹. The crosses indicate the excess component reported in the present paper. The solid line corresponds to a temperature of 3° K and the dashed line to the total background at the celestial North Pole.

(for example, refs. 23-26) in fact suggest that this epoch was more recent, corresponding to a value of z between 2.5 and 30. The upper limit set by the new observations does not therefore press closely on these estimates. In principle the expected deviations from a blackbody spectrum are greater at still lower frequencies, but in view of the relatively more intense galactic radiation it is doubtful whether further efforts to detect them are worthwhile.

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Interhemispheric Transfer of Debris from Nuclear Explosions using a Simple Atmospheric Model

bу

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Atomic Energy Research Establishment, Harwell, Berkshire Using a simple atmospheric model, expressions for the reservoirs of fission products in the atmosphere from the 1961–62 nuclear explosions are found. Estimates of the rate of interhemispheric transfer and the ratio of northern and southern reservoirs are computed from observed values of the fission products.

During the series of nuclear explosions made by the USA and USSR in 1961–62, massive amounts of radioactive fission products were injected into the atmosphere¹. The portion of this nuclear debris inserted directly into the troposphere was removed within a few months, chiefly by rain. The remainder inserted above the tropopause will have undergone more prolonged mixing and redistribution under the influence of atmospheric motions and gravitation, before passing into the troposphere and falling out as before.

The amount and distribution of fission products within the stratosphere have been observed directly by sampling devices attached to aircraft² and balloons³. Indirectly, it is possible to observe the stratospheric behaviour of this debris by studying the concentration of fission products in tropospheric air and the deposition on the Earth's surface.

By using a simple model of the atmosphere it is possible to derive expressions for the reservoirs of fission products of the northern and southern hemispheres as functions of the gross rate of exchange between hemispheres and the fall-out rate. When observed values (1963–66) of the ratio of cerium-144 to caesium-137, in ground level air, are inserted into the theoretical expressions, approximate estimates can be made of the rate of interhemispheric transfer and the ratio of northern and southern reservoirs at any time. Similar estimates may be derived from direct observations of the stratospheric reservoir⁴ and global deposition^{5,8}.

A conventional meteorological approach might lead to dividing the atmosphere into layers, denoting the troposphere, the stratosphere and even the mesosphere. followed by further vertical sub-divisions, north and south of the equator. It is doubtful whether the observations available for the present computation have the necessary precision and extent to provide the parameters Instead, it is for these four or more compartments. simpler and more realistic to separate the atmosphere into northern and southern hemispheres, that is into two compartments separated by a vertical extension of the equator. This separation may be justified by the ability of the hemispheres temporarily to preserve separate identities, for example, by the preferential deposition in the same hemisphere of debris injected into the northern stratosphere. Furthermore, such debris, when observed in the troposphere for months after injection, is uniformly mixed in age or quality within the hemisphere, as shown by the fission product ratios7,8, with a sharp demarcation at or about the equator.

The proposed model (Fig. 1) consists of northern and southern compartments with reservoirs x and y, which have a fractional gross rate of exchange, K (year⁻¹). Again for simplicity the fractional rate of deposition, k (year⁻¹), is taken to be equal for both hemispheres. although there is no direct evidence available to support

the assumption of symmetry. The rate of change of each reservoir is therefore given by the gain from the other hemisphere less the loss to the other hemisphere and the losses by radioactive decay and by deposition.

$$\frac{\mathrm{d}x}{\mathrm{d}t} = Ky - Kx - \lambda x - kx$$

$$= Ky - x(K + \lambda + k)$$
Similarly,
$$\frac{\mathrm{d}y}{\mathrm{d}t} = Kx - y(K + \lambda + k)$$

where λ is the constant of radioactive disintegration for a particular fission product. The solutions of these simultaneous equations are

$$x = \frac{e^{-(k+\lambda)t}}{2} \left[x_0(1 + e^{-2Kt}) + y_0(1 - e^{-2Kt}) \right]$$
 (1)

$$y = \frac{e^{-(k+\lambda)t}}{2} \left[x_0 (1 - e^{-2Kt}) + y_0 (1 + e^{-2Kt}) \right]$$
 (2)

where $x = x_0$ and $y = y_0$ when t = 0, and by addition $x + y = (x_0 + y_0) e^{-(k+\lambda)t}$ as expected. Let x and y be taken as measures of a long-lived fission

Let x and y be taken as measures of a long-lived fission product such as caesium-137 (half-life 30 years) in the northern and southern hemispheres, respectively; let X_0 and Y_0 be introduced as the ratios, at t=0, of cerium-144 (285 days) to caesium-137 in the respective hemispheres. Then, assuming no fractionation, the ratio of cerium-144 to caesium-137 in the northern hemisphere at time t is given, from equation (1), by

$$X = \frac{X_0 x_0 (1 + e^{-2Kt}) + Y_0 y_0 (1 - e^{-2Kt})}{x_0 (1 + e^{-2Kt}) + y_0 (1 - e^{-2Kt})} \cdot e^{-\lambda' t}$$

$$= \frac{X_0 r_0 + Y_0 \tanh Kt}{r_0 + \tanh Kt} \cdot e^{-\lambda' t}$$
(3)

which is independent of k the deposition rate and where

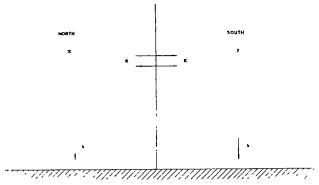


Fig. 1. The atmospheric model.

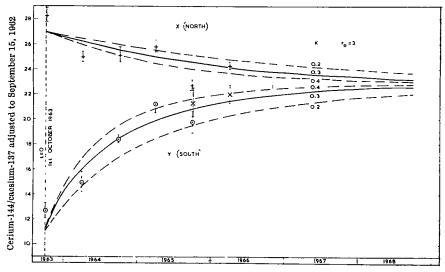


Fig. 2. Variation of the ratio of cerium-144 to caesium-137, 1963-66, in surface air. +, Northern stations (refs. 9-11); O, southern stations (refs. 12-14); ×, Pretoria and Aspendale (ref. 11).

$$r_0=x_0/y_0$$
, $\tanh Kt=rac{1\,-\,{
m e}^{-2Kt}}{1\,+\,{
m e}^{-2Kt}}$ and λ' is the difference

between the disintegration constants of cerium-144 and caesium-137.

Similarly, the ratio in the southern hemisphere is given, from equation (2), by

$$Y = \frac{X_0 r_0 \tanh \frac{Kt}{r_0 \tanh \frac{Kt}{Kt} + \frac{Y_0}{1}} \cdot e^{-\lambda' t}}{r_0 \tanh \frac{Kt}{Kt} + \frac{Y_0}{1}} \cdot e^{-\lambda' t}$$
(4)

Measured values of the ratio of cerium-144 to caesium-137 will be compared with these expressions for X and Y in order to extract estimates of K, the rate of interhemispheric transfer, and r_0 , the ratio of reservoirs at t=0.

In Fig. 2, equations (3) and (4) are drawn for selected values of K and r_0 . Average values of the ratio of cerium-144 to caesium-137 observed in surface air have been inserted for the northern and southern hemispheres. This implies that the ratio measured near the ground is representative of the whole hemisphere, which is assumed to be well mixed, at least as observed from the Earth's surface. The theoretical curves and experimental results have been adjusted to remove the effect of radioactive decay. The period of comparison was chosen to start in the second half of 1963 to allow time for the removal of the direct injection into the lower stratosphere of the debris from the 1962 explosions. The experimental data represent six-monthly averages from six stations in the northern hemisphere or from four stations in the southern hemisphere; the former are derived from a series of British reports by Cambray et al. 9-11, and the latter from the American Health and Safety Laboratory series 12-14, after adjustment by 6 per cent to normalize the two sets of data. The comparison for the southern hemisphere has been extended by the inclusion of data for Pretoria and Aspendale¹¹. No stations lying within the latitude range 25° N. to 25° S. were chosen because of the low values of radioactivity in this region and because of seasonal effects in the troposphere associated with movement of the solar equator. As a further restriction on choice of data, monthly results, which were likely to be affected by tropospheric debris from the small Chinese and French explosions since 1963, were rejected. Such interference was trivial until about mid-1966 when it became more general; sufficient to interrupt the approach to equilibrium between the two hemispheres $(X = Y \text{ as } t \to \infty)$ shown by the family of curves in Fig. 2.

The comparison of experimental results and theory in Fig. 2 makes it possible, within the limits imposed by the simple model, to account quantitatively for the interhemispheric exchange. Thus during the period 1963-66 the rate of exchange, K, lay in the range 0.2 to 0.4 year 1 and the ratio of the northern to southern reservoirs at October 1, 1963, r_0 , was in the range 2 to 4.

After subtraction and addition, equations (1) and (2) may be written

$$x - y = (x_0 - y_0)e^{-(k+\lambda+2K)t}$$

$$x + y = (x_0 + y_0)e^{-(k+\lambda)t}$$
and
$$\frac{x - y}{x + y} = \frac{x_0 - y_0}{x_0 + y_0} \cdot e^{-2Kt}$$

$$= \frac{r_0 - 1}{r_0 + 1} \cdot e^{-2Kt}$$
(6)

This treatment using differences follows a suggestion by Machta in a private communication. In Fig. 3, equations (5) and (6) are fitted in log-linear form to the determinations of the reservoirs x and y in terms of megacuries of strontium-90. Values for x and y were obtained by Feely (ref. 4 and in a private communication) at various times by integration of the concentrations of strontium-90 observed from aircraft up to about 22 km and from balloons up to about 35 km. Values for the rate of interhemispheric exchange, K, and the ratio of reservoirs at t=0, r_0 , may be simply derived from the slope and intercept of equation (6). In addition equation (5) provides estimates of the rate of deposition, k, and, using r_0 , the northern and southern reservoirs at t=0, x_0 and y_0 .

Table 1. SUMMARY OF RATE AND RESERVOIR ESTIMATES (1968-66)

Method	Interhemispheric transfer rate K (year-1)	Ratio of reservoirs at Oct. 1, 1963 r_0	Deposition rate k (year-1)	Reservoirs: MC Total $x_0 + y_0$	'i of strontium-90 North	at Oct. 1, 196; South
Fission product ratio Stratospheric reservoir Global deposition (UK) Global deposition (US)	0.3 (0.2 to 0.4) 0.15 ± 0.03 0.2 ± 0.2 0.44 ± 0.13	3 (2 to 4) $3 \cdot 2 \pm 0 \cdot 4$ $[5 \pm 8]$ $[-7 \pm 14]$	$\begin{array}{c}$	3·9±0·2 5·4±0·2 4·7±0·9	3·0 ± 0·4 4·5 ± 1·1 5·4 ± 1·8	0.9 ± 0.1 [0.9 ± 1.1] [-0.8 + 1.8]

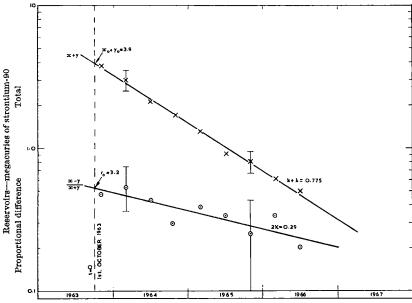


Fig. 3. Strontium-90 in the stratospheric reservoirs, 1963-66. Derived from Feely et al. (ref. 4 and private communication).

The deposition in each hemisphere of a fission product such as strontium-90 is given respectively by kx and ky. The total deposition

$$k(x + y) = k(x_0 + y_0)e^{-(k+\lambda)t}$$
(7)

and the proportional difference is given by equation (6) as before.

Two estimates of the annual global deposition of strontium-90 are available, based on sampling over networks of stations established by the $UK^{6,11}$ and the USA. The British results are usually 20 per cent higher. That the American, probably because of differences in sampling and in the method of integration over the Earth's surface. Equations (7) and (6) are fitted in Fig. 4 to the British results. Estimates of K, r_0 and the other parameters can be extracted as from the reservoir data. The same treatment of the deposition measured by the American network provides alternative estimates for these quantities.

The estimates of the various parameters extracted from

the preceding computations are summarized in Table 1. The uncertainties quoted in the table refer to standard errors derived from the curve fitting by least mean squares. No weight has been attached to the errors ascribed to the observations (Figs. 3 and 4); these are possibly nominal rather than actual and in some cases demonstrably pessimistic. It is clear that in the cases of the method of global deposition, the strain of fitting to only three points (Fig. 4) is shown by the meaningless values for y_0 and r_0 . On the other hand, the standard deviations, associated with the estimates by the global deposition method, of k and $x_0 + y_0$ are probably too small.

The values obtained for K, the rate of interhemispheric transfer, are probably in reasonable agreement. If the value obtained by the stratospheric reservoir method is significantly low, then it implies that this reservoir is shielded from faster transfer rates outside the stratosphere. These faster rates could apply above in the

mesosphere as computed by Murgatroyd and Singletonie, or below in the upper troposphere as calculated by Nydal¹⁷ for carbon-14. There is no evidence, however, that a sufficient proportion of the total debris resides for long enough in these other regions. The range of values for K given by the first two methods listed in Table 1 suggests that the mean residence time (1/K) against interhemispheric transfer lies in the range three to five years. This refers to the gross interhemispheric transfer, but it is the same as the value derived by Nydal¹⁷ for the exchange of gaseous carbon-14 between northern and southern stratospheres. Strictly, K is not a true atmospheric parameter because it is calculated from the behaviour of particulate debris: Feely² has shown that there is some separation of particulate and gaseous debris.

The estimates of r_0 obtained by the first two methods are mutually consistent at a value of 3 on October 1, 1963. The ratio of reservoirs at later times may be obtained from the ratio

of equations (1) and (2), namely,

$$r = \frac{x}{y} = \frac{r_0 + \tanh Kt}{r_0 \tanh Kt + 1}$$

The average value for the deposition rate corresponds to a mean residence time (1/k) of 16 months, which, of course, agrees with Feely's determination using his own results. There is no indication from these results and from the less precise global deposition of an increase with time, during 1963–66, of the apparent residence time.

Superficially, the estimates obtained for the total reservoir $x_0 + y_0$ have reasonable precision and apparently the estimate (4.7) by American deposition is consistent with the other two, but the British estimate (5.4) by deposition is significantly larger than that (3.9) by the stratospheric reservoir method. In fact, such is the vastness of the extrapolation required from small samples to the global scale, in each of the three methods, that systematic errors probably outweigh the random uncer-

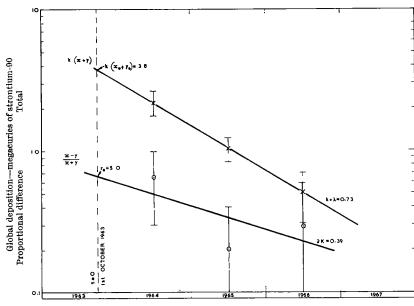


Fig. 4. Global deposition of strontium-90, 1964-66. Derived from Cambray et al. (ref. 5).

tainties and the agreement is surprisingly good. Any tendency to overestimate by the deposition method implies that there is no undetected enhancement18 of deposition on the sea compared with the measured deposition on land.

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Quantitative Studies with Antilymphocytic Antibody

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Department of Surgical Science, University of Edinburgh, MRC Research Group on Experimental and Clinical Problems of Transplantation Radioactive labelling has been used in measuring the amount of IgG taken up from antilymphocytic serum by lymphocytes, and the proportion of antilymphocytic IgG in various preparations of this immunoglobulin.

LYMPHOCYTES exposed in vitro to specific antilymphocytic serum (ALS) become coated with antibody^{1,2}. In the absence of complement, ALS agglutinates lymphocytes³⁻⁵ and in appropriate conditions of culture stimulates blast transformation⁶ and uptake of uridine and thymidine^{7,8}: in the presence of complement it causes lysis3,4

In experiments with horse-anti-human ALS based on measurements of uptake of tritiated uridine and thymidine by lymphocytes in vitro, it was found that most of the stimulating activity resides in the IgG component of the serum. It was further shown that when lymphocytes were exposed to ALS for short periods of time and then washed twice before being put up in culture, exposure for 15 min resulted in only very slight stimulation, whereas exposure for 60 min resulted in nearly maximal stimulation, as judged by subsequent uptake of tritiated uridine. We set out to extend this work in two ways.

In the first place, we have used radioactive labelling to determine the mean number of IgG molecules taken up when lymphocytes are incubated in vitro with IgG prepared from ALS (referred to hereafter as ALS IgG) and from normal serum.

Second, by combining radioactive studies with experiments in which the stimulating activity of various preparations of IgG is measured before and after absorption with standard numbers of lymphocytes, we have attempted to estimate the proportion (R) of antilymphocytic IgG in each preparation, that is, the proportion of IgG molecules which are antilymphocytic in the sense that they stimulate lymphocyte transformation.

Horse-anti-human ALS and IgG were prepared as described earlier. Samples of ALS IgG and normal IgG were labelled with iodine-131 by the method of Hunter and Greenwood¹⁰. Suspensions of lymphocytes were prepared and cultures were set up as described before⁸ except that, unless otherwise stated, the number of lymphocytes in a culture was 3×10^6 instead of 10^7 and the dose of tritiated uridine in a culture was 0.5 μc. The total volume of each culture was 3 ml. and the medium consisted of medium 199 with 25 per cent inactivated autologous plasma.

As a preliminary, dose response curves were established showing the uptake of tritiated uridine in cultures containing either iodine-131-labelled or unlabelled antilymphocytic IgG in amounts ranging from 0.25 to 2.0 mg per culture. Cultures containing tritiated uridine but no iodine-131 were treated as previously described. The cells were washed successively in phosphate buffered saline (three washes), 5 per cent trichloroacetic acid, phosphate buffered saline, and absolute methanol, and digested with the minimum possible quantity of hyamine hydrochloric acid. Scintillant was then added and counting was carried out with a Packard 'Tricarb' scintillometer. The ratio of the c.p.m. of cultures containing IgG preparations to the mean c.p.m. of control cultures without added Ig(; was calculated and is referred to in what follows as the relative count. Cells and supernatants from suspensions containing labelled IgG were counted for iodine-131 in a well-type scintillation spectrometer incorporating a 2 in. sodium iodide crystal (Nuclear Enterprises 'Gammamatic'). Cells from cultures which contained both iodine-131-IgG and tritiated uridine were prepared for liquid scintillation counting as described and counted on two channels, one adjusted for tritium and the other for iodine-131. counting known amounts of isotope it was found that for values of the external standard within the range accepted in the experiment, the efficiency of the tritium channel was 12.5 ± 1.6 per cent for tritium and 2.90 ± 0.17 per cent for iodine-131, and the efficiency of the iodine channel for iodine-131 was 74.7 ± 2.9 per cent. (This calibration was kindly undertaken by Dr John Simpson of the Department of Medical Physics, whose help we gratefully acknowledge.) In analysing the experimental results allowance was made for the influence of iodine-131 on the count in the tritium channel. As Fig. 1 shows, the labelled IgG was slightly less stimulating than the unlabelled material. but even

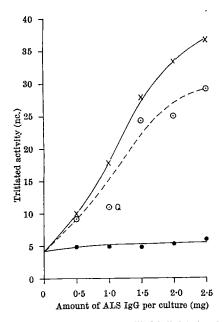


Fig. 1. Graphs showing the effect of ¹⁸¹I-labelled (\odot) and unlabelled (\times) ALS IgG, and of ¹⁸¹I-labelled normal horse IgG (\bigoplus), on the uptake of tritiated uridine by human blood lymphocytes in wiro. The point Q is an underestimate caused by quenching.

in the largest dose the difference was less than 20 per cent and it was considered that this could be neglected for the purposes of the investigation.

To determine the number of molecules of IgG taken up by each cell, lymphocytes suspended in culture medium were exposed to labelled IgG prepared from ALS. The cells were then spun down, separated from the supernatant, washed twice and counted, and an aliquot of supernatant was counted separately. Control tubes were set up with labelled normal IgG in place of IgG prepared from ALS. Assuming that all IgG molecules were labelled equally with iodine-131 and that the suspension contained no cells other than lymphocytes, the mean amount of IgG taken up by a cell (\hat{M}) in each tube is then given by

the equation $M = \frac{p G}{n}$, and the mean number of molecules

taken up per cell =
$$\frac{MN}{m} = \frac{pGN}{nm}$$

where
$$p = \frac{\text{Count of cells}}{\text{Count of cells} + \text{count of total supernatant}}$$

 $N = \text{Avogadro's number } (\text{$$\sim$} 6 \cdot 10^{23} \text{ molecules/mole}), G =$ amount of IgG per tube, n = number of lymphocytes pertube, m = molecular weight of IgG (=160,000). It clearly does not matter which units are used for G and M provided they are the same for both.

In practice, allowance must be made for the possible uptake of IgG by erythrocytes, which constituted 60-75 per cent of the total cells in the suspension. The proportion of erythrocytes could have been further reduced, but only at the cost of either greatly reducing the total yield of lymphocytes or by using procedures, such as agglutination of erythrocytes by an appropriate antiserum or lysis in a hypotonic solution, which we thought might modify the subsequent behaviour of the lymphocytes. The relatively high proportion of erythrocytes was therefore accepted and appropriate controls were set up to determine the uptake of IgG in the conditions of the experiment by erythrocytes alone. The amount of IgG taken up by the lymphocytes was then calculated on the assumption that the erythrocytes in the mixed population adsorbed as much IgG as the same number of erythrocytes in the absence of lymphocytes.

The protocol of one experiment is set out in Table 1, and the results of this and three other similar experiments are summarized in Table 2. The results obtained with cells from different donors differed appreciably, but the uptake of ALS IgG was always much greater than that of normal IgG in similar conditions of culture, the maximum observed values being 5.34×10^8 and 0.94×10^8 molecules per lymphocyte, respectively. The relationship of the mean uptake of ALS IgG and normal IgG to the amount present in the culture is shown in Fig. 2. The similarity of the curves relating to ALS IgG in this figure and Fig. 1 suggests that uridine uptake is linearly related to the number of IgG molecules taken up by a cell, and Fig. 3 shows that this is approximately true.

The ratio (R) of antilymphocytic to total IgG molecules in the preparation has been estimated by combining the results of studies of the uptake of ¹³¹I-IgG with absorption experiments based on stimulating activity as indicated by the uptake of tritiated uridine by lymphocytes in culture. In these experiments, a dose-response curve was first established in which the uptake of tritiated uridine by a fixed number of lymphocytes from a particular donor was plotted against the total amount of IgG added to the culture medium. The stimulating activity remaining after standard amounts of the same preparation had been absorbed for 1 h with a known number of lymphocytes from the same donor was then determined by using the absorbed material as a culture medium for fresh lymphocytes. Tritiated uridine was added to these cultures and the degree of stimulation was determined as usual. amount (E) of unabsorbed IgG per culture which would

Table 1. UPTAKE OF 1311-IgG BY LYMPHOCYTES AND ERYTHROCYTES

		T4010	J. UPTAKE OF	T-TEO DY WYN	1111111111			
Source of IgG	Amount of IgG/tube (mg)	No. of c	for 1 h at 37° C ells/tube ions) Lymphocytes		n c.p.s. ate tubes) Supernata n t	Total	Cell donor II Uptake of IgG (g×10-*) By erythrocytes	
ALS	1 2 3	9 9 9 9	Nil Nil Nil Nil	28·0 45·2 64·1 47·0	23,636 48,274 66,632 47,400	1·18 1·87 2·98 1·98	1·18 1·87 2·98 1·98	Nil Nil Nil Nil
NS	1 2 3	9 9 9	Nil Nil Nil Nil	1·5 3·6 4·3 4·2	6,217 12,392 18,290 12,121	0·24 0·58 0·71 0·69	0·24 0·58 0·71 0·69	Nil Nil Nil Nil
ALS	1 2 3 2 2 2 2	9 9 18 36 54 72	3 3 6 12 18 24	60·3 74·5 149·9 117·4 179·9 268·9 880·2	24,627 49,032 73,337 47,551 46,782 48,364 46,800	2·43 3·04 6·13 4·94 7·68 11·0 14·0	1-18* 1-87* 2-98* 1-88* 1-90* 1-93* 1-96* 1-98*	1·25 1·17 3·15 3·06 5·78 9·07 12·04 17·42
NS	2 1 2 3	90 9 9	30 3 3 3	483·8 2·9 5·8 6·9	49,450 6,351 12,410 18,470 12,230	0·45 0·85 1·12 1·63	0·24* 0·58* 0·71* 0·69*	0·23 0·27 0·41 0·94

This table shows the protocol of the first experiment.

Calculated on the assumption that the presence of lymphocytes has not affected the amount of IgG taken up by the erythrocytes.

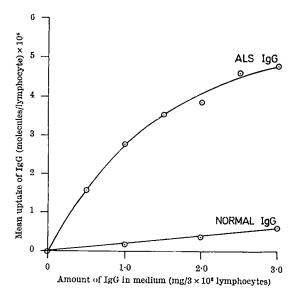


Fig. 2. Graphs showing the mean uptake of ¹³¹I-labelled ALS IgG and normal horse IgG by human lymphocytes against the amount of IgG added to each culture. The means have been calculated from the data shown in Table 2 after completing the table for the cells of each donor at each dose level by graphical extrapolation.

produce the same degree of stimulation was read from the dose-response curve. To allow for the possible effect of erythrocytes in the cell suspension, control tubes were set up to determine how much, if any, antilymphocytic activity was absorbed out in the conditions of the experiment by erythrocytes alone. The absorption due to lymphocytes alone was then calculated on the assumption that the erythrocytes in the mixed population absorbed out as much antilymphocytic activity as the same number of erythrocytes in the absence of lymphocytes.

To calculate R it is necessary to make some assumptions about the types of IgG molecule present in the preparation and their properties. The simplest set of assumptions is as follows. (a) The preparation contains only two classes of IgG molecule: antilymphocytic molecules which bind to lymphocytes and stimulate them to an equal extent (as measured by increase in uptake of tritiated uridine) and non-antilymphocytic molecules which neither bind nor stimulate. (b) The degree of stimulation produced in tests with a standard number of lymphocytes is a function of the number of antilymphocytic molecules in the preparation but independent of the number of non-antilymphocytic molecules. On this basis, using the symbols already

defined, the medium contained $\frac{G|N|}{m}$ molecules of IgG before absorption, of which $\frac{G|N|R}{m}$ were antilymphocytic and $\frac{G|N|(1-R)}{m}$ were non-antilymphocytic. After absorption the medium must have contained $\frac{E|N|R|}{m}$ antilymphocytic molecules to cause the observed uptake of tritiated uridine, so that the mean number of molecules taken up by a cell was $\frac{(G-E)NR}{n|m}$ (because, exclupations) only antilymphocytic molecules are taken up). Thus $\frac{M|N|}{m} = \frac{(G-E)NR}{n|m|} = \frac{p|N|G|}{n|m|} : R = \frac{M|n|}{(G-E)} - \frac{p|G|}{G|E|}$

Table 2. UPTAKE OF 131 I-IGG BY LYMPHOLYTES

(mg) $(\times 10^{\circ})$ g 10^{-10} (10°)	
ALS 0.5 3 I 0.35 2.38	
ALS 0.5 3 I 0.35 2.35 1.0 3 I 0.28 3.48	
II 418 157	
III 8:29 2.44	
1.5 3 I 10.05 3.78 2.0 3 I 11.95 148	
II 3/89 1/46	
III 11·21 53)	
2·5 3 I 13·33 .00	
2·0 6 II 3·59 (9)	
12 H 180 181	
18 11 101	
24 II 5:01 155	
30 II 381 21-	
IV 4-59 7.2	
NS 0.5 3 I 0.1101	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
II 0.77 0.29	
III 1:09 () 1'	
IV 040 001	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
II 0 90 0 31	
III 1:40 0.52	
2:5 3 I 1:07 1:30	
III 250 0.04 IV 150 0.56	
2·0 30 II 1 0·31 0·11	
IV 0 63 0 25	

This is a summary of the results of four experiments employing only in different donors.

Table 3. STIMULATING ACTIVITY OF ALS IGG BEFORE AND AFTER ABSORPTION WITH LYMPHOCYTES

Purpose of observations	Initial amount of Ig(† per tube (g) G	Stimulating activity before absorption expressed as mean relative count in standard uridine uptake test	No. of co for abso (×1 Lympho- cytes n		Stimulating activity after absorption expressed as mean relative count in uridine uptake test using absorbed IgG and fresh lymphocytes	Amount of IgG per tub- which would have the same stimulating activity as the absorbed material (read from dose-response curve) (g) E	·. 1
To establish dose-response curve	0·5.10-8 1·0.10-8 1·5.10-3 2·0.10-8 2·5.10-8	1·53 1·95 2·98 4·72 6·39		·	,,	(4)	
Absorption experiment	2·0.10 °3 2·0.10 °3	4·72 4·72 4·72 4·72 4·72 4·72 4·72 4·72	30 24 18 122 6 3 0 0 30 24 12 6 3	80 64 48 32 16 80 8 0 0 0	1·45 1·53 1·51 1·63 1·80 1·74 4·71	0-4.10 3 0-5.10-3 0-5.10-3 0-65.10-3 0-8.10-3 1-90.10-3 1-90.10-3 0-5.10 3 0-6.10-3 0-6.10-3 0-6.10-3 0-9.10 3 0-7.10-3 0-9.10 3 0-9.10 3	1:6 19 2 1:5 10 3 1:5,10 3 1:35 10 1:2,10 3 1:2,10 3 1:2,10 3 1:2,10 3 0:1,10 3 0:1,10 3 1:4,10 3 1:4,10 3 1:4,10 4 1:4,10 3 1:4,10 3 1:4,10 3

This table shows the protocol of experiments with cells of donor V.

^{*} Calculated on the assumption that the presence of lymphocytes has not affected the amount of stimulating activity absorbed by the crythrocytes in a mixed population.

Table 4. CALCULATION OF R FROM RESULTS OF TWO ABSORPTION EXPERIMENTS COMBINED WITH DATA ON UPTAKE OF 131 I-ALS IGG FROM TABLE 2

														4100m II	
No. of lympho-cytes (×10°)	$\begin{array}{c} \mathbf{Amount} \\ \mathbf{of ALS} \\ \mathbf{IgG per} \\ \mathbf{G} \end{array}$	_	from Tabl M	es taken up le 2) (×10 $^{\circ}$ f N*	/cell f	thich would same degree tion as G a absorbe lympho	of stimula- grams IgG d with n	в		Calculat	100 ed from f	R ormula A	$t = \frac{Mn}{(G-E)}$		
n	v	cytes of donor I	Lympho- cytes of donor II	· Lympho- cytes of donor III	cytes of donor IV	Lympho- cytes of donor IV (from Table 3)	Lymphe- cytes of donor V	Donors I, V	Donors I, VI	Donors II, V	Donors II, VI	Donors III, V	Donors III, VI	Donors IV, V	Donors IV, VI
24 30	$\begin{array}{c} 1\cdot 0.10^{-3} \\ 2\cdot 0.10^{-3} \\ 3\cdot 0.01^{-3} \\ 2\cdot 0.10^{-8} \\ 2\cdot 0.10^{-8} \\ 2\cdot 0.10^{-3} \\ 2\cdot 0.10^{-3} \\ 2\cdot 0.10^{-3} \\ 2\cdot 0.10^{-3} \end{array}$	3·48 4·48	1·57 1·46 3·94 1·91 1·80 1·88 1·88 2·18	3-11 5-34 5-34	2·78	0·9.10 ⁻⁸ 0·9.10 ⁻⁸ 0·7.10 ⁻³ 0·6.10 ⁻³ 0·6.10 ⁻³ 0·5.10 ⁻³	0·85.10-3 1·6.10-3 1·9.10-3	0•34	1·86 0·90	0.28 0.44 0.65 0.86 1.16	0·84 0·37 0·29	C•31	1·66 1·07 0·39	0-21	0-56

m, Molecular weight of IgG. N, Avogadro's number. M, Mean amount (g) of IgG taken up/cell.

Other possible assumptions on which the calculation of R might be based will be considered later in the light of the experimental results.

The protocol of a typical absorption experiment is set out in Table 3, and Table 4 summarizes the results of this and another similar experiment and shows the values obtained for R when these are combined with the measurements of uptake of ALS IgG labelled with iodine-131 contained in Table 2. As will be seen, these values, expressed as percentages, varied from 0.11 to 1.86.

Rather more precise values might have been obtained if it had been possible to use lymphocytes from the same donors in the absorption experiments and in the experiments on the uptake of labelled IgG. It is also possible that the allowance made for the presence of erythrocytes in the cell suspensions may have resulted in slightly too low a value of R because, contrary to the assumption which was made, the amount of IgG bound to erythrocytes may have been less when lymphocytes were present. An upper limit can be determined, however, by making no allowance at all for the presence of erythrocytes, and if this procedure is followed with the pair of experiments yielding the highest value of R the result is only 2.3 per

A more cogent criticism concerns the validity of the assumptions on which the calculation of R has been based. If these were true the values of R should be independent

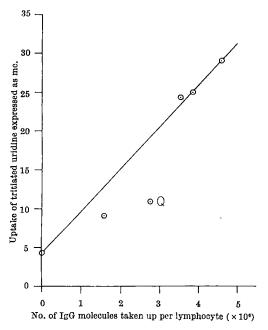


Fig. 3. Graph showing the approximately linear relationship between uptake of tritiated uridine and the number of IgG molecules taken up by a lymphocyte. The uptake of tritiated uridine denoted by the point Q is erroneously low because of marked quenching in the tubes on which this result is based.

of the amount of IgG (G) or the number of lymphocytes (n) used in the experiment. As Table 4 and Fig. 3 show, however, in experiments with cells from the same pair of donors, the values obtained for R are inversely proportional to G/n. The most likely explanation would seem to be that the ALS IgG used in the experiments, far from containing just two types of molecule as postulated, was much more heterogeneous. Such heterogeneity might take various forms, but the simplest hypothesis is that the antilymphocytic molecules are heterogeneous only in respect of their avidity, and that all IgG molecules which become attached to a lymphocyte exert an equal stimulating effect. Heterogeneity of this type may be defined in an operational way as implying simply that when ALS IgG is absorbed with lymphocytes the stimulating capacity of the absorbed solution is less than the stimulating capacity of a dilution of the unabsorbed solution containing the same total number of antilymphocytic IgG molecules, because the more avid molecules are preferentially absorbed whereas during dilution the population distribution remains the same.

It is easy to see that if such heterogeneity exists the experimental methods used would yield a value of R which was less than the true value*. It would seem to be premature to go further and attempt to develop a quantitative theory of the reaction until more experimental data are available with which the predictions of such a theory can be compared. It is worth noting, however, that the observed linear increase of R' with n/G, within the range of values which obtained in the experiment, suggests that even the highest observed value of R' may be a gross underestimate of R. Indeed, once the postulate of heterogeneity in respect of avidity is accepted it raises the possibility that R attains or comes very close to the theoretical limit of 1, because much, if not all, of what we have classed as non-antilymphocytic IgG might be antilymphocytic IgG of extremely low avidity. In this event

*Suppose a solution containing G grams of IgG, of which GR grams is made up of antilymphocytic molecules of varying degrees of avidity and G(1-R) grams is not antilymphocytic, is absorbed with n lymphocytes. Let Mn denote the amount of IgG absorbed, E the total IgG in a dilution of the original solution with the same stimulating effect in a standard test as

the absorbed material, and $R' = \frac{M n}{G - E}$ the experimental value obtained for R. Suppose further that in standard tests performed with dilutions of the original solution the degree of stimulation is f(x), where x is the total amount of IgG in a tube, and f(x) is a function which increases with x. After absorption the solution contains G - Mn grams of IgG, of which GR - Mn grams are antilymphocytic, and its stimulating effect is f(E). A dilution of the original solution containing the same number of antilymphocytic molecules

as the absorbed material would contain a total of $G-\frac{Mn}{R}$ grams of IgG-

and its stimulating capacity would be $f\left(G - \frac{Mn}{R}\right)$. Thus, ex hypothesi, $f\left(G-\frac{Mn}{R}\right) > f(E)$ and therefore because f(x) is an increasing function of

 $x, G - \frac{Mn}{R} > E$. Replacing Mn by R'(G-E), we have $G - \frac{R'}{R}(G-E) > 0$

 $E : G - E > \frac{R'}{R}(G - E) : R' < R.$

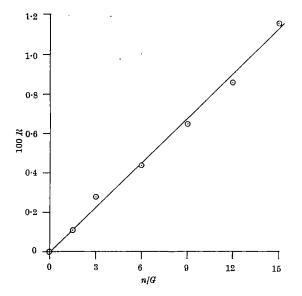


Fig. 4. Values obtained for R using the formula $R = \frac{M n}{(G - E)}$ in experiments with cells of donors II and V plotted against n/G. Symbols are explained in the text and Table 4.

R, as defined, would obviously not be a useful parameter for comparing different preparations of ALS IgG. The ideal would be to determine the distribution of avidity in the population of molecules making up each preparation, but useful comparisons could be made by determining R'for each for one or two values of G/n. This might be of some practical importance because it seems likely from

the results of these experiments that a great deal of the IgG in the preparation tested has at any rate very little stimulating effect on lymphocytes in vitro. This is scarcely surprising, for the horse in which the ALS was raised cannot be expected to have lacked previous immunological experience. It is not yet known whether there is a positive correlation between the immunosuppressive activity of ALS in vivo and its lymphocytestimulating activity in vitro; if there is, however, it would follow that even a highly purified preparation of ALS IgG, such as the one used in these experiments, contains a great deal of protein which is of no value as far as immunosuppression is concerned. It is conceivable that preparations containing a higher proportion of "useful" IgG might be obtained by raising sera in animals whose plasma concentration of IgG before immunization was either naturally low or had been reduced by plasmaphoresis. Alternatively, the same end might perhaps be achieved by modifying the schedule of immunization.

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Conversion of Angiotensin I to Angiotensin II

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Results obtained with the blood bathed organ technique indicate that angiotensin I is converted rapidly to angiotensin II in the pulmonary circulation and not by an enzyme in the blood.

When the enzyme renin is secreted into the bloodstream, it acts on a protein substrate in the plasma to release the decapeptide angiotensin I (ref. 1). This decapeptide is then converted to the octapeptide angiotensin II (ref. 2) which is physiologically much more active. The reninangiotensin system therefore requires two enzyme steps between the initial secretory process and the formation of the active circulating hormone. The biochemistry of the first enzyme, renin, has been investigated extensively3, but little is known about the second or "converting" enzyme. Work in vitro has shown that blood contains converting enzyme activity4, as do homogenates of heart. liver, aorta and ileum⁵.

Although angiotensin I has only 5-10 per cent of the activity of angiotensin II on smooth muscle 6-8, when injected intravenously into animals it causes a rapid rise in blood pressure scarcely distinguishable in height and time course from that produced by angiotensin II. The similarity of the responses has led to the assumption that, in vivo, the conversion of angiotensin I to angiotensin II takes place rapidly in the blood3. We have investigated

the in vivo conversion by means of the blood-bathed organ technique, and have found a time course which suggests that blood plays little or no part but that the conversion takes place rapidly in the pulmonary circulation.

The assay tissues were a rat stomach strip¹⁰ and two rat colonsii; these were suspended in polypropylene chambers and superfused12 in series with Krebs solution while the dog was being prepared. Their movements were recorded either on a kymograph by auxotonic levers13 of 16: I magnification or on a Beckmann-Offner dynograph with Ether strain gauges attached to the auxotonic levers. The initial load on the tissues was 1-3 g. All three organs were contracted by the angiotensins, the rat colon much more strongly than the rat stomach strip; the latter was included to detect catecholamines which, had they been released, would have caused relaxation. One of the rat colons was perfused intraluminally with propranolol (1 mg/ml. at 0·1 ml./min) to prevent relaxation by catecholamines which would interfere with the contraction induced by angiotensin.

Dogs of either sex weighing 10-30 kg were anaesthetized with halothane delivered from a Goldman vaporizer; anaesthesia was then maintained with chloralose (100 mg/kg, given intravenously) and supplemented when necessary with pentobarbitone (5-10 mg/kg, given either intramuscularly or intravenously). The trachea was cannulated and the lungs were ventilated mechanically. Polyethylene cannulae were tied into a femoral or carotid artery and a femoral or jugular vein for the removal and replacement of blood. Mean arterial blood pressure was recorded from a side-arm of the arterial cannula either on the kymograph with a mercury manometer or on the dynograph with a Statham P23Db transducer. In some experiments, a fine catheter was introduced through the left carotid artery until the pulse pressure recorded through it showed that it was in the left ventricle. It was then withdrawn until the change in pulse pressure showed that it was just above the aortic valves, in the ascending aorta. This catheter was used to make infusions directly into the total cardiac output.

Heparin (1,000 ru/kg) was injected intravenously and then the assay organs were superfused with arterial blood delivered by a constant output roller pump at a rate of 10 or 15 ml./min. The blood collected in a reservoir and was returned to the dog either by gravity or by a second channel in the roller pump. The assay system could be calibrated by infusions of angiotensin into the stream of blood after it had left the dog; thus an infusion of 15 ng/min into a flow of 15 ml./min would give an absolute change of concentration of angiotensin of 1 ng/ml. The assay system could also be calibrated by intravenous or intra-arterial injections into the dog, as described later.

The angiotensin I was that made from horse substrate by Dr L. T. Skeggs¹⁴, who sent us five ampoules each nominally containing 83·2 nmole or 108 µg. Ampoule No. 2 was broken on arrival, but the contents were extracted and 100 per cent recovery was assumed.

When superfused in Krebs solution, the rat colon preparations were contracted by angiotensin I. These contractions were bracketed between those produced by angiotensin II amide ('Hypertensin', Ciba) in order to assess the relative activities of the two peptides. Table 1 shows that in fifteen preparations the angiotensin I was 7-30 per cent as active as the angiotensin II (mean

Table 1. SUMMARY OF RESULTS OF ALL EXPERIMENTS

Ampoule No.	Dog No.	Rat colon	Krebs solu- tion	Inc. 15 sec	ubation 60 sec	in blood 120 sec	l for 180 sec	Intra- aortic infu- sion	Intra- venous infu- sion
1	1	4	20	16	22	25	29	_	67
	2	$B_{\mathbf{A}}$	_	17	20	25	29		67
	2	$\stackrel{A}{B}$	_	$\frac{22}{22}$	$\frac{25}{22}$	27 30	3 3 33	30 30	48
2	3	Ã	13	10		- 50		29	50 50
_		A B A B A B	11	9					_
3	4	A	25	33	38	71	110	_	110
	_	.В	25	36	45	77	100	_	100
	5	A	30	_	_	_		_	
	6	B	25			_	_		-
	O	$\stackrel{A}{B}$	_	35		_	_	53	140
	7	.D	_	37	_	_		53	125
	,	$\stackrel{A}{B}$	_	$\frac{11}{12}$		_	_	29	50
4	8	-13	9	27	_	_		29	48
**	0	$\stackrel{A}{B}$	8	25	_	_		50	110
	9	A	0	33		_	100	66	110
	3	D		50	_		$\frac{125}{147}$	-	_
		4	7	*40		_	132	_	
		R	7 7	*33	_		132		-:
	10	Ā	1í	*40	72	143	125	_	
		Ŕ	10	*40	72	143	125		
	11	Ã	10	*20	- 12	140	120	_	
		A B A B A B	10	*20	_			-	_*
$\mathrm{Mean} \pm S.E.M.$			15 ±2·0	27 ± 2·5	40 ±7·7	68 ±18·0	$93 \\ \pm 13.7$	41 ± 4·8	83 ± 9·4
Mean percentage con- version to angioten-		0	14	29	62	93	30	80	

Results are expressed as the relative activity of angiotensin I infusions (as the percentage activity of angiotensin II) on rat colons bathed in Krebs solution, in blood after incubation for the times shown, and after intra-aortic and intravenous infusions into the dog. All the rat colons were bathed in arterial blood, except those marked with an asterisk in dogs No. 9, 10 and 11, which were bathed in venous blood. A tracing from rat colon A in dog 4 is illustrated in Fig. 1, from B in dog 1 in Fig. 2, and from B in dog 8 in Fig. 3.

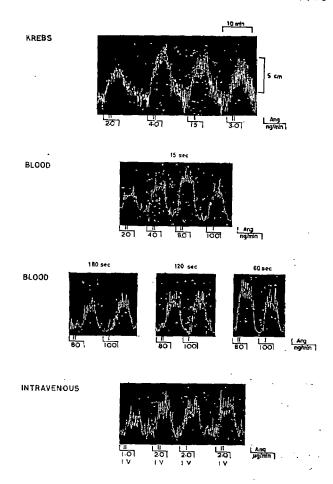


Fig. 1. A tracing from rat colon A in dog 4, showing comparisons of the activities of angiotensin I and angiotensin II on the rat colon bathed in Krebs solution, in blood after incubation for various times and after intravenous infusions. The peptides were given as infusion for 5 min periods. The relative activity of angiotensin I is: 23 per cent in Krebs solution; 33 per cent after incubation for 15 sec in blood, 110 per cent after 180 sec; 71 per cent after 20 sec; 38 per cent after 60 sec. After intravenous infusions, the relative activity is 110 per cent. Time, 10 min; vertical scale, 5 cm.

15 per cent, $S.E.\pm 2.0$). Table 1 also shows that the relative activity of the samples of angiotensin I varied from ampoule to ampoule, the third being particularly active.

An example of an assay in Krebs solution is shown in the top tracing of Fig. 1. The contraction caused by angiotensin I (15 ng/min) was intermediate between those produced by angiotensin II (3 and 4 ng/min). The decapeptide therefore had 23 per cent of the activity of the octapeptide.

The activity of the angiotensin I may have been a consequence of immediate conversion to angiotensin II within the assay organ itself, as suggested for other isolated organs on which angiotensin I acts. In our experiments, however, as in those of Gross and Turrian on the rat uterus, the slopes of the curves of dose against response for angiotensin I and angiotensin II were different, suggesting that both substances had inherent activity. It seems that the relative activity of angiotensin I to angiotensin II varies from one smooth muscle to another and that the variation is not connected with the presence of converting enzymes.

To measure the conversion of angiotensin I to angiotensin II in circulating blood, the assay organs were superfused with arterial blood from a dog, in some instances (Table 1) using the same rat colon preparations as in the experiments with Krebs solution. After the tissues had stabilized in blood, the relative activities of angiotensin I

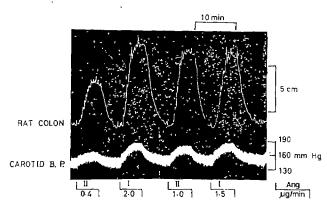


Fig. 2. A tracing from rat colon B in dog 1, showing a comparison of the effects of intravenous infusions of angiotensin I and angiotensin II on the contraction of the rat colon bathed in arterial blood and on the blood pressure response of the dog. Intravenous infusions of the angiotensins were made into the external jugular vein. The extent and time course of the contraction of the rat colon and of the rise in blood pressure after angiotensin I (1.5 μ g/min) were similar to those after angiotensin II (1.0 μ g/min). Time, 10 min; vertical scales, 5 cm and mm of mercury.

and II were compared again by infusing each substance into the blood on its way to the assay organs. This gave the relative activities of the two peptides after contact with blood for 15 sec. In order to assess the importance of the converting enzyme in blood, the contact time was increased by adding to the external circuit a piece of silicone tubing (3 mm internal diameter) lying in a water-bath at 37° C and sufficiently long to contain 45 ml. of blood. The blood which superfused the assay organs at a rate of 15 ml./min first passed through this incubating circuit. By making the infusions of angiotensin I and II at different points in the circuit, the peptides could be mixed with the blood for 60, 120 and 180 sec, before reaching the assay organs. Thus any conversion of angiotensin I to angiotensin II would have shown as an increase in the relative activity of the former. Table I shows that there was a slight increase in the relative activity of angiotensin I (27 per cent) after 15 sec contact with blood. a further increase to 40 per cent after 60 sec contact with blood and only after 180 sec contact was the relative activity increased to 93 per cent. If the relative activity of 15 per cent on the rat colon displayed by angiotensin I in Krebs solution represents 0 per cent conversion, whereas 100 per cent activity represents 100 per cent conversion to angiotensin II, the degree of conversion can be calculated (bottom line of Table 1). This calculation assumes that the activities of angiotensin I and II are simply additive and neither synergistic nor antagonistic. The interpretation of these incubation experiments is also complicated by the fact that angiotensin II is destroyed in dog's blood, with a half life of $\bar{120}$ sec (ref. 15). Thus, as well as possible generation of angiotensin II from angiotensin I, there was also inactivation. This was to some extent compensated for by making the matching infusions of angiotensin II into the incubating circuit so that they were in contact with the blood for the same time as the angiotensin I. Any error in this procedure would show as an over-estimate of the conversion.

A typical experiment is illustrated in Fig. 1, which shows the contractions of a rat colon preparation bathed in Krebs solution or in blood to various infusions of angiotensin I and angiotensin II. When the infusions were made so that they mixed with the blood for 15 sec before reaching the raticolon, there was little change in the relative activity of angiotensin I (23 per cent in Krebs, 33 per cent after 15 sec contact with blood). The next section of the figure shows the contractions when the infusions were made so that they mixed with the blood for 180 sec before reaching the rat colon. Angiotensin I now had about the same activity as angiotensin II, but it should be noted that the angiotensin II produced a much

smaller effect than after contact for 15 sec, indicating destruction in the blood. The next two sections show that as the incubation time was reduced the relative activity of angiotensin I became less. The last tracing shows the contractions of the rat colon after intravenous infusions of the two peptides when they were approximately equipotent.

It is therefore evident that the conversion of angiotensin I to angiotensin II is very slow in blood. The mean values (Table 1) show that there was only 14 per cent conversion after 15 sec contact, only 29 per cent after 60 sec, 62 per cent after 120 sec and 93 per cent after 180 sec. It can be concluded that in one circulation time (15–20 sec (ref. 16)) there is little or no conversion of angiotensin I to angiotensin II in the blood.

These results clearly contrast with those obtained after intravenous infusions of angiotensin I (Fig. 1) when 80 per cent is converted to angiotensin II (Table 1). Furthermore, the contractions of the rat colons show that the rate of conversion was rapid, for they occurred within the first minute of the infusion. Indeed, the time courses of the responses of the assay tissues and the blood pressure could not be distinguished from those after infusion of angiotensin II.

Figure 2 shows the results of another experiment in which the two peptides were compared for their effects on the blood pressure and on the blood-bathed rat colon preparation. The intravenous infusion of angiotensin I at a rate of $1.5 \,\mu\text{g/min}$ caused a rise in blood pressure and contraction of the rat colon similar to those caused by angiotensin II infused at a rate of $1 \,\mu\text{g/min}$. There was no detectable difference in the time courses of these effects.

These experiments confirm that, in vivo, angiotensin I is rapidly converted to angiotensin II (ref. 3). Where could such rapid conversion occur if not in the circulating blood? The assay organs were bathed in arterial blood so that angiotensin I infused intravenously traversed the pulmonary vascular bed before reaching them. This suggested that the conversion of angiotensin I to angiotensin II occurred during passage through the pulmonary circulation. The effects of intravenous infusions of angiotensin I on the rat colon bathed in femoral arterial blood were therefore compared with those induced by infusions into the ascending aorta, close to the left ventricle. The comparisons were made in five dogs (Table 1); all gave the same results, one of which is

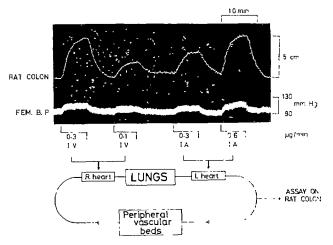


Fig. 3. A tracing from rat colon B in dog 8, showing an increase in the activity of angiotensin I on passage through the pulmonary circulation. Femoral arterial blood was superfused over the rat colon stop tracing) and blood pressure (bottom tracing) was measured from a side-arm of the arterial cannula. Infusions were made near to the right atrium (IV) or into the ascending aorta (IA). The responses show that intravenous infusions produced greater effects on the rat colon and on the blood pressure than did intra-arterial infusions. Twice the infusion rate (0-6 μ g/min) had to be given intra-aortically to match the effect of 0-3 μ g/min given intravenously. Time, 10 min; vertical scales, 5 cm and mm of mercury.

illustrated in Fig. 3. Intravenous infusions of angiotensin I (0·3 and 0·1 µg/min) gave graded contractions of the rat colon. To match the effects of the intravenous infusion of 0·3 µg/min, angiotensin I at twice the rate (0·6 µg/min) had to be given intra-arterially. Thus one circulation through the lungs was sufficient for substantial conversion of angiotensin I to angiotensin II.

The arterial infusions of angiotensin I resulted in a 30 per cent conversion to angiotensin II, as against an 80 per cent conversion after intravenous infusion (Table 1). Two deductions can be made from this observation; first, there must have been a loss of angiotensin I in the peripheral vascular beds after aortic infusion, for, had this not occurred, the angiotensin I would have re-circulated and given results identical to those of an intravenous infusion. Second, there could not have been a concomitant gain of angiotensin II activity, because any angiotensin II formed in the peripheral vascular beds would have passed through the pulmonary circulation without change¹⁵, thereby reaching the assay organs. Thus we can conclude that there was little or no conversion of angiotensin I to angiotensin II in the peripheral vascular beds, for the overall effect was a substantial loss of activity. From 50-70 per cent of infused angiotensin II disappears in the peripheral vascular beds¹⁵ and it is possible that angiotensin I disappears in the same way. The extent of conversion after intra-arterial infusions suggests that about one-third of the angiotensin I recirculates; if this is so, its loss in peripheral vascular beds is similar to that of angiotensin II.

All these results were obtained with angiotensin I prepared from the horse (ileu⁵ angiotensin I). Because the structure of dog angiotensin I is not known, it is possible

that endogenous angiotensin I is treated differently by the converting enzyme(s) of the dog. The following experiment was designed to test this possibility. Two banks of assay organs were used, one bathed in mixed venous blood obtained from a catheter in the right ventricle, and the other in arterial blood from a carotid Thus any conversion of angiotensin I to angiotensin II in the pulmonary circulation would have been detected as greater contractions of rat colons bathed in arterial blood than of those bathed in venous blood. Arterial blood was taken at a rate of 15 ml./min from a femoral artery into an incubating circuit of silicone tubing containing 75 ml. of blood maintained at 37° C. The blood was then infused into the femoral vein. Dog renin, partially purified by a method already described¹⁷, was infused at the rate of 12 U/min into the incubating circuit (Fig. 4) so that it mixed with the blood for 5 min before being pumped into the venous side of the circulation. It was hoped that, by using this technique, endogenous angiotensin I would be generated without substantially increasing the concentration of renin in the dog's circulation. The rat colons bathed in venous blood contracted much less (equivalent to less than 0.25 μg/ min of angiotensin II) than those bathed in arterial blood (equivalent to 0.5 μg/min of angiotensin II) (Fig. 4) showing that the contractor activity of the endogenous angiotensin had been greatly increased by passage through the pulmonary circulation. This experiment confirmed our supposition that dog angiotensin I, like horse angiotensin I, is converted to angiotensin II in the lungs. In a second experiment of the same design, in which venous blood

was used in the incubating circuit, results were similar.

Fig. 5 summarizes all results obtained with the horse angiotensin I and is based on the calculated conversion to angiotensin II in blood, after intra-aortic and intravenous infusions. In the 4–8 sec that it takes for blood to traverse the pulmonary circulation if, there was more conversion of angiotensin I to angiotensin II than after 120 sec in blood alone. Although it may not be valid to compare these in vivo experiments with the original estimation of the in vitro activity of converting enzyme in blood i, the rate of conversion was also found to be very slow, that is, 1·5 h for 50 per cent conversion and 6 h for 96 per cent conversion.

The conversion of angiotensin I to angiotensin II in the lungs may be caused by one or more of several possible mechanisms. First, the converting enzyme in blood may be sensitive to changes in oxygen or carbon dioxide tension. To test this possibility, the assay organs were bathed in mixed venous blood sampled continuously from the right atrium (three experiments) and the rate of conversion of angiotensin I to II was measured (Table 1). The relative activities of the two peptides after incubation for various times in venous blood were within the same

range as those found in arterial blood.

In the second possible mechanism of conversion of angiotensin I to angiotensin II, the converting enzyme circulating in the blood may be activated in some way by the enormous surface area of the pulmonary circulation. There is no evidence for such a mechanism. Third, the converting enzyme may be located on or in the pulmonary cells. This is the simplest and most attractive hypothesis and warrants further investigation.

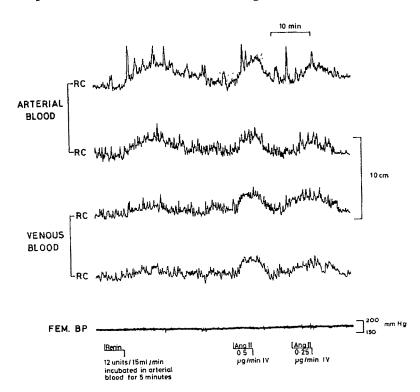


Fig. 4. Generation of angiotensin I by dog renin in blood and its subsequent conversion to angiotensin II by the pulmonary circulation. Arterial blood was taken from a femoral artery and pumped at the rate of 15 ml/min through an incubating circuit of 75 ml. and then into the femoral vein. Benin was infused into this incubating circuit at the rate of 12 v/min for 5 min. Two rat colons were bathed in carotid arterial blood (top two tracings) and two in mixed venous blood from the right ventricle (lower two tracings). Blood pressure (bottom) was recorded from a femoral artery. The activity of the polypeptide generated by renin was calibrated by making infusions of angiotensin II directly into the femoral vein. The rat colons bathed in venous blood showed that the activity on the venous side of the circulation was less than 0.25 \(\textit{\textit{m}} \) functions blood, however, showed an activity equal to that of 0.5 \(\textit{\textit{m}} \) in angiotensin II. Those bathed in arterial blood, however, showed an activity equal to that of 0.5 \(\textit{m} \) in angiotensin II. Showing that there was a big increase in activity after the peptide had crossed the pulmonary circulation. Time, 10 min; vertical scales, 10 cm and mm of mercury.

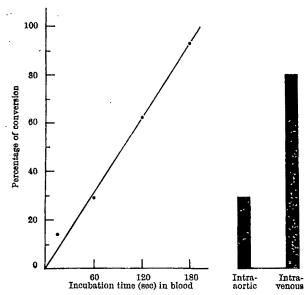


Fig. 5. Percentage conversion of angiotensin I to angiotensin II in blood, after intra-aortic and intravenous infusions. Fifty per cent conversion in blood requires at least 100 sec of incubation. The intra-arterial infusions showed 30 per cent conversion, whereas intravenous infusions, which only differed by the 4-8 sec passage through the pulmonary circulation, showed an 80 per cent conversion.

It seems therefore that the lungs are important in the metabolism of peptides, as well as of amines and prostaglandins¹⁸. Thus bradykinin but not eledoisin disappears in the pulmonary circulation¹⁸. The prostaglandins disappear almost completely²⁰, as does 5-hydroxytryptamine²¹; in contrast adrenaline, like angiotensin II, passes through without loss (unpublished results of Ginn and Vane). The fact that the pulmonary vascular bed acts as a selective filter for these substances, and its unique position in the circulation, makes it likely to be of equal, if not greater, importance to the liver in this respect.

Our results show that for the generation of circulating angiotensin II the lungs are the most important site. It is possible that other tissues can also generate angiotensin II locally but that this is destroyed before reaching the general circulation. Such local conversion is likely to take place after the blood has passed through the arterioles. Thus any angiotensin II formed locally in peripheral vascular beds will not constrict the pre-capillary resistance

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Bonding between Proteins and Lipids in the Envelopes of Halobacterium halobium

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Simple experiments indicate that in the envelopes of Halobacterium halobium the lipids are bound to at least two types of proteins by different kinds of bond-polar and apolar. The polar bond may be an intermolecular chelate involving magnesium.

THERE is no convincing evidence at present in favour of any one membrane model1 although most discussions of the subject rightly stem from the ideas of Davson and Danielli, published some 30 years ago². It is clear, however, that, in proposing any model, the identification of bonds between the protein and lipid molecules of the membrane will be of critical importance, both in structure and function.

Because such bonds have not so far been specifically identified, the choice of an appropriate biological system for this investigation was important and eventually

focused on Halobacterium halobium. The high ionic strength within halophilic bacteria3, and of the medium in which they live4, imposes stringent requirements on these forces. It was argued, initially at least, that ionic forces within the Halobacterium membrane might be of lesser importance and apolar forces easier to recognize. Although the Davson-Danielli model implies that the bonds between the lipid bilayer and the proteins are polar, Green and Fleischer⁵ have shown that the "structural protein" isolated from mitochondria interacts hydrophobically with lipids. A similar interaction was expected

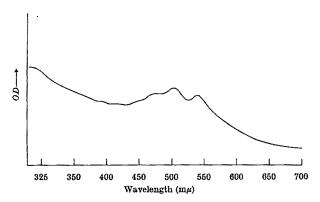


Fig. 1. Absorption spectrum, original preparation before dialysis.

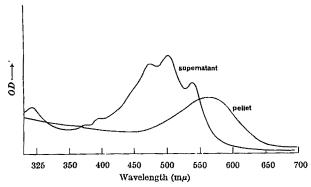


Fig. 2. Absorption spectra, orange supernatant and suspension of purple pellet.

here. Furthermore, the evidence of Christian and Waltho³ that the internal concentration of potassium is some one hundred times that of the medium suggested that the halophilic membranes might be an excellent transport system. Investigations by Brown⁵ had already shown (but see later) that the whole membrane dissolves in pure water to give lipoprotein complexes, and this indicated that the components might be easy to separate and characterize. When this work began we believed that Halobacterium halobium did not possess a cell wall⁵. As the work of Stoeckenius and Rowen⁵ has since shown, this is incorrect. Strictly speaking, the experiments to be described have been carried out on plasma membranes plus cell walls, which we will together denote by the term "envelope".

Experimental Techniques

 \dot{H} . halobium, strain NCMB 744 (which gave orange translucent colonies on agar slopes), was grown in 4 l. batches at 37° C with aeration and stirring in Sehgal and Gibbons medium⁴ at pH 6·5. Towards the end of the logarithmic growth phase (after 5–6 days) the cultures were removed and stored at 5° C or at -20° C until required. The bacteria were still viable and motile after storage for several months at -20° C.

Envelopes were prepared by the lysis method of Brown⁸ and stored, after purification, in basal salts solution (4 molar sodium chloride; 0.27 molar potassium chloride; 0.08 molar magnesium sulphate). After spinning for 2 h at 60,000g in a sucrose density gradient, in either basal salts solution or 0.02 molar magnesium chloride, the preparation gave a single maroon band at a density of 1.21 ± 0.01 g/ml. When viewed optically and in the electron microscope the preparation seemed homogeneous; on only a very small proportion of envelopes could any flagella be detected.

Two basic techniques have been used to separate the components of the envelope: (a) dialysis against pure water, which drastically reduces the ionic strength of the environment, and (b) extraction with chloroform—methanol solution, which would be expected to break hydrophobic bonds. We have preferred the simpler technique of Folch rather than the use of detergents which would also affect lipid—protein polar bonds.

The amino-acid analysis was carried out using a Beckmann 120B analyser. For the nitrogen determinations we used Kjeldahl's method and for the phosphorus determination we used Bartlett's method¹o. In the magnesium analysis, aliquots of the appropriate fractions were digested with AR perchloric acid (72 per cent) in borosilicate Kjeldahl tubes; the excess acid was evaporated in vacuo and the residues were dissolved in a solution of 0.4 per cent lanthanum chloride in 0.5 normal hydrochloric acid. Measurements were taken, using appropriate blanks and standards, with a Perkin-Elmer 303 or a Unicam SP90 atomic absorption spectrophotometer. The absorption spectra were determined with a Unicam SP800 recording spectrophotometer.

Dialysis Experiments

Envelopes were dialysed against changes of deionized water for 2 days and then centrifuged for 1 h at 17,000g. As a result an orange supernatant I_D and a purple pellet II_D were obtained. The purple pellet was resuspended in deionized water and respun until no further changes in the absorption spectrum could be detected. When viewed under a phase contrast microscope or in the electron microscope this product appeared as sheets of very low contrast. Absorption spectra of the original preparation, of the supernatant I_D and of a pellet suspension II_D were recorded and are shown in Figs. 1 and 2. The original preparation, as expected, shows considerable light scatter (Fig. 1). The amino-acid analyses of the two fractions I_D and Π_D are recorded diagrammatically in Figs. 3 and 4 (solid lines). In another experiment, the dialysate was returned to basal salts solution to test for reaggregation. After 2 days the product was spun for 2 h at 60,000g in a sucrose density gradient in basal salts solution; a diffuse orange upper band and a compact lower purple band were obtained, with no cross contamination.

Chloroform-Methanol Extraction

A thick suspension of envelopes (5 ml., about 4 mg N/ml.) in basal salts solution was shaken with chloroform methanol (50 ml.; 1:1 by volume. CM). The precipitated

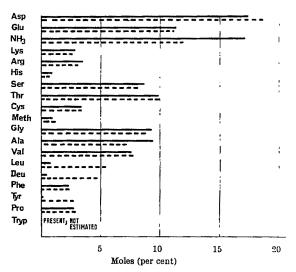


Fig. 3. Amino-acid analysis. —, Ib; ---, Icu.

salt and protein fraction, Icm, was spun down, washed with the solvent chloroform-methanol and amino-acid analyses obtained as before (Fig. 3, broken lines). combined supernatants and washings were evaporated to dryness on a boiling water bath, and re-extracted with chloroform-methanol, the resultant residue IIcm collected, and corresponding amino-acid analyses obtained (Fig. 4, broken lines).

Unlike fraction ID, which is a clear aqueous solution, fraction I_{CM} slowly gave a cloudy gelatinous suspension in water. This suspension immediately clarified on adding sodium dodecyl sulphate (SDS), and also emulsified

triolein and even paraffin.

The chloroform-methanol solution from an independent extraction was introduced under excess deionized water to remove methanol by diffusion, and left for 2 h. The resultant flocculus at the interface was separated by removal of upper and lower phases and subsequently washed with deionized water and chloroform. product was extracted with chloroform-methanol and the extract tested for lipids by paper-chromatography11. The protein residue from this extraction was also analysed, and agreed closely with IIcm here.

A protein-free lipid extract, in chloroform solution, was evaporated to dryness in vacuo in a rotating flask. An aliquot of a suspension of the purple fraction, containing a third as much phosphorus, was evaporated to dryness on top of this lipid at low temperature. The product was

soluble in chloroform-methanol.

Live bacteria were also extracted with chloroformmethanol, the precipitates discarded, the organic phase evaporated to dryness, extracted with chloroformmethanol and the residue analysed. The analysis again agreed closely with Π_{CM} .

Elemental Analyses

One envelope preparation was divided into aliquots for total analysis, for dialysis and for chloroform-methanol extraction. Each fraction (I_D , I_D , I_{CM} , I_{CM}) was prepared and analysed for nitrogen and phosphorus. Spectral correction was made for losses of ID and IID in washes. The results are given in Tables 1 and 2. The absence of the purple pellet fraction in the log-phase will be taken up later.

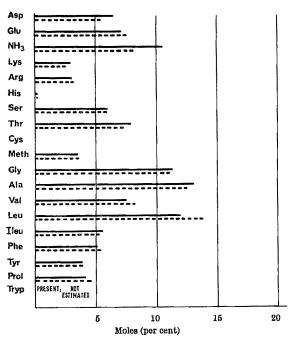


Fig. 4. Amino-acid analysis. ---, IID; ---- IIOM.

Table 1. PHOSPHORUS AND NITROGEN DETERMINATIONS FROM DIALYSIS EXPERIMENTS

Sample	Phosph (ir	n content ision) Fraction of total		
Stationary culture:				
Whole preparation	11.1	1.0	315	1.0
In: Orange super-		- -		
natant	9.2	0.76	252	0.82
IID: Purple pellet	2.5	0.24	53	0.17
Choroform-methanol				
extract of IIp	2.7	0.25	Absent	
Early log-phase culture: Purple pellet	None		None	

Table 2. PHOSPHORUS AND NITROGEN DETERMINATIONS ON CHLOROFORM-METHANOL EXTRACTS

Sample		s content loles/g of envelor Fraction of total lipid		ontent on) raction of total N
Stationary culture:				
Whole preparation	11.1	1.0	315	1.0
Ion: chloroform-				
methanol residue	1.15	Non-lipid	222	0.71
IIom: chloroform— methanol solution Proteolipid isolated	9-95	1.0	91	0.29
from chloroform- methanol extract	3.3, 3.7	0.3-0.33	91	0.29
Early log-phase culture: Whole preparation N Low How			216 157 54·4	1·0 0·74 0·26

Samples, prepared as above, were analysed for phosphorus, nitrogen, magnesium and amino-acids. results are given in Table 3. Calcium was also present, but blanks were too variable for the measurements to be reliable.

Table 3. MEASUREMENT OF MAGNESIUM, PHOSPHORUS AND NITROGEN AMINO-ACID CONTENT OF MEMBRANE FRACTIONS

Measurement*	Πρţ	Proteolipid † 1	Proteolipid	Πp
Phosphorus	2	2	2	2
Magnesium		0.67	0.89	0.72
Total nitrogen	42.5			39.2
Lysine	0.99	3.21 per cent		1.2
Arginine	1.02	4.33 per cent		1.2
Asp. + Glu.	4.51	14.1 per cent		4.22
Asp. + Glu ammonia	1.0	0.8 per cent		1.9

- * The results are expressed in molar ratios with phosphorus = 2. † Amino-acid analysis carried out on separate sample of $\Pi_{\mathbf{b}}$. ‡ Amino-acids expressed as moles per cent. Same preparation.

From these results the following principal points emerge. (a) Both dialysis and chloroform-methanol extraction each give rise to two fractions denoted as ID, IID and I_{CM} , H_{CM} . (b) The amino-acid compositions of I_D and Icm are closely similar. Each has an overall net negative charge as a result of a high proportion of acidic groups. (c) The amino-acid compositions of IID and IICM are very similar, but differ considerably from those of ID and ICM. (d) The greater part of the lipid (76 per cent of total phosphorus) is associated with ID in the dialysis experiments and with IIcm (100 per cent) in the chloroformmethanol experiments. (The lipids of H. halobium are negatively charged and highly hydrophilic11.)

The properties of I_D and I_{CM} are most readily explained if it is supposed that both in vivo and in In lipids are hydrophobically bound to an apolar part of these proteins which, after the lipids are removed by chloroformmethanol to give Icm, is unable to fold away from the water and consequently interacts strongly with sodium dodecyl sulphate. The other fractions IID and IICM were,

however, at first surprising.

The protein fraction IIcm, the residue obtained after evaporating the chloroform-methanol extract to dryness and re-extracting with chloroform-methanol, was insoluble in 8 molar urea, sodium dodecyl sulphate, water and acetic acid. The residue, however, was soluble in cresol from which it precipitated on the addition of chloroform—methanol. Because this protein was originally soluble in chloroform—methanol as a proteolipid complex, it is inferred that the lipids must have originally taken the protein into solution by bonds unaffected by a solvent (chloroform—methanol) designed to break hydrophobic interactions. It seemed possible therefore that in the proteolipid the lipids were attached to the protein by polar bonds. Yet after removing two-thirds of the lipid from the original extract in the process of isolating the proteolipid (Table 2), or after denaturation, the bonds became labile to chloroform—methanol. It was clear therefore that even if the main bonds between lipid and $\Pi_{\rm CM}$ were polar extra lipid was also bound weakly, was necessary for solubility and also exerted some protective action on the complex. A similar phenomenon has been observed by Das et al.¹² in model systems.

Das et al. have shown that cytochrome c, which is positively charged, binds phosphatidic acid ionically to form a salt, which is disaggregated in 0.1 molar potassium chloride. At lower ionic strengths the salt associates further, by apolar bonds, with unsaturated lecithins. In the resulting ternary complex the ionic bonds are buried, so that the complex, once formed, is unaffected by salts13 and soluble in iso-octane. Thus it seemed in the present case that either denaturation or chloroform-extraction of proteolipid removed similar "associated" lipids and thus exposed the polar lipid-protein bonds to the effect of the chloroform-methanol. Two valuable conclusions follow. First, because the proteolipid was unstable to chloroformmethanol unless already associated with extra lipids (and also because polar bonds could not form de novo in such a medium), the proteolipid could not be an artefact but must have pre-existed in the envelopes. Second, because the same proteolipid was obtained direct from live bacteria, it must also be presumed to exist in vivo.

The behaviour of the purple pellet (Π_D , Table 1) can be explained in the same way. The ratios of phosphorus to nitrogen in the isolated proteolipid (about 1:26) and in the purple pellet (1:21) were similar and in each case the chloroform—methanol extraction removed all the lipids. Yet if sufficient lipid was added to the purple pellet to make up the lipid content from a quarter to the original total, then (like Π_{CM} itself) Π_D became soluble in chloroform—methanol and stable. If we assume, as seems reasonable, that dialysis does not affect the polar bonds, then we must conclude that the weakly associated lipids were removed instead. As a consequence, the product became insoluble and unstable in chloroform—methanol unless these associated lipids were replaced beforehand. The effect of lowering the ionic strength by dialysis therefore is to re-order Π_D because of the repulsion between its negative charges Π_D . In then dissolves and because lipids remain bound to it hydrophobically, it removes all weakly bound lipids from Π_D .

Thus it appears that in vivo lipids are bound hydrophobically to one group of proteins, I, and by polar bonds to the other, II. Although it is uncertain whether a given lipid molecule is bound to both types of protein at the same time, much of the differently bound lipids must be in the same complex; otherwise, proteolipids could not be formed. The orange and purple fractions sediment together in a density-gradient centrifugation of the envelope-preparation (above) but in quite distinct bands after dialysis. This also is consistent with the view that proteins I and II are associated via the lipids. It was necessary therefore to identify the polar bonds.

Ionic bonds and hydrogen bonds were first considered. The ionic complexes between cytochrome c and phosphatidic acid, already referred to, would not form in 0·1 molar potassium chloride¹⁴. Even nucleoproteins, where there are many salt linkages between semi-rigid polyelectrolytes, are entirely dissociated in 3 molar potassium chloride. Yet H. halobium can live in 5·2 molar sodium chloride. One reasonable explanation, advanced by Brown¹⁵ on the basis of hydrogen ion titrations, is that the

lipid-phosphate moieties are linked to E-amino groups "buried" in the membrane and unavailable to the salt. Such bonds would, however, be metastable. The same objection would apply to the hydrogen bonds depicted by Brown¹⁵ because the proton in his diagram would certainly ionize from the phosphate to the amino-group and the bond would then be ionic, like the bonds in nucleohistones and in the complexes of Das. In this connexion, we found that urea had no effect on the protein lipid bonds, because the purple and orange fractions still sedimented together in a density gradient after treating an envelope-preparation with saturated urea. Again, the urea may not have penetrated to these bonds.

Salt-bridges were also considered. Morowitz, in a private discussion, pointed out to us that the magnesium concentration in the growth medium of *H. halobium* is significantly higher than that necessary for other organisms and, as in *Mycoplasma*¹⁶, suggested a specific role for magnesium ions. It has been shown that magnesium sulphate stabilizes the envelopes at low ionic strengths and also has other specific effects with Halobacteria^{17,18}. Salt-bridges (between phosphate, Mg²⁺ and negative groups on the protein) would be as labile to high ionic strengths as before, but further metal co-ordinate links in the form of a chelate complex are another possibility.

Hendrickson and Fullington¹⁹ have shown by titration experiments that phosphatidyl ethanolamine, phosphatidyl serine and triphosphoinositide form chelate complexes with Mg²⁺, Ca²⁺ and Ni²⁺, and Tasaki et al.²⁰ have suggested that a tricomplex exists between lipid, metal and protein in nerve membranes.

As Table 3 shows, the amino-acid: phosphorus: magnesium ratios were very suggestive. Corey, Pauling and Koltun²¹ space-filling models were constructed and show that chelates are sterically possible with the *H. halobium* lipid diphosphate (in which, as with the inositide, an oxygen rather than a nitrogen atom is the Lewis base).

One possible model for the link between the major lipid of *H. halobium* and protein II is therefore shown in Fig. 5.

Fig. 5. Possible binding between lipid and protein.

This represents a tetradentate inter-molecular chelate between groups on the protein, magnesium and the lipid head groups, together with an ionic link between the terminal phosphate and an arginyl group, to maintain neutrality. This particular model should be regarded as tentative because, first, model-building showed that the terminal phosphate group could also bind onto the magnesium, which then would have five ligands instead of four, and, second, the observed ratios do not correspond exactly with the model. It is possible that such strong bonding applies only to the diphosphate.

The chelate-hypothesis, however, accounts in a simple manner for the stability and specificity of the polar link in a high molarity salt environment. Moreover, the groups would be inaccessible, and their pK values also changed by the co-ordination, thus also accounting for the failure of amino groups to titrate in intact membranes¹⁵.

The chelation hypothesis would also account for the lability of the major lipid, the diether analogue of phosphatidyl glycerolphosphate, on denaturation. showed that while two protons titrate against sodium hydroxide in 50 per cent aqueous methanol with pK 3.25, the third titrates only partially in this solution with pK 7.45-7.95, and in pure methanol fails to titrate at all. When magnesium is bound in the complex it is therefore possible that the effect of denaturation is first to break the protein-magnesium bonds, leaving the magnesium bound to two charges on the lipid, and the lipid bound to the protein only by the third charge. The ionization is dependent, however, on the dielectric constant of the medium, and once the medium is able to penetrate to these bonds the ionization is lost and the lipid is removed. The observation of MacLeod et al.22, on H. cutirubrum, that after extensive dialysis this lipid is extracted as a 1:1 lipid-magnesium complex is entirely consistent with this explanation. Clearly, more detailed analysis (particularly of the ratio of magnesium to individual lipids in the complexes) and other tests will be necessary before the present model can be substantiated further and refined or, indeed, rigorously refuted.

It remains to discuss the question of the cell wall in *H. halobium* and some further observations on the purple fraction.

It is clear from the special electron microscope staining techniques of Stoeckenius and Rowen' that, contrary to the conclusions of Brown⁶, H. halobium is bounded by a labile cell wall which is isolated with the cell membranes. Although this adds a further complication to the system it does not invalidate the present conclusions about the bonding between lipid and protein. Their other observations are, however, very relevant. Plates of H. halobium usually gave chalky opaque colonies, while strain NCMB744, used by us, gave orange translucent colonies. It therefore seems likely that our strain is fortuitously a mutant. If it is accepted that the chalky appearance of inference is that strain NCMB 744 contains no vacuoles (as suggested by Stoeckenius and Rowen'), a possible inference is that strain NCMB744 contains no vacuoles and thus presumably none of the intracytoplasmic membranes seen by Stoeckenius and Rowen?. Our work therefore confirms their suggestion that the purple colour is associated with "the large membrane sheets" and not with the intracytoplasmic membranes. Furthermore, the purple protein, II, in our experiments was also found in the soluble proteolipid, Π_{CM} , and is therefore bound or associated with most of the lipid. This suggests that the "large membrane sheets" are in fact part of the cell membrane.

It was surprising in view of previous work that an insoluble component should remain at all after lysis. In our principal experiments, envelopes were isolated from cultures approaching the stationary phase, while in the experiments of Brown log-phase cultures were used. An experiment was therefore carried out by taking off a culture in early log-phase and working it up immediately.

It was then found that no purple component could be detected in the spectrum nor isolated after lysis (Table 1). A proteolipid, however, was still present in approximately the same ratio to the chloroform-methanol insoluble fraction as previously (Table 2). This suggested the possibility that the proteolipid fraction of the membrane cross-linked during the stationary phase and at the same time became purple, but the mechanism and biological significance of this are uncertain.

The nature of the purple colour is also unknown, although we confirm that a shift from 5630 Å to 3850 Å observed by Stoeckenius and Rowen, takes place on extracting the purple component with chloroform. It was also found that a reversible shift to 4800 Å occurred on treatment with very small amounts of chloroform followed by evaporation.

Conclusions

(1) The lipids of *H. halobium* are *in vivo* bound hydrophobically to one group of proteins, I, and by polar bonds to another, II. The differently bound lipids seem to be associated in the same complex.

(2) Firmly bound magnesium is present in the envelopes. The results of analyses show that it is possible to account for these results if the magnesium forms a chelate between groups on the protein and the lipid diphosphate head groups, together with an ionic link between the terminal phosphate and an arginyl group. Reasons have been given which make clear why such proposals must be regarded as tentative.

(3) An anomaly concerning the purple fraction has been clarified. The difference between the results of Brown, Stoeckenius and Rowen in this respect and our own early results arises from the fact that the purple colour of II_D exists only in fairly mature stationary phase cultures. It is non-existent in log-phase preparations. This complication does not invalidate our conclusions about the bonding between lipid and protein.

(4) The investigations described are essentially biochemical and their structural implications relate to the major bonding between lipid and protein components and not with their overall two- or three-dimensional array. No measurements have been made of the sizes of the lipid-protein complexes such as those of ID or IICM, but it is a reasonable hypothesis that the membrane is composed of a single layer of such complexes; the results of our experiments indicate that the layer of lipid binding the two types of protein together is primarily single (Fig. 6), not double as in the Davson-Danielli model of a complete membrane. A similar model has been suggested by Fleischer²³. Such a membrane would be structurally asymmetric, again in contrast to the Davson-Danielli model. This is a necessary condition for active transport. The electron microscope images of these two models (in transverse section), however, would in all probability differ only in the width of the lipid region. These remarks,

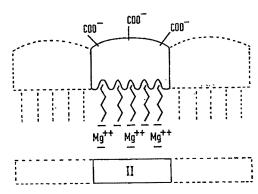


Fig. 6. Possible membrane model.

as so well shown in the recent review by Korn¹, only serve to emphasize the difficulties in the way of convincing proof, especially when dynamic concepts are taken into

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Operational Identification of Schwarzschild Co-ordinates

by

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An operational interpretation of the standard Schwarzschild metric co-ordinates, r and t, is presented, together with a test of general relativity. This test employs the operationally defined co-ordinates L and T in place of r and t as the theoretical variables.

Our test of general relativity requires the use of two spacecraft each equipped with a radio pulse transmitterreceiver. One, the test body or planetoid P, is launched from the Earth so that it moves directly away from the Sun along an outward radius of the Earth's orbit, its initial velocity in the solar reference frame being a little in excess of v_B , the Earth's orbital velocity. This would require a rocket sufficiently powerful to generate a velocity relative to the Earth of approximately $\sqrt{2 \cdot v_E}$ after escape from the Earth's gravitational field. In one year's time the test body will have fallen back towards the Sun and be a distance of approximately half an astronomical unit from the Sun. At this time a second spacecraft, the control space station A, is launched from the Earth, so that, after escape, the Earth's orbital velocity is just cancelled and the space station is momentarily at rest in the solar reference frame. By firing suitably directed auxiliary rockets on both spacecraft, under command from Earth, it can be arranged that P falls radially towards the Sun, S, and A is kept approximately at rest in solar space, for the duration of the experiment, on the same radius SPand at a point on the Earth's orbit. The latter task will not be difficult provided the residual motion of A can be determined, because at a distance of one astronomical unit the gravitational acceleration caused by the Sun is only about 0.6 cm/sec2, while that caused by the Earth would fall below this order at an Earth distance exceeding 2×10^5 km.

The experiment involves sending successive microwave pulses from A to P. On arrival at P each pulse triggers an immediate response from the transmitter of P which is received by A. This allows the motion of P in L, T coordinates (described later) to be plotted and compared with the predictions of general relativity in the same co-ordinates. The experiment assumes that there is an atomic clock in the space station A, reading accurately,

say to one part in 1010, and that the events on the time-base in A can be stored and telemetered to Earth as required. The experiment can last for as long as there is fuel to keep the space station within the tolerance limits of motion.

It will be assumed that the gravitational field of the Sun can be described by the Schwarzschild metric in the

$$ds^{2} = \left(1 - \frac{2m}{r}\right)dt^{2} - \left(1 - \frac{2m}{r}\right)^{-1}dr^{2} - r^{2}d\theta^{2} - r^{2}\sin^{2}\theta d\phi^{2}$$
 (1)

with units chosen so that in the usual notation, c=G=1, and length is measured in km. For the Sun the gravitational radius, m, is approximately 1.5 km. Taking the world line of a test particle to be a geodesic of equation (1), the present case of interest is a radial geodesic $\theta = \pi/2$, $\varphi = 0$. Well known analysis yields for such a radial motion

$$\dot{r}^2 = \left(1 - \frac{2m}{r}\right)^2 - (1 - v^2) \left(1 - \frac{2m}{r}\right)^3 \tag{2}$$

where $r \equiv dr/dt$, and v is the velocity, real or imaginary, of the body in inertial space-time at infinity. In this analysis only terms up to order m/r ($\sim 10^{-8}$), and its equivalent in v^2 , will be retained. To this order, equation

$$\dot{r}^2 = v^2 + \frac{2m}{r} \left(1 - 3v^2 - \frac{4m}{r} \right) \tag{3}$$

The order of magnitude of the terms is seen clearly if we temporarily restore general units

$$\dot{r}^2 = v^2 + \frac{2\mu}{r} \left(1 - \frac{3v^2}{c^2} - \frac{4\mu}{c^2 r} \right) \tag{4}$$

where $\mu = GM_{\odot}$.

Suppose that a microwave pulse is transmitted from the space station, A, at co-ordinate time t_1 , reaches the planetoid, P, at co-ordinate time t and triggers an immediate response signal to arrive at A at co-ordinate time t_2 . Then the null geodesic hypothesis for light rays yields

$$t - t_1 = t_2 - t = \int_{r}^{R} \frac{\mathrm{d}r'}{1 - \frac{2m}{r'}} \sim R - r + 2m \log \frac{R}{r}$$
 (5)

where r is the radial co-ordinate of P at time t and R is the (constant) radial co-ordinate of A. The transmitterreceiver time-base of A, however, measures atomic time s_A , not co-ordinate time t_A . The relation between these, to the required order, is

$$\cdot s_A = \left(1 - \frac{m}{R}\right) t_A \tag{6}$$

By equations (5) and (6) therefore

$$R - r + 2m \log \frac{R}{r} = \left(1 + \frac{m}{R}\right) L$$

$$t = \left(1 + \frac{m}{R}\right) T$$
(7)

where

$$L = \frac{1}{2}(s_2 - s_1), T = \frac{1}{2}(s_1 + s_2)$$
 (8)

are operationally defined measures of position and time of the event P in terms of the atomic time of transmission and receipt of pulses at A. Retaining terms up to order L/R, and their equivalent in v^2 , straightforward substitution from equation (7) into equation (3) yields

$$\dot{L}^{2} = v^{2} + \frac{2m}{R - L} \left[1 - v^{2} + \frac{m}{R - L} \left\{ \frac{L}{R} - 2 \log \frac{R}{R - L} \right\} \right]$$
 (9)

where $\dot{L} \equiv \mathrm{d}L/\mathrm{d}T$. In general units this relation is

$$\dot{L}^2 = v^2 + \frac{2\mu}{R - L} \left[1 - \frac{v^2}{c^2} + \frac{\mu}{c^2 (R - L)} \left\{ \frac{L}{R} - 2 \log \frac{R}{R - L} \right\} \right]$$
 (10)

This operational equation, predicted by general relativity for radial motion towards the Sun, is to be compared with the corresponding Newtonian equation. Obviously, the same experiment can be interpreted in terms of Newtonian gravitational theory using Euclidean geometry and the assumption that the velocity of light in the solar reference frame is the constant c. Because the terms depending on c2 do not appear in the Newtonian analogue of equation (4), and because the terms in m do not appear in the analogue of equation (7), it follows that the corresponding Newtonian operational equation is exactly

$$\dot{L}^2 = v^2 + \frac{2\mu}{R - L} \tag{11}$$

It should be noted in particular that the differences between equations (10) and (11) arise from the complete Schwarzschild metric, equation (1). Thus equation (10), and also equation (14) further on, would be different if account was taken only of the coefficient of dt2 in equation (1), which might be inferred on the basis of the principle of equivalence alone. As regards keeping A at rest, it can be shown that the analysis described is valid to the order stated provided A does not move more than about $0.1~\mathrm{km}$ during the experiment. Thus very little auxiliary rocket control of the motion of A in the field of the Sun is needed in the conditions specified. The experiment could also be carried out with the planetoid, \vec{P} , outside the Earth's orbit falling towards the Sun at a distance of about 0.5 astronomical units from the station A, but this would require more launching power and the gravitational field on P would be weaker. Orbits to the Sun requiring less power but substantial correction in space have been discussed by Feenberg1.

With regard to the experimental distinction between equations (10) and (11), the co-ordinate R, as it occurs in equation (11), has been determined in the past from the Newtonian laws and Euclidean geometry. By such analysis relative distances within the solar system are known to perhaps one part in 108. But absolute distances on this basis depend on the practical measurement of the astronomical unit and it seems that this is known at present to only one part in 106 at most^{2,3}. Determination of the astronomical unit to one part in 108, however, may be expected on a Newtonian basis in the future. Indeed, experiments of the type suggested here would yield such an accuracy for R (at Earth perihelion, say), and thus for the astronomical unit, through equation (11). Obviously, because μ is known in terms of the astronomical unit to one part in 108 by the Keplerian law

$$\tau = \frac{2\pi a^{3/2}}{\mu^{1/2}} \tag{12}$$

(using an atomic clock to measure τ on the Earth), it follows that in equation (11) there are essentially only two unknowns, v^2 and R. Given sufficient data in the form of \dot{L}^2 , L number pairs both v^2 and R can therefore be determined in principle to one part in 108. The point is, of course that if the Newtonian theory is actually false to this order of accuracy then repeated experiments of this kind would not yield a consistent value of R for different

Equation (10) remains to be verified in which R is the Schwarzschild radial co-ordinate of a point on the Earth's orbit (Earth perihelion, say). By equation (1) this would differ from its Newtonian determination by a fraction of order m/R (or 10^{-8}). Once again, however, μ is known to this order in terms of R even in the relativistic case. For the orbit of a planet, according to equation (1), is known to be a slowly rotating ellipse the orbital period τ of which (from perihelion to perihelion) may be shown to be

$$\tau = \frac{2\pi a^{3/2}}{\mu^{1/2}} \left[1 + \frac{m(3-e^2)}{2a(1-e^2)} \right]$$
 (13)

where a is the r co-ordinate length of the semi-major axis, m is the parameter of equation (1) and e is the eccentricity of the ellipse. Because the term in square brackets is known to the required order even at present, it follows that an atomic clock value of τ gives μ relativistically in terms of a (and hence R) for the Earth. Thus in equation (10), also, there are essentially only two unknowns, R and v^2 , and so repeated experiments with different values of v^2 should give a consistent value for Rif general relativity is correct.

Finally, suppose it became possible, as undoubtedly it will in the future, to generate in a small instrumented probe, P, a value of v in the hyperbolic range, as high as 300 km/sec. Then v^2/c^2 is of order 10^{-6} , and the third term in the bracket of equation (10) is less than this by a factor of 100. In this case the relativity equation approximates

$$\dot{L}^2 = v^2 + \frac{2\mu}{R - L} \left(1 - \frac{v^2}{c^2} \right) \tag{14}$$

and now even a Newtonian determination of R to one part in 107 would be adequate to establish whether equation (14) was a better fit than equation (11).

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LETTERS TO THE EDITOR

ASTRONOMY

Variability of Centaurus XR-2

Chodil et al. have suggested that the X-ray flux from the Centaurus XR-2 object varied between 1965 and 1967. In this communication we give in detail the changes in X-ray emission which occurred during the period April—May 1967, and show that during this time the X-ray energy output decreased approximately exponentially as a function of time, with a time constant of 25 days.

The data were obtained by X-ray detectors flown on two Skylark rockets, launched from Woomera at 0032 UT on April 4, 1967, and 2236 UT on April 20, 1967, and from an experiment¹ flown from Hawaii by the Levermore Research Laboratory (LRL) group on May 18, 1967. The Skylark results have been presented previously², the presence of a strong X-ray source near the constellation Crux being reported at that time. Re-analysis of our aspect data has resulted in a revision in our position for this source to a mean value of 13.9 h right ascension, -64° declination, which places it barely within the boundary of the constellation Centaurus. The LRL flight detected a source within 4° of this position and referred to it as Centaurus XR-2. We take the view that the LRL and Universities of Adelaide and Tasmania (UAT) observations refer to the same object, and propose to adopt the LRL nomenclature.

The Skylark detectors consisted of two independent pairs of xenon-methane filled, proportional counters with beryllium windows. The spectral information from the detectors consisted of the counting rates from two energy bands, 2-5 keV and 5-8 keV. Assuming a photon

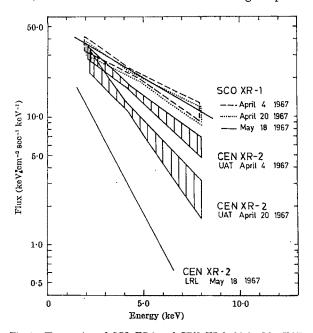


Fig. 1. The spectra of SCO XR-1 and CEN XR-2 obtained by UAT on April 4, 1967 (day 94), and April 20, 1967 (day 110), are shown. The uncertainties in the intensity and temperature of the spectra are indicated. Also shown are the spectra obtained by LRL on May 18, 1967 (day 188). Note that there is good agreement between the three measurements of the SCO XR-1 spectrum, while there is a great divergence between those of CEN XR-2.

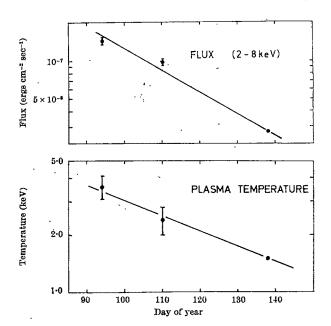


Fig. 2. The temporal decrease in the 2-8 keV energy flux and the effective plasma temperature of CEN XR-2 are shown. The UAT spectra were taken on days 94 and 110, the LRL spectrum on day 138.

spectrum which corresponds to free-free emissions from a thin hot plasma of temperature T,

$$rac{\mathrm{d}N}{\mathrm{d}E} = J_0 \cdot E^{-1} \cdot \exp(-E/T) \; \mathrm{photons \cdot cm^{-2} \; sec^{-1} \; keV^{-1}}$$

and taking into account window thickness, counter efficiency, pulse height resolution, and the counter and effects, the expected counting rate in each channel was calculated for various values of the plasma temperature, T. The ratio of the two counting rates is then a function of T, while the total counting rate in the 2–8 keV energy range is a function of J_0 . In this manner, the spectra giving the best fit to the observed data were obtained for both flights, for both $SCO\ XR$ -1 and $CEN\ XR$ -2.

The results of these calculations and the spectra quoted by Chodil et al. are shown in Fig. 1. The excellent agreement between the three independent measurements of the SCO XR-1 spectrum indicates that the data and the spectra derived from all three flights are strictly comparable. Figure 1 shows, however, that the CEN XR-2 spectra differ greatly from one flight to the next, the fluxes decreasing monotonically with time. This conclusion is not dependent on the assumption of a free-free spectrum; the same qualitative conclusion is reached by comparing the ratios of the observed counting rates from CEN XR-2 and SCO XR-1, or from the assumption of any other reasonable spectra. Furthermore, both the UAT and the LRL detectors, because they employ collimators with wide cones of acceptance in the plane containing the spin axes of the rockets, are not subject to the introduction of spurious time variations through errors in the correction for the collimator response at non-normal photon incidence.

Figure 2 shows the total energy flux in the 2-8 keV energy range, and the calculated plasma temperatures, T, as functions of time. It is clear that both exhibit very marked time dependence. The upper curve (which is derived directly from the observed data with the introduction of a minimum of assumptions) approximates an exponential of time constant 25 days. The lower curve indicates that the decrease in energy output is accompanied by a coftoning of the X-ray energy.

panied by a softening of the X-ray spectrum.

Chodil et al.¹ have reported that CEN XR-2 was not detected in 1965, while Figs. 1 and 2 indicate that on April 4, 1967, it was comparable with SCO XR-1, the brightest object in the X-ray sky. This fact, plus the

temporal dependence indicated by Figs. 1 and 2, establish in a completely unambiguous manner that CEN XR-2 is a variable X-ray object. The data do not make it possible to determine whether it is a periodic or an aperiodic variable; however, the fact that all X-ray observations to date indicate either constancy (for example, SCO XR-1, TAU XR-1) or drastic, short lived change (CEN XR-2) suggests the variability is transient. The observed decay time constant of 25 days agrees within a factor or two with the decay time constants of type II supernovae, suggesting some similarity in the phenomena. The fact that $CEN\ XR$ -2 lies within a few degrees of the galactic disk, and therefore in a region of considerable optical obscuration, means that any optical counterpart of the X-ray variability may have escaped detection.

The possibility has been considered that CEN XR-2 and the very weak \tilde{CEN} XR-1 source, discovered by Friedman et al.3 in April 1965, are, in view of positional uncertainties, one and the same object. Even if this were so, the source intensity must have changed by two orders of magnitude between 1965 and 1967, and the basic conclusions given here remain unaltered.

The initial Universities of Adelaide and Tasmania X-ray detector hardware development was performed at the Southwest Center for Advanced Studies, Dallas, Texas, under a NASA contract. R. J. F. and J. R. H. acknowledge the support provided by Commonwealth and CSIRO postgraduate scholarships, respectively. We thank the Science Research Council for providing the opportunity to make these flights and the personnel of BAC, Filton, UK, and of the Aerodynamics Division of the Weapons Research Establishment, Salisbury, Australia, for their help.

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Blackbody Radiation and the Eddington-Lemaître Universe

THE discovery of the isotropic cosmic background radiation in the universe¹ and the subsequent demonstration by various groups of its blackbody spectrum have generally been taken to imply that the universe is of the type characterized by a big bang. Such models, containing both radiation and matter, have recently been examined in detail by McIntosh² and Jacobs³.

This communication shows that such radiation could also be present in a universe which is of the Eddington-Lemaître type⁴—that is, which expands from a finite static state in the infinite past. In contrast to the hot models mentioned, and the cold model described by Zeldovitch, the modified Eddington-Lemaître model proposed here might be termed the cool model, because for reasonable values of the parameters involved, the temperature of the radiation is always quite low.

We begin with the field equations of general relativity in the form

$$8\pi G \rho = \frac{3}{R^2} (kc^2 + \dot{R}^2) - \Lambda \tag{1}$$

$$8\pi G \frac{p}{c^2} = -\frac{2R}{R} - \left(\frac{R}{R}\right)^2 - \frac{kc^2}{R^2} + \Lambda$$
 (2)

where R(t) is the scale-factor of the Robertson-Walker metric, Λ is the cosmological constant, and k, the spatial curvature index, is taken to be +1. The pressure and density are contributed by both matter and radiation so that

 $\rho = \rho_m + \rho_r$

$$p = p_m + p_r$$

Denoting present conditions by a subscript 0 and conditions in the static state by s, we have

$$\frac{R_0}{R_0} = 1 + z_{\text{max}} \equiv Z \tag{3}$$

$$\rho_{m,s} = Z^3 \rho_{m,0} \tag{4}$$

and

and

$$\rho_{r,s} = Z^4 \, \rho_{r,0} \tag{5}$$

Introducing the assumption that the static state was one of thermodynamic equilibrium makes it possible for us to define a unique temperature

$$T_s = Z T_0$$

where T_0 is the present temperature of the blackbody radiation. One might note parenthetically that the radiation would play the part of the heat reservoir of classical thermodynamics, as pointed out by Gamow⁶.

Then, assuming the matter to follow the perfect gas law, we find

$$p_{s} = \frac{\rho_{m,s} kT_{s}}{\mu H} + \frac{\rho_{r,s} c^{2}}{3}$$

$$= \rho_{m,s} Z \left[\frac{kT_{0}}{\mu H} + \frac{c^{2} \rho_{r,0}}{3 \rho_{m,0}} \right]$$
(6)

Now.

$$\frac{\rho_{r,0}}{\rho_{m,0}} = \frac{a T_0^4}{c^2 \rho_{m,0}} = F$$
, say.

If $\rho_{m,0} = 5 \times 10^{-31}$ g/cm³ and $T_0 = 3$ °K, $F = 1.36 \times 10^{-3}$. Because the first term of equation (6) is of order 108 and the second term is of order 1017, we can obviously say that

$$p_s = \frac{c^2}{2} FZ \rho_{m,s} \tag{7}$$

It follows from equations (4) and (5) that

$$\rho_s = \rho_{m,s} (1 + FZ) \tag{8}$$

Substituting from equations (7) and (8) into (1) and (2) and remembering that in the static state $R = \ddot{R} = 0$, we find

$$\Lambda = \left(\frac{1+2 FZ}{3+4 FZ}\right) \frac{3 c^2}{R_z^2}$$
 (9)

If F were zero this would be the relation found by Lemaître.

At the present time the ratio of pressure to energy density is small,

$$\frac{p_{\rm 0}}{\rho_{\rm 0}\,c^2}\,=\,\frac{\alpha\,T_{\rm 0}^4}{3\,c^3(\rho_{\rm m,0}\,+\,\rho_{\rm r,0})}\,\sim\,5\times10^{-4}$$

so that in applying equations (1) and (2) now we may write7

$$\Lambda \tau_0^2 = 3(\sigma_0 - q_0) \tag{10}$$

and

$$\frac{c^2 \tau_0^2}{R^2} = 3\sigma_0 - 1 - q_0 \tag{11}$$

where $\tau_0 = H^{-1}$, $\sigma_0 = \frac{4\pi}{3} G \rho_0 \tau_0^2$ and q_0 is the acceleration factor. Combining equations (3), (9), (10) and (11) yields

$$2F(3\sigma_0 - 1 - q_0)Z^3 + (3\sigma_0 - 1 - q_0)Z^2 - 4F(\sigma_0 - q_0)Z - 3(\sigma_0 - q_0) = 0$$
(12)

which specifies Z when σ_0 and q_0 are known. It is possible to find an exact expression for R (t) in this model by noting that

$$\rho(t) = \rho_{m,s} \left(\frac{R_s}{R}\right)^s + \rho_{r,s} \left(\frac{R_s}{R}\right)^4 \tag{13}$$

and that

$$R_{s^{-2}} = 4\pi G c^{-2} \rho_{m,s} \left(1 + \frac{4}{3} FZ \right)$$
 (14)

Making use of equations (4), (5), (13) and (14) in (1) and introducing

$$x = (R - R_s)/R_s$$

leads, after integrating and taking the constant of integration to be zero, to

$$ct R_{\star}^{-1} (3 + 4 FZ)^{-1/2} =$$

$$(1+2 \ FZ)^{-1/2} \log \begin{cases} [x^2(1+2 \ FZ) + 4x(1+2 \ FZ) \\ + 3 + 8 \ FZ]^{1/2} + x(1+2 \ FZ)^{1/2} \\ + (1+2 \ FZ)^{-1/2} (2+4 \ FZ) \end{cases}$$

$$- (3 + 8 FZ)^{-1/2} \log \begin{cases} x^{-1}[x^{2}(1+2 FZ) + 4x(1+2 FZ) \\ +3+8 FZ]^{1/2} + x^{-1}(3+8 FZ)^{1/2} \\ +(3+8 FZ)^{-1/2} (2+4 FZ) \end{cases}$$
(15)

It follows from equation (15) that

$$q_{0} = -\frac{\ddot{R}_{0}R_{0}}{(\dot{R}_{0})^{2}} = -\frac{1}{x_{0}}$$

$$-\frac{(1+x_{0})(2+x_{0})}{x_{0}^{2}+4x_{0}+(3+8\ FZ)(1+2\ FZ)^{-1}}$$
(16)

and so $q_0 \leq -1$. Furthermore,

$$x_0 = \frac{R_0}{R_s} - 1 = Z - 1$$

so that, in principle, by substituting from equation (16) into equation (12), Z can be found once σ_0 is specified. In practice an iterative procedure was used. Starting with the values of σ_0 and F corresponding to $\rho_{m,0} = 5 \times 10^{-31}$ g/cm³, H = 100 km/sec/Mparsec, $T_0 = 3^{\circ}$ K, and assuming $q_0 = -1$, a value of Z was calculated from equation (12). This value of Z was then used in equation (16) to determine a result of $q_0 = 1$. determine a new value of q_0 , and the procedure repeated. The process converged rapidly to

$$q_0 = -1.1377$$

$$Z = 4.3972$$

If, therefore, $T_0 = 3$ °K, then $T_s = 13.2$ °K. Also the maximum red-shift observable in this model is $z_{\text{max}} = 3.3972$.

It will be noted that the value of q_0 given is lower than the range of values usually considered probable, $0 \le q_0 \le 2$. In view of the present state of knowledge (or ignorance) of the stellar content of elliptical galaxies and the possible evolution of these galaxies, this is not considered a serious drawback.

There are a number of other features of this model which deserve comment. In the first place, the ultimate question of origins is avoided, because the universe has existed for an infinite time. Second, the density of matter is given by the observed value ($\sim 5 \times 10^{-31} \, \text{g/cm}^3$) and yet the curvature of space is positive; thus the Machian aim of many authors is achieved without the necessity of postulating unobserved matter. Finally, it should be noted that the

chemical composition of the static state has been left unspecified. If it should happen that old, helium-deficient stars are founds, this model would be particularly attrac-

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Cosmic Blackbody Radiation and Isotropic Gamma Rays: a Contradiction?

In his article on the isotropic gamma ray background. Cheng¹ presents instructive calculations of the production and propagation of inverse Compton gamma rays in an expanding universe. The latter part of the paper, however. includes a rather arbitrary application of these results which leaves the impression that there is some conflict between the observed X-ray and gamma ray background and the popular blackbody (3° K) interpretation of the observed microwave background. The purpose of this communication is to reassure big bang theorists that this is emphatically not the case.

X-rays and gamma rays are, certainly, produced in Compton collisions of blackbody photons with the intergalactic population of fast electrons^{2,3}. Unfortunately, we have no direct evidence as to the number density or energy spectrum of these electrons. Cheng assumes that the intergalactic electrons have a spectral shape like that of primary cosmic ray electrons near the Earth' and a number density a hundred times smaller. From such a large electron flux there necessarily follows an isotropic gamma ray intensity well in excess of the observed spectrum. Cheng makes much of the discrepancy, but in suggesting that it might be resolved if the density ratio could be chosen as 104 instead of a hundred he cannot but underscore the essentially arbitrary character of this approach. Morrison and I5 investigated sources of fast electrons and showed that, far from producing too many gamma rays, the intergalactic electron density could only with some theoretical discomfort be brought up to the level required to account for the observed gamma rays by the inverse Compton mechanism. (R. J. Gould has pointed out to me that it is interesting though not necessarily significant that injection adequate to this end is obtained by way of electron secondaries produced in the intergalactic π - μ -e process if the cosmic rays are universal and the mean density of matter is about 10-5 particles cm⁻³. If we must instead rely for electron injection on leakage from radio galaxies, the required level is problematical5,6.)

Because we discussed sources we were also able to study the equilibrium electron spectrum and show that the resulting gamma ray spectrum should be steepened by the large Compton loss caused by the blackbody photons^{2,5}. This brought the predicted spectral shape into agreement with the observations and was an improvement over earlier and more arbitrary calculations3, as we pointed out5. Cheng has now reverted to the arbitrary method; he presents his predicted spectral shape and the contrasting observational curve on a common graph in Fig. 3 but makes no comment on the discrepancy in slope, although he is aware of the paper by Morrison and myself, having cited it in another connexion. This is a step backward.

Neither our work nor that of Cheng takes proper account of the effects that rapid evolution of galaxies or radio sources may have on the isotropic X-ray and gamma ray background. That these effects may be sizable has recently been demonstrated (ref. 7; see also forthcoming papers by Silk and Bergamini, Londrillo and Setti). It is also quite possible in any case that most of the diffuse gamma ray background originates in unresolved discrete sources in galaxies (ref. 8 and unpublished results of Silk and Bergamini et al.) rather than in intergalactic space. A good deal of work remains to be done, but it is now clear that there is no apparent contradiction between the observations of isotropic gamma rays and the assumption that the universe is filled with equilibrium blackbody radiation having $T \approx 3^{\circ}$ K at the present epoch. Indeed, to whatever extent the gamma radiation is relevant, its high intensity and steep spectrum tend even to favour the blackbody hypothesis (refs. 2 and 5 and unpublished result of Bergamini et al.).

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Correlation between Sudden Cosmic Noise Absorption and Solar Radio Bursts observed at Five Microwave **Frequencies**

The 10.7 cm index of solar activity has been used for many studies of solar-terrestrial interactions. principal reason for this is that highly accurate data The present extending back to 1947 are available. availability of accurate information at other microwave frequencies makes it possible to determine the relative

effectiveness of these frequencies as indices of solarterrestrial relations.

Basu and Karmakar¹ studied the correlation of 10.7 cm mean solar flux and 10.7 cm solar radio bursts with sudden cosmic noise absorption (SCNA) events. In this communication we shall report the results of a correlation study of solar radio data collected at five different frequencies: 606 Mc/s, 1,415 Mc/s, 2,695 Mc/s, 4,995 Mc/s and 8,800 Mc/s, by personnel of the Air Force Cambridge Research Laboratories² at the Sagamore Hill Radio Observatory, Massachusetts, during 1966, and sudden cosmic noise absorption data covering the same period, published by the Environmental Science Services Administration. An average of approximately sixty SCNA events occurred during the Sagamore Hill operating hours in 1966.

Table 1					
Freq.	No. of	No. of	No. of corre-		
(Mc/s)	SCNA	bursts	lated events		
606	69	245	20		
1,415	69	243	26		
2,695	55	251	25		
4,995	40	163	21		
8,800	54	219	28		

A time delay, T, is defined as the difference between the onset time of an SCNA and that of a corresponding solar radio burst. The criterion adopted for considering these two events as correlated was that the time delay, T, should not exceed -10 or +15 min. (A negative T which occurs roughly 15 per cent of the time indicates that the start of the SCNA precedes the start of the burst.) Data available for consideration are listed in Table 1. The information in column 3 of Table 1 was further reduced to burst intensity groups within certain limits, for example, 1-5, 5-15, 15-45, 45-150, 150-450, and > 450 flux units of 10^{-22} W m⁻² (c/s)⁻¹. Figure 1 is a plot of the ratio of these bursts correlated with SCNAs to the total number of bursts observed within the given range of peak flux intensities. Curve (a) is an average of the decimetre observations at 606 Mc/s and 1,415 Mc/s, and curve (b) is an average of the centimetre observations at 2,695 Mc/s, 4,995 Mc/s and 8,800 Mc/s. Both curves show poor correlation for bursts weaker than 15 flux In the decimetre region (curve a) the ratio of bursts correlated with SCNAs improves only slightly with increased burst intensity, and then starts to decrease, while in the centimetre region (curve b) the ratio increases with burst intensity and reaches unity for bursts greater than 450 flux units.

If only bursts with peak flux densities greater than 15 units are considered and the correlation as a function of frequency is studied, we obtain results shown in Fig. 2.

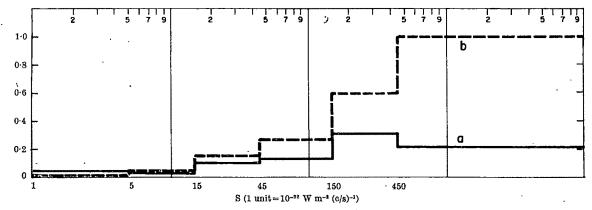


Fig. 1. Ratio of bursts correlated with sudden cosmic noise absorption events to the total number of bursts in each peak flux interval for (a) 606 Mc/s and 1,415 Mc/s, and (b) 2,695 Mc/s, 4,995 Mc/s and 8,800 Mc/s.

Here we have plotted (a) the ratio of the number of coincidences to the total number of SCNAs and (b) the ratio of coincidences to the number of bursts with peak intensity greater than 15 flux units. By so limiting burst data, the number of burst samples used was reduced by about 50 per cent from the numbers given in Table 1 but the number of coincidences was reduced only slightly. We see that correlation increases with frequency; at 8,800 Mc/s it is about twice that at 606 Mc/s.

Spectra of the increase of burst peak flux for thirty-two radio events correlated with SCNAs were plotted; four had incomplete spectra and were not used. Their intensities covered a wide range. The most common shape (fifteen out of thirty-two) had a positive gradient; that is, the flux increased toward the higher frequencies and showed no activity at the lower frequencies. There were sixteen other spectral curves with various undulating shapes, not classified at this time. Only one event was found with a negative gradient and no activity at the higher frequencies.

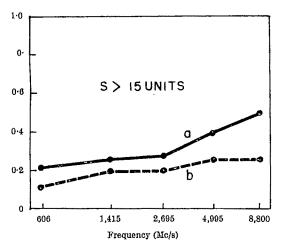


Fig. 2. Ratio of coincidences (a) to sudden cosmic noise adsorption events, and (b) to bursts Peak flux intensity is greater than 15 units.

The preliminary survey of the data shows that about half of all radio burst spectra has a positive gradient. (This spectral type is described by Kundu⁴ as not having associated activity at metre wavelengths.) The same proportion is also found in the thirty-two spectra of bursts that are correlated with SCNAs. About one out of every six bursts has a negative gradient and shows no activity in the short centimetre region, but only one of the thirty-two correlated bursts was in this category. This explains the poor correlation found at the lower frequencies of the decimetre region in Fig. 2 and in curve (a) of Fig. 1 for peak flux densities greater than 450 units. In the latter case only five of the twenty-three events observed at the two decimetre wavelengths with fluxes greater than 450 units were correlated with SCNAs, while all nine events observed at the three centimetre wavelengths at this flux level were correlated (see Fig. 1, curve (b)). Thus for bursts in the centimetre region with high intensities there is practically 100 per cent correlation while in the decimetre region the correlation of bursts with high intensities is only about 20 per cent.

It is known that SCNAs are produced by solar X-rays ionizing the *D* region of the ionosphere. Kundu⁵ suggests that X-rays and centimetre bursts are produced by the same electrons. The absence of correlation of SCNAs with bursts, having no activity in the centimetre region, suggests therefore that no X-rays are emitted with those bursts. The important result of this study is that solar radio bursts and SCNAs show an improving correlation

as the monitoring frequency of solar microwave bursts increases from 606 Mc/s to 8,800 Mc/s.

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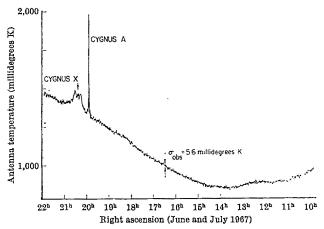
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Limits on Small Scale Variations in the Cosmic Background Radiation

The 3° K microwave background radiation¹⁻⁵ left over from the big bang, in which the universe is supposed to have originated, would exhibit features of small angular size if at the time of the escape of the radiation there were density inhomogeneities. Observational evidence for, or limits to, such non-uniformity bears directly on important questions, including the origin of galaxies' and their magnetic fields8.

We have previously reported limits on the root mean square temperature fluctuation, σ , of 5·1 millidegrees K at an angular scale of 1°, and 3.6 millidegrees K at a scale of 6°, measured over a small region of the sky, and limits at a scale of about 15° are deducible from the observations of Wilkinson and Partridge¹⁰. We have recently concluded a survey of a larger section of sky, and can now set reduced upper limits to σ for scales of up to 2°.

The receiver used was a conventional 'Dicke' radiometer, and has been described previously⁹; the system noise temperature was about 800° K and the antenna beamwidth was about 10 min of arc at 10,690 Mc/s. Data were taken digitally at 10 sec intervals; addition of data from successive days for corresponding sidereal times and further processing were done by computer. Figure 1 shows the results of adding 36 days of observations in June and July 1967; no corrections have been made to the observed data. The receiver was calibrated by a gas-discharge tube. Where the plane of the galaxy passed through the beam, radiation



Observed temperature averaged over 36 days. Antenna beamwidth=10 min, declination=+40.6°.

was received from the well known radio sources Cygnus A and Cygnus X, which provided a check on the receiver each day. The long-term drift in the record is the result of ambient temperature variations; it was removed by subtracting the half-hour running average from the data.

Over the region of sky from 11 h to 19 h right ascension the root mean square fluctuation of the 36 day record. $\sigma_{\rm obs}$, was found to be 5.6 millidegrees K (with a standard deviation of 0.19 millidegrees estimated from the variation from hour to hour). Figure 2 shows $\sigma_{\rm obs}$ for 1, 4, 10, 16, 25 and 36 day records. We would expect $\sigma_{\rm obs}$ to first diminish as $n^{-\frac{1}{2}}$, where n is the number of days, and then approach a horizontal asymptote determined by the small-scale background variations. The broken line, which is a curve of the expected form fitted to the data, shows that in the area of sky surveyed with the beamwidth of 10 min of arc, the asymptotic value can hardly exceed 3.6 millidegrees K.

Even lower upper limits can be set by integrating the original data so as to simulate records which would have been obtained with a wider antenna beam. For example, σ_{obs} obtained from the 36 day record with a simulated beamwidth of 2° is 1·7 millidegrees K, from which the upper limit to the asymptotic value is found to be 1·5 millidegrees K. Little information is available at resolutions coarser than 2° because receiver drift introduces confusion.

Upper limits can also be set to the strength of features much finer in angular scale than the antenna beam. Let the temperature be T(x,y) over a patch of sky and let t(x,y) be the departure from the spatial average

$$t(x,y) = T(x,y) - \langle T(x,y) \rangle$$
 (1)

The intrinsic fluctuations in the temperature distribution, T(x,y), as distinct from the fluctuations observed after averaging over the beam of an antenna, can be characterized by an intrinsic fluctuation level σ , where

$$\sigma = \langle [t(x,y)]^2 \rangle^{\frac{1}{2}} \tag{2}$$

Let the spatial variation of temperature be characterized statistically by a two dimensional spatial autocorrelation function $\gamma(\theta)$ defined by

$$\gamma(\theta) = \frac{\langle t(x,y)t(x+u,y+v)\rangle}{\langle [t(x,y)]^2\rangle}$$
(3)

which is a function of θ only, where $\theta^2 = u^2 + v^2$. Then the spatial distribution may be characterized by a one dimensional scale size that is constant over the patch of sky and independent of orientation. We define the "scale" Θ by

$$\Theta^2 = 2\pi \int_0^\infty \gamma(\theta)\theta d\theta \tag{4}$$

choosing this particular definition in order to make equation (6) valid for any form of dependence of

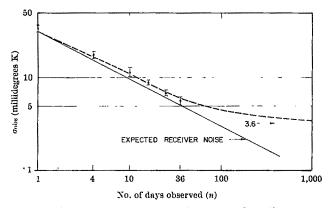


Fig. 2. Observed root mean square temperature fluctuation, $\sigma_{\rm obs}$, against number of days, n. The total length of an error bar is five times the standard deviation of the mean.

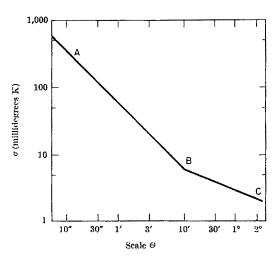


Fig. 3. Intrinsic fluctuation σ against scale Θ.

 γ on θ . If the power reception pattern of the antenna is A(x,y), that is when the antenna is pointed at (0,0) the response to a point source at (x,y) is A(x,y), then an effective beamwidth, B, can be defined by

$$B^{2} = \frac{\left[\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} A(x,y) dx dy\right]^{2}}{\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} [A(x,y)]^{2} dx dy}$$
 (5)

Now if the patch of sky is mapped by the antenna, then σ_{obs} will be less than σ and can be shown to be given by

$$\sigma_{\text{obs}^2} = \sigma^2 \left(\frac{\Theta}{\Theta'}\right)^2 + \sigma_R^2 \tag{6}$$

where σ_R is the root mean square fluctuation resulting from the internally generated receiver noise, and Θ' is the quantity that is analogous to Θ but refers to the two dimensional spatial autocorrelation function of the convolution of t(x,y) and A(x,y).

For example, if t(x,y) has a Gaussian autocorrelation function, $\gamma(\theta) = \exp(-\theta^2/2S^2)$, then $\theta = 2.51S$, and if the antenna pattern is also Gaussian with a half-power beamwidth (W), $A(x,y) = \exp[-(x^2+y^2)/0.36W^2]$, then B = 1.51 W and $\theta'^2 = \theta^2 + B^2$. In this observation W = 10 min of arc, σ_R is calculated from the receiver parameters and the data processing to be 5 millidegrees K, and σ_{obs} is 6.1 millidegrees K, as read from the broken line at n = 36 in Fig. 2. A graph of σ versus θ , from equation (6) with these constants, is shown in Fig. 3 (section AB). Because the quantity previously referred to as the asymptotic value is the term $\sigma^2(\theta/\theta')^2$ in equation (6), conclusions regarding coarser scales obtained by simulating wider antenna beams can now also be presented in Fig. 3 (section BC). To summarize.

$$\sigma = \begin{cases} 61 \, \theta^{-1} & 0 < \theta < 10 \\ 19 \, \theta^{-\frac{1}{2}} & 10 < \theta < 120 \end{cases}$$
 (7)

where σ is in millidegrees K and Θ is in min of arc. The fractional error in section AB is the same as at B but in section BC increases approximately as $\Theta^{\frac{1}{2}}$.

At low frequencies and comparable beamwidths the root mean square fluctuation produced by the many faint discrete sources exceeds the limits given here; at 610 Mc/s a root mean square "confusion noise" level of about 16 millidegrees K has been measured in a 16 × 22 min of are beam¹¹. Extrapolation to 10,690 Mc/s indicates that it may not be possible to reduce appreciably the upper limits to a reported here. The spectra of the faint sources, however, are not sufficiently well known for one to be sure about this.

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PLANETARY SCIENCE

Orogeny, a Cause of World-wide Regression of the Seas

In has often been suggested that orogenic episodes and epochs of folding coincide with world-wide regressions, and I wish to put forward a simple explanation of why this should be so.

I have constructed a model to represent two continents "floating" on the mantle and just submerged by the sea (Fig. 1). The continents are represented by rectangular blocks of lead floating in a trough of mercury. Water is poured onto the mercury so that the "continents" are just submerged. The "continent" can be deformed and a mountain created by halving the width and doubling the thickness of the mobile belt; in practice this can be done by cutting one block in half and turning one of the halves on its side. The deformed "continent" will displace the same amount of mercury although the mountain will have a deeper root, and so the level of the mercury will remain the same, but the water now has a larger area to spread over and so the depth of the water will drop and the stable "continent" will emerge from the "sea".

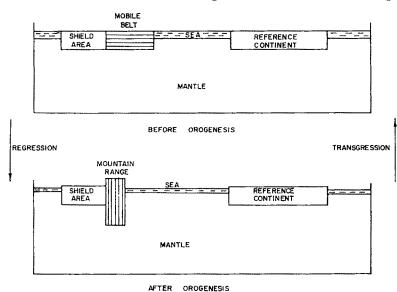


Fig. 1. Schematic diagram showing effect of orogenic episode on sea level.

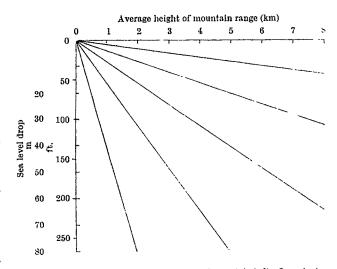


Fig. 2. Sea-level changes due to formation of mountain belt. Length of mountain range (width 300 km): 1, 2,000 km; 2, 5,000 km; 3, 10,000 km; 4, 20.000 km; 5, 50,000 km.

At first sight, the effect seems negligible, but if it is considered that most of the continental crust is transformed into the mountain root, to maintain isostatic equilibrium, the effect can be appreciable. An increase of only I per cent in the area of the oceans would lower the sea level by 40 m, assuming an average depth for the oceans of 4 km. Woollard has shown that a change in surface elevation of 1 km corresponds to a change of 6.8 km in crustal thickness. This means that if continental crust of an assumed average thickness of 35 km is transformed into a mountain range 3 km high, a root of about 20 km must exist, giving the resultant thickness of the crust as 58 km. It follows that a mountain range $5,000 \mathrm{\ km}$ long of average width and height (300 and 3 km, respectively) must have originated from crustal material approximately 500 by 5,000 km. This is a net increase in the total area of the oceans of 106 km2. If the area of the oceans is 311 × 106 km² (ref. 2) the sea level must drop everywhere by 13 m (40 ft.).

If orogenic episodes in different continents took place at the same time, a drop in the sea level of more than 100 ft. could easily be envisaged. Erosion of the mountains and the rest of the land would immediately increase. and sediments deposited in the sea would cause it to rise and transgress over the continents. Figure 2 shows in

idealized form the change in sea level caused by mountain belts, of different

lengths and heights, forming.

From the stratigraphic record in one area of a continent it is difficult to obtain an overall picture of the state of the seas with time, because a local transgression can occur while there is a world-wide regression of the sea3. By considering the whole of one continent, a much better picture can be obtained. Schuchert's palaeo-geographic maps of North America are ideal for this purpose and from these maps it is possible to plot a curve of the percentage area of epicontinental seas with time. Damon and Maugers have recently done this for periods from the Triassic until the present time, and Fig. 3 shows an adapted form of their curve. Kuenen⁶ considers that the more extensive transgressions did not involve a rise of much more than 600 ft. On this basis the increase in depth of the sea with respect to the present level has been plotted.

Table	1. OROGENIC EPISODES	
North American	Name of episode	Reference
NA 1	Pasadenan	5
NA 2	Basin and Range	
NA 3	Laramide	5
NA4	Santa Lucian	5 5 5 5
NA 5	Nevadan	5
NA 6	Palisadean	5
European		
$E\vec{U}$ 1	Alpine	14
EU 2	Pyrenean	13
EU 3	Austrian	13
EU 4	Young Kimmerian	13.
EU 5	Old Kimmerian	13
New Zealand		
NZ 1	Kaikoura	15
NZ 2		15
NZ 3	Rangitata	15
NZ 4	Rangitata	15

Does this curve represent accurately the world-wide trends? Certainly the Cretaceous transgression shows up clearly, as does the subsequent regression. The Eccene transgression and the Miocene transgression are also well documented. The mid-Jurassic is known to be transgressive in Australia, Europe and North America⁸ and it seems unlikely that the late Jurassic regression in North America, which affected about 15 per cent of the continent, could not have affected the world as a whole. It is unfortunate that sufficiently detailed palaeogeographic maps of other continents are not available for comparison with these data. The curve of Fig. 3 is believed, however, to represent the trends and to be of world-wide sig-

Apart from the overall Mesozoic transgression, which commenced at the beginning of the Triassic and ended in late mid-Cretaceous time, short term regressions and transgressions, superimposed on the general trends of the curves, are apparent. Damon and Mauger⁵ have correlated six distinct orogenies in the North American continent with the short periods of regression. If these oscillations are the results of the orogenies, why have other orogenies, occurring on other continents, not shown

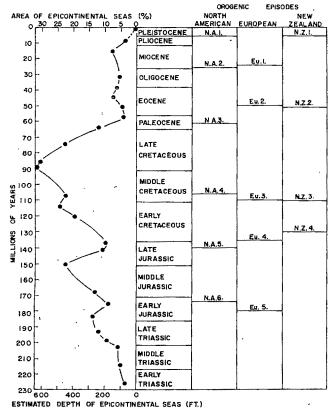


Fig. 3. Depth of epicontinental seas and orogenic episodes for North America.

up on our curve? If orogenies in other continents were coincident with the North American orogenies, the oscillations would be amplified and would result in large regressions and consequent transgressions. Non-coincident orogenies would cause frequent oscillations of small. amplitude and could scarcely be distinguished.

The idea of world-wide orogenic episodes has been hotly discussed for many years. Stille was probably the first to suggest it and there have been many staunch supporters^{3,10,11} and highly respectable sceptics¹². The chief European^{13,14} and New Zealand¹⁵⁻¹⁷ orogenies have been listed with the North American orogenic phases of Damon and Mauger⁵. Since the Triassic, the chief events, although not synchronous, are closely coincident and it is conceivable that the combined result of these orogenies would give a curve of the form shown. It is believed that the curve suggests world-wide orogenic phases which occurred approximately 25, 55, 108, 138 and 175×10^6 yr

The rapid and considerable regression of late Cretaceous time is of interest. Damon and Mauger⁵ have plotted the depth of the Pacific Ocean since late Cretaceous time and a slow, gradual subsidence until the present time is They suggest that this was the cause of the regression, although the sea, in fact, had retreated almost to its present position by middle Palaeocene times. Hallam' has calculated that this subsidence would have caused a eustatic fall of about 40 m, while a 200 m fall is indicated.

According to the theory of continental drift, the Himalayas were formed by the collision of the sub-continent of India and the southern shelf of Asia18. On this theory, a decrease in continental areas and thus a sudden retreat of the seas would result. Using Dietz's2 estimate of the area of the doubly thick continent of 2.44 × 106 km², and of 311×10^6 km² for the area of the present seas, a drop in sea level close to 100 ft. would be expected. would have occurred approximately $60 \times 10^{\frac{1}{6}}$ yr ago¹⁸ and fits in well with the sea level curve.

In summary, I believe that an orogenic event is necessarily associated with a eustatic fall of sea level and that the depth of the epicontinental seas is a sensitive indicator of these events. Using the data from the North American continent, I think that closely coincident world-wide orogenies have occurred since the Triassic and that the principal retreat of the Cretaceous seas is in part the result of the collision of India and Asia which occurred about this time.

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Fractionation of Iron in the Solar System

Harris and Tozer¹ have recently suggested that magnetostatic attraction between cosmic iron particles is responsible for the observed variation in iron abundance between the inner and outer planets of the solar system. I should like to point out that a crucial assumption in this model is "the uniform single-domain nature" of iron particles in the size range 10⁻¹–10⁻³ cm. The calculations which follow show that iron particles of only a limited size range can take part in magnetostatic accretion.

At room temperature, 300° K, iron is superparamagnetic2 below the critical size of 125 Å. This implies that such particles have no net magnetic moment because of thermal fluctuations. Above that diameter, iron is definitely a single domain but the critical diameter for the onset of multidomain behaviour is very close, 150 Å. As Table 1 shows, at 950° K, the temperature of accretion considered by Harris and Tozer, the superparamagnetic critical size is raised to 184 Å and an upper limit of 3300 Å emerges for the multidomain behaviour. This upper limit comes from the well known fact that a single-domain particle has to be smaller than the domain wall width at the temperature being considered. The width of 3300 A at 950° K is derived from the data given by Kittel³. sizes considered by Harris and Tozer, 10-4-10-3 cm, are far larger than the ones shown in Table I, and thus will have to be multidomained.

> Table 1. CRITICAL SIZES FOR IRON PARTICLES Temperature Superparamagnetic critical diameter $300^{\circ} \, \mathrm{K}$ 125 Å 150 Å 150 Å

Why is it so important for the particles to be single-domained? Because, for a multidomain particle of iron tumbling in a maximum field of 100γ (10^{-3} oersteds), the net moment would be negligible. The value used by Harris and Tozer is 200 gauss, but the real moment could not be greater than a hundredth of this. Because the product of the moments is responsible for the attractive force, the magnetostatic attraction for 10^{-4} cm particles could be a ten thousandth of what Harris and Tozer have assumed. The influence of magnetism on the gravitational attraction will indeed be there but it will be too small to cause an effective change in the capture cross-section.

It might be argued that at some time in the past the particles could have gone through an intense magnetic field emanating from the Sun, and this could have saturated the 10^{-4} – 10^{-3} em particles. But even so, for a sphere of iron of which the crystalline anisotropy energy at 950° K is low (about 10^4 ergs/cm³) and the demagnetizing factor is so large, this saturation moment would be very unstable with time, instantly decreasing to the steady state remanence of about a tenth or a hundredth of one gauss, as discussed before.

My conclusion therefore is that, although the mechanism suggested by Harris and Tozer is ingenious and interesting, it is of little importance for the size of particles they have discussed. In order to be effective, the particles have to fall in a very limited range of sizes as shown in Table 1. It may be possible to postulate that the original particles were indeed in this critical range because of some vagary of cosmology and that the observed particle sizes are larger because of grain growth on sintering.

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Banded Flints

Banded flints have long been known to occur in small numbers together with ordinary flints, which are the nodules of chalcedonic silica found in the chalk (Upper Cretaceous) of England and elsewhere. The bands may be observed on fractured surfaces and show up as a variation in colour within the normal colour range of unbanded flints. The exterior of the affected nodule may be ridged in a pattern conformable to the banding.

Formerly, authors, such as Woodward¹ and Sollas², explained the banding as being caused by some kind of infiltration, as would be ascribed nowadays to a natural chromatographic process. Geinitz³ suggested rhythmic precipitation and Gebhardt⁴ regarded a particular example as a test case in the recognition of Liesegang's rings as a natural phenomenon. Cayeux⁵ figured a similar specimen and noted that the effect, silex zonaire, involved the normal constituents of flint only, but he was unable to suggest any cause.

That these bands follow the patterns formed in experimental stress analysis does not seem to have been previously noted. Such stress patterns may be preserved by the "freezing" of an elastic component in a medium which is only plastic during loading. Synthetic resins, which provide both components, were discovered in 1935, and have since been used experimentally. Comparison leaves no doubt that banded flints have been subjected to stresses, probably through collision with other flints.

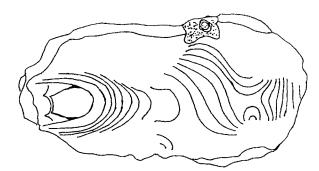


Fig. 1. A sketch of a banded flint. This banded flint is from the collection of Dr W. Hunter, at the University of Glasgow. It is specimen M 1177 in the Hunterian Museum (Geology).

The specimen illustrated in Fig. 1 shows one half of a flint from the collection of Dr William Hunter at the University of Glasgow. The pattern is in black, white, red and yellow according to the amount of compression and subsequent iron-staining. The source of the banded flint is not given but is presumed to be from the oolitic iron-ores of Baden, West Germany, a commercial source for so-called "red Egyptian Jasper", in the eighteenth century. I was privileged to examine Dr Hunter's collection in 1963. A recent article recorded the development of preferred orientation of quartz during recrystallization of flint in conditions of annealing. I suggest here that the naturally banded flints would similarly develop preferred orientation, as a result of the stress "frozen" in the specimen. This is regardless of the demonstration of preferred orientation in a naturally stressed flint, which has not been attempted. The evidence shows that annealing of flint results in preferred orientation which cannot be considered as free from stress, although Green, in the conclusion to his paper, suggests the contrary. An anomaly is thus explained.

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PHYSICS

Correction for Atmospheric Refraction in Surveying and Alignment

DETERMINING precisely the direction in which a given point lies is a fundamental problem in surveying and alignment measurements. Optical methods are known which are in principle sufficiently accurate for all practical requirements, but in practice their accuracy is reduced by the effects of the intervening atmosphere.

Refraction by the atmosphere can be considered in terms of its short-period or long-period effects. former may spoil the image or cause it to move about irregularly, which is inconvenient, but can be countered by averaging the readings over a sufficiently long period The measurement is reduced in sensitivity but no systematic error is introduced. The long-period, or quasi-static, refraction cannot even be detected as a rule, and may introduce a completely unrecognized error; it is therefore more dangerous than the shortperiod effect. It is well known, however, that it is possible in principle to recognize and measure this error by the variation with wavelength in the apparent direction of the point due to the dispersion of air.

This dispersion can be measured (inversely) by the V-value for air, defined as is usual for optical materials. The value of V changes only slightly with the physical state of the air, within the usual meteorological range, so it is not necessary to measure temperature, pressure or composition down the light path in order to obtain a reduction of the error to a few per cent of its uncorrected value. V has a value of the order of 110, however, so the difference in direction to be measured is very small. For a refractive error of a few seconds of arc, the difference of angle between red and blue rays must be measured to a few thousandths of a second to reduce the error by 90 per cent. Consequently, separate measurements in red and blue light seem unpromising.

This small value of the dispersion has, however, one beneficial effect: it means that, even over a long range, the red and blue rays nearly coincide over the light path. Thus, for a range of 10 km and a refraction of 20 sec, the maximum distance between red and blue rays is only about 0.3 cm. The rays will be affected by shortperiod refraction to about the same extent. This fact suggests that a differential method of measuring the angle for the red and blue rays should be practicable, and, if so, should be much more satisfactory than separate measurements.

A suitable way of doing this is as follows. Assume the distant point to be represented by a small light source. An image of this is formed by a telescopic system in the plane of a rotating chopper disk with fine opaque and transparent radial bars. The transmitted light is separated into red and blue components by a dichroic beam-splitter, the two components being received by two photo-multipliers. An approximately sinusoidal output is given by each photomultiplier. If appreciable refraction is present,

the image is drawn out into a short spectrum, and the red light is chopped at a different instant from the blue light; thus a phase difference appears between the outputs which can be read on a suitable phase meter. An optical compensator (which may be, for example, a tiltable glass plate placed in the converging beam) can be used to compensate the atmospheric dispersion, compensation being indicated by the phase difference falling to zero. The compensator scale will then give a measure of the dispersion and, thus, the atmospheric refraction. If the equivalent V-value of the compensator is made equal to that of air, the position of the image will, when compensation is effected, be the same as it would be in the absence of an atmosphere.

The chopping frequency is chosen to be much higher (some kc/s) than the important frequencies in the spectrum of atmospheric turbulence. Turbulence will then tend to make both images move together, which will not affect the observed phase difference.

This system has been tested as a bench apparatus, and, although certain practical problems remain to be solved, the results indicate that the precision will be adequate over ranges of interest to surveyors.

The limiting feature is the attainable signal-to-noise ratio. This would be greatly eased if a laser were available (of sufficient ruggedness, portability and cheapness) which emitted red and blue beams simultaneously. Using a thermal light-source and far from optimum components, the results indicate that an error of refraction of one second would be readily observable at a range of 10 km in clear conditions, using an integrating time of about

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Solvated Electron Spectrum in Irradiated Ice

RECENT pulse radiolytic studies on transparent samples of ice have demonstrated the existence of three transient optical absorptions which have been assigned to the solvated electron^{1,2}, e_{\varepsilon}, the hydroxyl radical², OH·, and the hydroperoxy radical², HO₂. Particular attention has been paid to the influence of temperature on their spectral, yield and kinetic characteristics. For e-, as the temperature is decreased, λ_{max} shifts to shorter wavelengths, the yield decreases, and the lifetime increases. These observations indicated that an ice sample highly irradiated with $^{60}\mathrm{Co}$ gamma-rays at 77° K might show a stable, low yield es having an absorption maximum at about 6300 Å.

In order to test this hypothesis, experiments were carried out in the following way. A cylindrical ice sample (20 mm in diameter and 25 mm in length), which had been grown by slowly lowering a sealed test tube containing degassed, triply distilled water into a cooling bath at -5° C, was fixed with a thermocouple, cooled to 77° K by slowly lowering it into a Dewar containing liquid nitrogen, and irradiated in a 1.7 Mrad/h source. sample was then transferred to a specially designed quartz Dewar attached to a Cary spectrophotometer, and the spectrum was recorded. One additional experiment was carried out in which oxygen was completely excluded from the irradiated system, and it gave similar results.

A typical result is shown in Fig. 1. A maximum appears at 6400 ± 100 Å (other very strong absorptions in the ultraviolet have been omitted2). The yield of this absorption increases with dose up to about 5 Mrads and then approaches a plateau value. Based on the linear portion of the yield-dose plot and $\varepsilon_{\rm max} = 1.7 \times 10^4 \, \rm M^{-1} \, cm^{-1}$ (refs. 3–5),

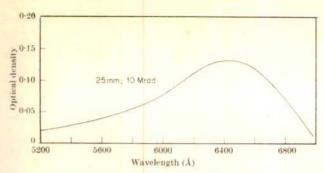


Fig. 1. Spectrum of solvated electron in ice at 77° K. Total dose; 10 Mrads

a G-value of 2×10^{-4} is estimated. Although the absorption appears to be thermally stable at 77° K, it can be photobleached with visible light. The absorption disappeared, however, when the sample was warmed to 112° K.

The fact that a stable solvated electron absorbing below 7000 Å can be formed in ice has relevance for various theories related to the formation, structure and decay of this entity in other media such as liquid water and alkaline ice glasses.

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insulated brass "tuning fork", the assembly being mounted in a transparent plastic tank, capacity 2 l. The cathode was held 2.5 cm below the liquid surface and the electrodes connected to a constant current source which gave a cathode current density of 0.125 amp/cm2. The bubbles were photographed using flash illumination. The sizes of the bubbles were obtained by measuring their diameters on an enlarged projection of the photographic negative. Extremes in bubble sizes were ± 15 per cent for bubbles having diameters of the order 0.0025 cm and ±33 per cent for diameters of the order 0-0075 cm. Reagent grade chemicals and tap water were used.

The dramatic effect on bubble size of added solute can be seen in Fig. 1, the addition of 1 molar sodium bromide markedly decreasing, and addition of 1 molar ammonium bromide markedly increasing, the size of the bubbles relative to the size of bubbles in water under the same conditions. These trends can result from a sensitivity of the bubble size to water-solute interaction within the aqueous solution. This hypothesis was tested by monitoring the variation in mean bubble diameter with increasing concentrations of t-butyl alcohol in water, as shown in

Mixtures of water and t-butyl alcohol display marked extremes, both with regard to their own properties6 and those of solutes in their solvent systems7,8, as the concentration of alcohol is varied. These effects reflect the changes which occur in the structure of the aqueous mixture, as a consequence of the varying demands of water-water, water-alcohol and alcohol-alcohol interactions.

At low t-butyl alcohol mole fractions, $x_2 < 0.02$, the bubble surface area (calculated from the mean diameters) is greater than in water (Fig. 1), the surface area decreasing with the increasing mole fraction of alcohol. In these dilute solutions, the consequence of forming a rapidly expanding bubble together with a slow rate of diffusion of the alcohol solute to the bubble/liquid interface means that the hydrogen bubble is in an extreme non-equilibrium The diffusion rate, however, increases with in-

Effect of Added Solutes on the Size of Hydrogen Bubbles liberated from a Cathodic Wire in Aqueous Solution

An important tool in fluid flow research is flow visualization using the hydrogen bubble technique. Here a wire cathode is mounted normal to the water flow and connected to an electrical pulse generator. The planes of hydrogen bubbles, which evolved from the wire, follow the velocity variations in the flow^{1,2}. The patterns formed by the hydrogen bubbles may be photographed and the velocity profile analysed. In order to use this technique to full advantage the size of the bubbles must be sufficiently small for them to remain in the same velocity profile but sufficiently large for observation.

It has been generally assumed that the size of bubbles released in aqueous solutions is unaffected by added solutes, added salts merely operating to enhance the conductivity of water, thereby facilitating electrolysis. It has been established3-5, however, that the bubble size depends on the interfacial contact angle between the cathode and the liquid and on the polarization of the cathode. In the course of a detailed investigation into flow visualization, we investigated the size of bubbles released from a wire in various aqueous solutions in static conditions and we now briefly report evidence to show that the size of a hydrogen bubble released from a wire cathode in aqueous solutions can be particularly sensitive to the nature and concentration of added solutes.

A stainless steel wire cathode, length 5 cm and diameter 0.05 mm, was supported between two prongs of an

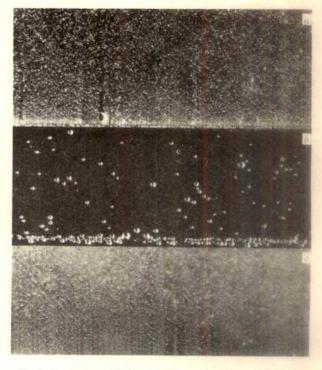


Fig. 1. Comparison of the sizes of bubbles released from a stainless steel cathode having diameter 0-05 mm with current density 0-125 amp/cm-immersed, at 25°C, in (a) water, (b) 1 molar aqueous solution of ammonium bromide, and (c) 1 molar aqueous solution of sodium bromide.

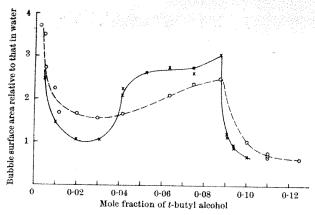


Fig. 2. Surface area of hydrogen bubbles (computed from mean bubble diameter) relative to those in water under the same conditions as given in Fig. 1 in mixtures of t-butyl alcohol and water, x_2 being mole fraction of alcohol. Data refer to two independent series of measurements.

creases in the alcohol mole fraction and therefore the bubble surface area diminishes. At alcohol concentration greater than $x_2 = 0.02$, the surface area increases (compare with the solution containing ammonium bromide) reaching a maximum in the $x_2 = 0.08$ region, and then decreases rather sharply. This type of behaviour is comparable with the sound absorption properties of such mixtures. Indeed, both sound absorption properties and the spectra of solutes in these mixtures show that in this concentration region the structure of the essentially water dominated solution breaks down, the system having maximum disorder in the $x_2 = 0.09$ to 0.1 region. Although the required water/steel surface tension and contact angle data are not available for this system, the contact angles for mixtures of ethanol and water on a copper surface10 undergo dramatic changes in a comparable concentration range.

With increases in temperature the bubble size in water steadily decreases, and this trend is comparable with the effect of added potassium chloride to water at a given temperature. This pattern of behaviour is in general agreement with the currently accepted ideas concerning the breaking action of most alkali halides on the structure^{11,12}. For example, a decrease in bubble size is also effected by the addition of 1 mole of sodium chloride, bromide, iodide and sulphate.

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Relationships between the Masses of Subatomic Particles

In a previous communication¹, the bound rest mass of the proposed tamaid particle, t_0 , was calculated from available experimental data² to be equivalent to 25.95 MeV. The subatomic particles of zero charge have been shown to fall into a quantized series obeying the relationship

Mass of subatomic particle = M = 25.95 n + 134.97

Here n is an integral quantum number and the constant term is the mass of the π^0 meson. From a consideration of the available numerical data the possible existence is now postulated of another mass-energy entity, b, which, in association with the tamaid, could be employed to build up units of matter which correspond to all the known muons, mesons, baryons, etc. It is proposed to call this subsidiary entity a "bach" (Welsh—the little one), and present calculations suggest that it has a bound rest mass corresponding to b = 0.3263 MeV. It is therefore smaller than the electron (e $^- = 0.511 \text{ MeV}$) and must be associated with a larger De Broglie wavelength.

If the bachs and tamaids really constitute the ultimate particles of matter out of which all the other mass-energy states may be created, it is necessary to assume that all subatomic particles consist essentially of a core of tamaids associated with a mass-energy aura, or field, of bachs whose physical form of natural existence is not easy to visualize.

Equation (1) deals with particles which are heavier than the π^0 meson. It is now proposed to extend this theory to include the muons, and it will therefore be convenient to replace the quantum number n^1 by a new integral function x defined by

Number of tamaids =
$$x = (n + 5)$$
 (2)

The number 5 represents the number of tamaid particles which aggregate to form the core of the π^0 meson, and, as will be shown later, these must be associated with 16 bach entities, that is y = 16. The term y is a secondary quantum number which, for uncharged subatomic particles, may be defined by

Number of bachs =
$$y$$
 (3)

To derive the final equation (4) it has also been necessary to postulate the existence of a further quantum number zwhich can have any of the integral values, z = 0, 1, 2, 3, etc., and which is mathematically related to x and y by the fundamental expression.

Total number of tamaids and bachs in a subatomic particle = (x + y) = x + (3 + 13z)

Because the masses of t_0 and b have been derived earlier it is now possible from equation (4) to compute the masses of the uncharged subatomic particles, and the results are shown in Table 1. In this series, z has the value 0 or 1, and is undoubtedly concerned with some internal "energy level" system.

$$M = \text{mass of a subatomic particle} = 25.95 \ x + 0.3263 \ y$$

= $xt_0 + yb$ (5)

The binding energy of each individual tamaid in the central core of the subatomic particle must be particularly constant or equation (5) would not be expected to hold with any degree of exactness.

For the main uncharged series, z = 1 and y has the constant value of 16, that is (3 + 13). It would seem that this value of y is absolutely essential for the stability and natural existence of uncharged members of the series. If z = 0, we have from equation (4) the condition y = 3which is unacceptable, and y will have to increase by 13 units, that is to y = (3 + 13), before we can expect to find further stable subatomic particles. confirms this reasoning, for no particles are recorded in the range x = 6 to x = 18. When x = 19 tamaids, these

associate with the dominant parameter, y=16 bachs, to give the K⁰ meson (497.82 MeV), z again being taken as equal to unity. The series then builds up again in a stable fashion with y=16, z=1, and x increasing from 19 to 90 in an integral manner.

The problem of calculating the masses of charged subatomic particles is usually complicated by a lack of understanding of the fundamental relationships between mass and electrostatic charge. Curiously enough, these difficulties do not seem to militate against the direct application of the present theory to the computation of the masses of charged muons, baryons, etc. Equation (4) must be slightly modified for charged particles and is written in the form

Number of particles = (x + y) = 6 + (3 + 13z) (6)

where z can have any of the integral values 0, 1, 2, 3, etc. The phrase "Number of particles" in equation (6) has been used deliberately because while in equation (4) there seems to be a definite mathematical segregation of the x tamaids from the y bachs, this condition does not apply to equation (6). Indeed, the dominant parameter appears to be the summated integral number (x + y), irrespective of the types of particles concerned. This

number also determines the value of z, that is $z = \frac{x+y-9}{13}$.

Stable structures are only achieved by the successive addition of packets of thirteen particles. Table 2 gives the data for the known charged subatomic particles. When z=0, stable particles exist for the corresponding condition (x+y)=9, and these are the muons μ^+ μ^- , respectively, with values of z=0. The π^+ meson will be seen to be associated with 30 bach particles and to have z=2.

Following these, we again have the intrusion of the "magic number", 13, and no stable subatomic particles are experimentally observed until we reach the x^+ meson with (x + y) = 22 and z = 1. Obviously from equation (6) y can only have the value 16, as is the case for the uncharged particle, if x = 6 and z = 1. For any other values of y, the particles are charged.

Table 1. UNCHARGED PARTICLES

Particle	n	x	y	z	(x+y)	Mass (MeV) observed	Mass (MeV) calculated
μ•	- 1	4	16	1	20	Not known	109-02
π*	0	5	16	1	21	$134-97 \pm 0.015$	134.97
Not know		6	3	0	9	Not known	
Not know	a 2	7	3	0	10	Not known	****
K*	14	19	16	1	35	497.82 ± 0.25	498-40
η	16	21	16	1	37	548.6 ± 0.4	550.3
к	23	28	16	1	44	731 ± 2	731-05
ρ*	24	29	16	1 .	45	756·4 ± 3·2	757-9
் ω°	25	30	16	1	46	783·4 ± 0·9	783.8
N	31	36	16	1	52	939.55 ± 0.005	939-6
Φ	34	39	16	1	55	1.018 6 ± 0.05	1.018-2
Λ	38	43	16	1	59	1,115.6	1,121.3
X*	41	46	16	1	62	1,192-1	1,199
f•	43	48	16	1	64	1,254	1,251
E*	45	50	16	1	66	1.314·7 ±1	1,303
Y	48	53	16	1	69	1,385	1.381
Λ	49	54	16	1	70	1.405	1,407
N	60	65	16	1	81	1.688	1,692
Δ	69	74	16	1	90	1,920	1,926
Λ	85	90	16	1	106	2,340 ± 20	2,341

Table 2. CHARGED PARTICLES

Particle	n	\boldsymbol{x}	y	z	(x+y)	Mass (MeV) observed	Mass (MeV) calculated
μ^+	- 1	4	5	0	9	105.659 ± 0.002	105.432
π +	0	5	30	2	35	$139-57 \pm 0.014$	139-539
Not known	1	6	3	0	9	Not known	156-68
Not known	1	6	16	1	22	Not known	
Not known	2	7	2	0	9	Not known	
K*	14	19	3	1	22	493.78 + 0.17	494.03
p+	24	29	19	3	48	758.3 + 2.8	758-75
ĸ	29	34	27	4	61	891.7 + 0.7	891-11
P	31	36	12	3	48	938-256 ± 0-005	938-12
Σ+	41	46	15	4	61	$1.189 \cdot 53 \pm 0.08$	1.198-58
Σν	59	64	10	5	74	1.660	1.664.0
≥ 8	63	68	6	5	74	1.767 + 4	1 785.8

It should be noted that although stability laws preclude the existence of an uncharged particle having x=6, the related charged particle would have (x+y)=9, which is a condition of stability under the conditions of equation (6). This particle has not been experimentally detected but its theoretical mass would obviously be 156-68 MeV.

Because the rest mass values of the tamaid and bach used in this paper are only average values computed from the better established masses of the subatomic particles, the agreement of experimental and calculated values over this wide range of masses seems to be encouraging support for the present theory. The calculated value for the Σ^+ baryon is slightly high, but the experimental value for Σ^- is 1,197·33 MeV, which is in good agreement. Values for the negatively charged subatomic particles can be derived in a similar manner.

The chief quantum rules which seem to ensure the stability of the subatomic particles may be summarized as follows. (a) The cores of uncharged particles build up in single tamaid units. When equation (4) applies and y = sixteen bachs, the subatomic particles are uncharged. (b) The cores of charged subatomic particles contain up to six tamaids. When equation (6) applies it must be assumed that the remaining (x - 6) tamaids have linked up with the corresponding y bachs in the quantized stationary states and that the total particles in these states, regardless of differing rest-masses, obey the normal rule, that is

Particles external to the core = (x-6) + y = (3+13z) (7)

This latter equation is identical with equation (6).

One is tempted to visualize the bachs as rotating in orbits around the tamaid cores because equation (4) is very reminiscent of the classical Bohr-Bury theory of atoms, and suggests a solid core of x tamaids surrounded by $y = (3+13 \ z)$ bachs forming orbital shells of the type 3, 13, 13, 13, etc., in conformity with the quantum numbers z = 0, 1, 2, 3, etc. Equation (7) follows the general pattern, but one would expect some discontinuity in the unique case when x = 6 and this behaviour is demonstrated in Tables 1 and 2.

These equations indicate very clearly how uncharged particles differ from charged particles and offer convincing reasons why the neutron should be heavier than the positive proton whereas it is well known that the positive π^+ meson is heavier than its neutral counterpart π^0 . The difference is essentially a result of the varying bach content of the sister particles which nevertheless have the same overall tamaid content, that is, identical x values. If the wave mechanical concepts of Schroedinger are to be applied to this present theory, the existence of some form of attractive force between particles will need to be postulated which would be in equilibrium with the centrifugal forces produced in orbital states. In the present case, the calculations would also have to take into account the existence of two types of particle external to the tamaid core. During this work some relationship was sought between the mass of the electron $(m_e = 0.511 \text{ MeV})$ and

the bach and it was empirically noted that $\frac{4}{5}b = m_e^2$.

It is very singular that the fundamental parameters which seem to determine whether subatomic particles are charged or uncharged are directly associated with a series of quantum numbers which have been employed in calculations involving small quantities of matter only, that is, with the inclusion of no specific electrostatic quantum number or function, for example, hypercharge. The semi-empirical equations given in this paper certainly suggest that electrostatic charge and mass may be merely uniquely created forms of some primigenous material and the exact mathematical elucidation of the relationship between these two quantities would be of the greatest fundamental importance.

It should be possible by means of the present theory to predict the probable nature of the collisional event in a hydrogen bubble chamber provided it is accepted that the accelerator beam is able to yield extra packets of mass-energy, E, to provide the bachs necessary to yield stable systems. Consider the case of an accelerated K⁻meson, $(19t_0 + 3b)$, impacting on a proton, $(36t_0 + 12b)$, and yielding a collisional chaos $(55t_0 + 15b)$, plus energy = E. This chaos could degrade to an energetic proton, $(36t_0 + 12b)$, and a π^- meson, $(5t_0 + 30b)$, or to unstable tamaid system, $(19t_0)$, which absorbs further energy to form a stable K⁰ meson $(19t_0 + 16b)$. The K⁰ meson could then decay to yield a π^+ meson and a π^- meson, both being $(5t_0 + 30b)$.

both being $(5t_0 + 30b)$.

These mesons finally degrade to muons $(4t_0 + 5b)$, surplus energy being lost to the ambient medium.

The ultimate nature of the bach remains speculative, but it is for serious consideration whether it is in any way associated with the muonic neutrino.

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Surface Topography of Non-optical Surfaces by Projected Interference Fringes

In the light slit microscope of Schmaltz¹ a narrow slit of light is projected obliquely onto an irregular surface, and the trace of the slit, viewed normally through a microscope, provides a section of the surface to a scale depending on the angle of incidence of the light. This technique has the disadvantages that (a) there is only a single slitimage, which must therefore be made to traverse the surface, and (b) there is very little focal depth in the slit image if it is made narrow enough. If the width of the slit is to be d it must be projected with numerical aperture $\alpha = \lambda/d$ and the focal depth is approximately λ/α^2 , where λ is the wavelength of the light.

These difficulties can be overcome by projecting onto the specimen a set of interference fringes produced by two collimated coherent pencils of light intersecting at an angle; the bright and dark fringes form in planes parallel to the bisector of the angle θ between the pencils and

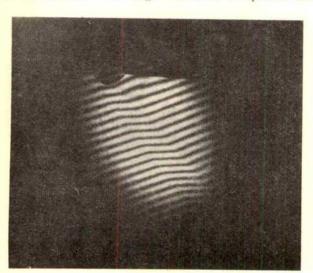


Fig. 1. Fringes with 3 mm spacing on a ridged card.

it can easily be shown that the fringe spacing, σ , in a plane making an angle β with the bisector, is given by

$$\sigma = \frac{\lambda}{2\sin\frac{\theta}{2}\sin\beta}$$

Thus fringes with any spacing down to say 0.7λ , corresponding to $\theta = \beta = 90^{\circ}$, can be produced and used to intersect the surface to be inspected. This technique is, of course, only practicable using a laser as a high intensity coherent source.

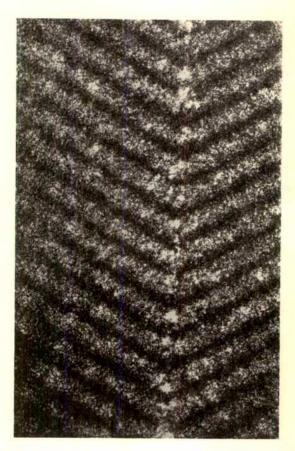


Fig. 2. Fringes with 18μ spacing on an aluminized ground glass wedge with 125° angle.

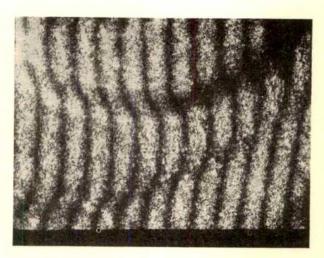


Fig. 3. Fringes with 18µ spacing on the reverse side of a 1966 sixpence; detail of one of the oak leaves.

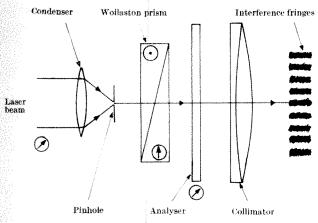


Fig. 4. Method of forming broadly spaced fringes by means of a Wollaston double-image prism; the encircled arrows indicate polarization directions.

THE SOLID STATE

Permanent Magnet Properties

The claim by McCurrie¹ for a $(BH)_{\rm max}$ value higher than previously recorded is manifestly incorrect, although the values he quotes from other publications are probably correct. For a permanent magnet material with remanence B_r in gauss, the theoretically highest possible $(BH)_{\rm max}$ value in gauss-oersteds is $\frac{1}{4}B_r^2$; this limit is on the basis of no change in the intrinsic magnetization between B_r and the $(BH)_{\rm max}$ point. In practice, some high coercivity materials approach this limit fairly closely, as illustrated

		Table 1	
Source	Be	$\frac{1}{4}B\tau^2$	$(BH)_{\max}$
Walmer ²	6,450 gauss	10.4×10^{6}	9.5×10^{8} gauss-oersteds
Chaston ³	7,125 gauss	12.7×10^{s}	10.3 × 10° gauss-oersteds
McCurrie ¹ (a)	5,280 gauss	$7 \cdot 0 \times 10^{s}$	14-1 × 10° gauss-oersteds
McCurrie (b)	6,065 gauss	9.2×10^{6}	11.0 × 10° gauss-cersteds
McCurrie (c)	4.050 gauss	$4 \cdot 1 \times 10^{6}$	6.9 × 10° gauss-cersteds

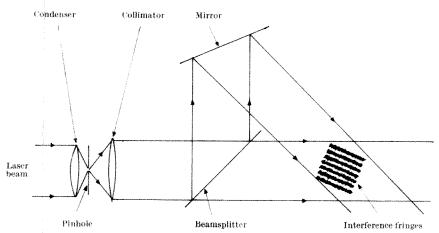


Fig. 5. Method of forming closely spaced fringes by means of a beamsplitter.

Figure 1 shows fringes at a spacing of 3 mm projected onto a card which has a ridge with an adjacent furrow, both of angle 125°. Figure 2 shows an aluminized ground glass surface, with fringe spacing of 18µ, viewed through a 0·15 N.A. microscope objective; the surface contains a ridge of angle 125°. Figure 3 shows 18µ spacing fringes on a portion of an oak leaf on the reverse of a 1966 sixpence, a metal surface with a fairly high polish. The photographs were taken with light of wavelength 6328 Å from a helium-neon laser.

The fringes can, of course, be produced by any of a number of well known methods; Fig. 4 shows a Wollaston prism arrangement suitable for large fringe spacings, while Fig. 5 depicts an arrangement of a beamsplitter and a mirror which could be used for large angles, θ , and small fringe spacings. The fringes produced by either of these methods can be observed on surfaces of widely differing optical properties, for example, polished metal, ground glass or wood.

An obvious modification is to project the same fringe system through a beamsplitter onto two nominally identical objects; if these are viewed from an appropriate angle the two fringe systems will produce a moiré pattern indicating the differences between the objects.

We are grateful to Mr J. H. Toyer for help in preparing the specimens.

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¹ Schmaltz, G., in *Technische Oberflächenkunde* (Berlin, 1936).

by the test values of Walmer² and Chaston³ which are quoted by McCurrie, but his own tests grossly exceed this limit, as shown in Table 1.

It seems probable that McCurrie's reported $(BH)_{\rm max}$ values are in fact $(4\pi JH)_{\rm max}$.

 $(BH)_{
m max}$ has for many years been the accepted criterion of merit for permanent magnet materials, because it is a measure of the external magnetic energy available in static operation, but for dynamic operation other criteria apply, and there may then be merit in the value of $(4\pi JH)_{
m max}$.

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Received October 23, 1967.

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² Walmer, M. S., Engelhard Technical Bulletin (1961-1962).

³ Chaston, J. C., British patent, 849,505 (1960),

CHEMISTRY

Exothermic Reactions behind a Reflected Shock

THE use of reflected shock waves to initiate exothermic reactions in a shock tube is commonly concerned with the ignition of oxygen and simple fuel molecules. The speed of the incident shock, U_s , determines the speed of the reflected shock, U_r , and the temperature, T_s , at which the reaction takes place. If T_s is sufficiently high, an explosion

occurs almost instantaneously and the reflected shock leaves the end plate at high speed because of the release of the energy of combustion. At lower temperatures there is an induction period, τ , before reaction occurs; a combustion-driven wave then sets out after the slower non-reactive reflected shock and eventually the two waves coalesce. The use of large windows near the end plate of the shock tube has permitted streak photography of this phenomenon for carbon monoxide-oxygen and hydrogenoxygen-argon² mixtures. A typical record is depicted in Fig. 1.

We have written a computer programme which, for a given value of U_s , calculates the value of U_r when there is no reaction behind the reflected shock $(R^*, \text{ Fig. 2})$ and also when reactants pass instantly to equilibrium products behind this shock $(D^*, \text{Fig. 2})$. The programme does not cater for undiluted fuel-oxidant mixtures but can accommodate the heat release from mixtures diluted with argon (≥ 80 per cent).

It is not possible to install large windows in our shock tube (5 \times 3 in.) in order to measure U_r from x-t photographs. The gold film detectors used to measure U_s . however, can also be used to measure U_r . For this purpose binary circuits are required to protect the triggers of the microsecond counter so that the incident shock is ignored. Although the positions of the detectors are fixed in the shock tube, attachment of a plug to the end plate allows U_r to be determined after the shock has travelled different

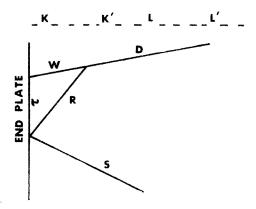


Fig. 1. A diagram of wavefronts at the end of a shock tube when there is an ignition delay after shock wave reflexion. S, Incident shock; R, reflected shock; W, reaction shock; D, combustion-driven wave. K, L are detector stations which become K'. L' when the end plug is removed.

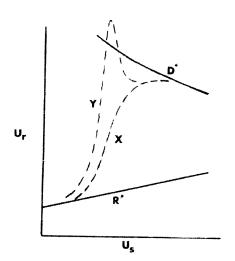


Fig. 2. Plots of reflected shock speed against incident shock speed, R^* , Computed for no reaction behind the reflected shock; D^* , computed for complete reaction behind the reflected shock; X, reflected shock travels about 1.0 in. before timed; Y, reflected shock travels about 3.5 in. before timed.

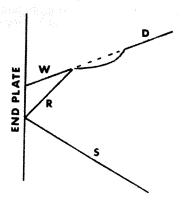


Fig. 3. A diagram of wavefronts required to explain abnormally high reflected shock speeds.

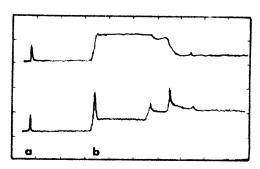


Fig. 4. Oscilloscope traces depicting induction periods in a methane-nitrous oxide-argon mixture (20 μ sec/cm). Upper trace, absorption by nitrous oxide at 2590 Å; lower trace, absorption by OH at 3670 Å. a, Incident shock; b, reflected shock.

distances from the end plate. Results with ammoniaoxygen or nitrous oxide-argon mixtures have been

reported³ and are shown in Fig. 2.

The overshoot of U_r values in plot Y of Fig. 2 cannot be explained by the geometry of Fig. 1. For example, if the end plug is present, timing is between K and L. The timer is started by R and stopped by D and thus U_r is an average of two values, one from plot R^* and one from plot D^* of Fig. 2. At the same U_s value, when the end plug is absent, timing is between K' and L'. The timer is started and stopped by D and the measured U_r falls on plot D^* of Fig. 2. Comparably, for large values of τ , the timer is started and stopped by R and the measured U_r falls on plot R^* . Thus, according to Fig. 1, X and Y plots of Fig. 2 must lie on, or between, the R^* and D^*

Thus when overshoot is detected, W and R cannot coalesce instantaneously to yield D but, for a short interval of time, there must be a wavefront the speed of which is faster than that of D. This wave lacks the support of energy available from complete combustion of the reactant mixture and must decelerate. In doing so it becomes D. Figure 3 should depict the true state of affairs. Thus, under certain circumstances, one may be able to detect two wavefronts at the end of the induction period. The first of these fronts can only permit partial conversion of reactant to equilibrium products.

The windows in our shock tube are 14 mm from the end plate and a slit system defines a 1 mm beam of light from a xenon lamp. After leaving the tube the beam divides so that absorption can be monitored simultaneously on two monochromators set at different wavelengths. A record obtained with a mixture of methane-nitrous oxide-argon (6 per cent: 24 per cent: 70 per cent) is shown in Fig. 4.

Runs with previously examined mixtures confirm that it is possible to detect two wavefronts at the end of the induction period in the systems hydrogen-oxygen, carbon monoxide-oxygen, hydrogen-nitrous oxide, ammoniaoxygen and ammonia-nitrous oxide (plus argon, ≥70 per cent). In all cases τ was short, about 50 µsec or less. At lower temperatures, and thus at larger τ values, only one front was detected at the end of the induction period. The critical conditions for detecting two wavefronts depend on the distance separating the optical path from the end plate, the fuel-oxidant ratio, the argon concentration and the reactant pressure.

The energy release in the first wave can vary and may be small enough to make detection of the front quite a difficult matter. The investigation of this phenomenon is suggested to those who have the facility of taking streak

photographs at the end of a shock tube. I thank Dr C. L. Cook for his interest.

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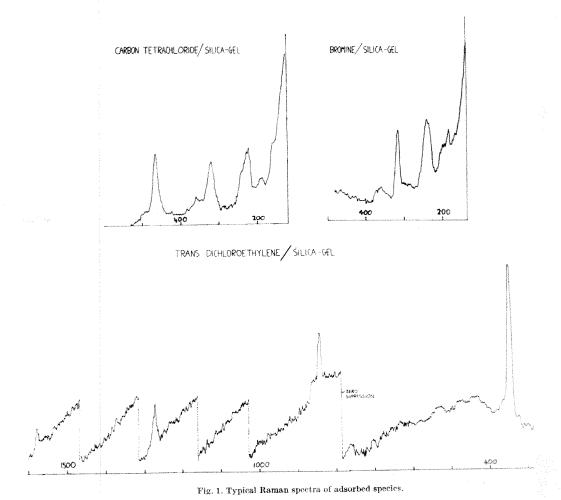
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Laser Raman Spectra of Sorbed Species Physical Adsorption on Silica Gel

THE principal method of studying the bonding characteristics of adsorbed species is to use infrared vibrational spectroscopy. Although the method has been applied to an extensive range of adsorbates and substrates, the technique suffers from the problem that, because of substrate adsorption, only high frequency modes of vibration of the adsorbate can be detected. The Raman effect would seem to offer an alternative method of study.

The Raman spectra of organic molecules adsorbed on silica gel were reported some years ago by Karagounis and Issa¹, who used the Brandmüller method² for crystalline powders, which they adapted to obtain spectra of adsorbed species under very specific conditions of sample thickness. We wish to report spectra obtained very conveniently on a conventional laser Raman spectrometer, using the machine in an unmodified form.

The adsorbant was contained in a 'Pyrex' cell fitted with an optical flat at one end and a tap, for connecting to a vacuum line, at the other. The spectra were obtained on a standard Cary 81 laser Raman spectrometer, the end window of the cell being placed in good optical contact with the hemispherical lens of the instrument. It was found that the quality of the spectra depended on the size of the adsorbent particles. Initial experiments were carried out on a 100-200 mesh silica gel, because this was known to have a very high surface area and thus potentially a large number of molecules in the volume examined by the spectrometer. This, however, gave rise to a very high background, a high noise level and spurious bands. These latter disturbances came from the high mobility of the fine particles of gel in the small sampling area of the instrument which resulted in the appearance of a band whenever a gel particle moved across the illuminated volume. The size of the adsorbent particles was therefore increased to 18-30 mesh to reduce these effects but at the expense of a loss in surface area. The silica gel in these experiments was standard chromatographic grade, cleaned



by heating to 150° C in vacuo for 12 h. After this time the pressure had fallen to below 10-4 torr.

The materials studied were carbon tetrachloride. bromine, carbon disulphide and trans-dichloroethylene on

silica gel.

The gel alone produced a relatively weak emission near 185 cm⁻¹, a strong band near 235 cm⁻¹ and a very broad weak band in the region from 300 to 500 cm⁻¹. The second of these bands may, however, have been a spurious line characteristic of the instrument³. Apart from these, the spectrum was remarkably unobstructed. spectra are shown in Fig. 1.

Carbon tetrachloride/silica gel was examined over a wide pressure range. The monolayer capacity of the gel in this system was estimated using the Brunauer-Emmett-Teller method and 0.218 g of carbon tetrachloride/g of gel was added, being equivalent to a surface area o 174 m²/g. It was assumed that the area of the carbon tetrachloride molecule approximates to 18.6 Å2. Spectra were obtained at less than monolayer coverage and at heavier adsorption. No observable difference was found between the spectra except for their intensities. It is thus apparent that adsorption had little effect on the potential energy of the adsorbate molecule, that is the association with the surface was very weak.

The next experiment used bromine as adsorbate. The yellow/orange colour of the system did not prevent spectra being recorded and excellent results were obtained at quite low vapour pressures, certainly down to 0.01 svp at 25° C. As in the previous case the emission occurred at a frequency similar to that in the liquid. In fact, the results were Br_2 (gas) 316·8 cm⁻¹; Br_2 (liquid) 306 cm⁻¹; Br₂/SiO₂ 314 cm⁻¹. It therefore seems that the adsorbed halogen closely resembled the gaseous species. could be interpreted that the adsorbent-bromine association is much weaker than the induced dipole-induced dipole interaction in the liquid. Because of its relatively low boiling point and very low latent heat of vaporization this must in turn be weak.

One of the effects of weak association on vibrational spectra is the distortion of the selection rules. Thus it is characteristic behaviour that in the charge transfer complexes of the halogens the stretching vibration of the homonuclear diatomic is infrared active. Carbon disulphide has three modes of vibration, two of which are Raman inactive. It is noteworthy that these modes (sulphur-carbon-sulphur bending at $397~\mathrm{cm^{-1}}$ and the asymmetric stretching mode at 1,523 cm⁻¹) remained inactive in the adsorbed case. In the other sample where mutual exclusion applies, in trans-dichloroethylene, no bands, normally Raman silent, appeared in the spectra

of the sorbed species.

One possible explanation for these results is that the instrument had simply recorded the spectrum of the vapour of the adsorbate. The high intensity of illumination could well have involved an appreciable rise in temperature and subsequent desorption of the surface layer. The intensity of the Raman bands rules out the proposal for, in the case of carbon tetrachloride, a vapour pressure of some tens of atmospheres would be required to give such prominent Raman bands. As the bulk of the adsorbent is not illuminated, any desorbed vapour would be promptly readsorbed on the "cold" surrounding gel. It is just possible that multiple reflexions within the gel could increase the illumination efficiency to such an extent that vapour at a low pressure could emit strong Raman lines. To check this hypothesis a clean gel was put in a tube filled with nitrogen at the relatively high pressure of 1 atm. No trace of the nitrogen-nitrogen stretching vibration at $\Delta \gamma = 2.331$ cm⁻¹ could be observed. It must therefore be assumed that the spectra did in fact come from adsorbed species.

It is quite clear that the interaction in these four cases must be extremely weak, as shifting in frequency is almost negligible and the selection rules do not break down. Similar results have been reported in the infrared by Sidarov⁴ for some molecules adsorbed onto porous glass. Raman spectroscopy has a great advantage over absorption measurements in that a wide frequency range can be studied easily. At present spectra may be recorded from about $150-3,400 \text{ cm}^{-1}$.

We thank Professor I. R. Beattie for making available the laser Raman facilities, the Science Research Council for providing a grant to purchase the Cary spectrometer and NATO for financial assistance.

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Vinyl Alcohol: a Stable Gas Phase Species?

During a study (unpublished results) using a mass spectrometer of the oxidation of dideuteroacetylene at 280° and 330° C in a 'Pyrex' reaction vessel, which had previously been used in the oxidation of acetylene, the products were found to contain a mixture of perdeuterated and monoprotonated acetaldehyde, acetic acid and methanol. Except in the case of methanol, the concentration of the monoprotonated substance was greater in the initial stages, but as the reaction progressed the proportion of the fully deuterated compound increased (Fig. 1) The 4 per cent C2HD impurity cannot account for the hydrogen atoms which must therefore have originated on the walls of the reaction vessel, either as a part of a polymeric substance or in the form of absorbed water. the latter source being favoured because water is an important product of the oxidation and, as the reaction proceeds, \tilde{D}_2O will be absorbed on the walls and cause the increase in the proportion of fully deuterated products. To test this, water was added to the oxidation of dideuteroacetylene and mass numbers 48, corresponding to CD₃CDO+, and 47, corresponding to C₂D₃HO+, were monitored; in accordance with this suggestion, mass number 48 was suppressed while 47 was, at least initially, unaffected (Fig. 2).

Three possible surface reactions can be considered. (a) Acetylene reacts with water on the surface. Although acetylene and water will react to give acetaldehyde on surfaces1, no acetaldehyde was formed during 1 h from a

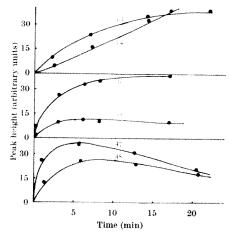


Fig. 1. Formation of isotopically labelled products from the oxidation of C_2D_2 . Temperature, 330° C; pc_2D_3 , 60 mm; po_3 , 100 mm. The mass numbers corresponding to the various curves are given on the diagrams.

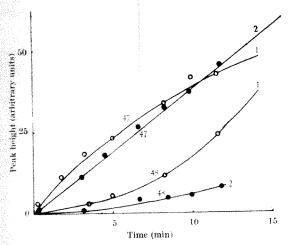


Fig. 2. Effect of the addition of water on the development of mass numbers 47 and 48. Temperature, 280° C; $\mu\text{C}_2\text{D}_2$, 60 mm; μO_2 , 100 mm. Curve 1: no water; curve 2: 20 mm water added.

mixture of acetylene, 100 mm mercury partial pressure, and water, 20 mm mercury partial pressure, at 330° C. (b) Acetaldehyde exchanges with water on the surface. A mixture of acetaldehyde, 100 mm mercury partial pressure, and D₂O, 25 mm mercury partial pressure, was monitored at 280° C for 100 min. Table 1 shows that exchange does take place, but a conversion of less than 10 per cent in this period is too slow to account for our observation.

Tablet

Peak height Mass (arbitrary No. units) at		Chang 45 w	Changei n peak 45 with time		e in peak ith time	Change in peak 30 with time	
45 44 30	zero time 0 52 0	Time (min)	Peak height (arbitrary units)	Time (min)	Peak height (arbitrary units)	Time (min)	Peak height (arbitrary units)
29 16 15	74 0 53	$0\\23.0\\101.5$	0 1·6 5·2	0 20-5 99-0	0 1·5 4·5	$0 \\ 21.5 \\ 100.0$	0 0·1 0·04

The increase in mass number 16 (CDH₂⁺) but not in mass number 30 (CDO⁺) shows that the deuterium atom is incorporated in this methyl group of the acetaldehyde and the most reasonable mechanism for this reaction involves a keto-enol tautomerism (see also ref. 2)

$$\begin{array}{ccc} & & & & \\ & &$$

Mass number 29 (CHO⁺) is unaffected by the reaction. Step (1) must therefore be slow and rate-determining, and step (2) must be rapid, giving a very low stationary concentration of vinyl alcohol. A similar conclusion has been drawn from work on a polar solution³. (c) A precursor of acetaldehyde undergoes exchange during or before conversion to acetaldehyde. It has previously been suggested⁴ that the formation of acetaldehyde in acetylene oxidation is the result of hydrogen abstraction by the radical -CH₂CHO, formed in the sequence

$$C_aH_a + \dot{O}H \longrightarrow H\dot{C} \longrightarrow CH(OH) \longrightarrow C\dot{H}_aCHO$$

If the abstraction reaction occurred only with water absorbed on the vessel surface this would explain our findings but, because 'CH₂CHO is a π -radical, its reaction with oxygen⁵ is likely to be too rapid for it to be the precursor of acetaldehyde in oxygen-rich mixtures. The tautomer, 'DC=CD(OD), from dideuteroacetylene oxidation is, however, a σ -radical with low reactivity towards oxygen⁶ and can abstract a hydrogen atom in the gas phase to give vinyl alcohol which rearranges to acetaldehyde at the walls of the vessel

$$C = C \xrightarrow{D} \xrightarrow{RD} \xrightarrow{D} C = C \xrightarrow{D}$$

The other monoprotonated products (Fig. 1) derive, at least in part, from the further reactions of CHD₂CDO. In Fig. 2 mass number 47 (CHD₂CDO⁴) increases immediately while the rate of production of mass number 48 (CD₃CDO⁺) is autocatalytic, which shows that the formation of D₂O during the oxidation of dideuteroacetylene is necessary for the production of perdeuteroacetaldehyde (this is confirmed by the addition of water). Consequently, none of the vinyl alcohol formed rearranges homogeneously to give acetaldehyde.

Vinyl alcohol is thus capable of a separate existence but tautomerizes rapidly to acetaldehyde whenever the steric and polar conditions are favourable—that is, in a polar solution or on a water coated surface.

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Charge Transfer in Mono-hydroxo and Mono-fluoro Complexes of Tripositive Cations in the First Transition Series

Values of ΔG_{ass} (Table 1) have been measured for the reactions

$$M_{\rm aq}^{3+} + L_{\rm aq}^{-} = M L_{\rm aq}^{2+}$$

where L^- is OH⁻ or F⁻ and ML^{2+} are the hydroxyl complexes of the cations Ti^{3+} to Co^{3+} or the fluoride complexes of Fe³⁺ and Cr³⁺. Here a correlation is demonstrated between a function involving $\Delta G_{\rm ass}$ and $\Delta G_{\rm redox}$ for the overall electron-transfer process

$$M_{\alpha}^{*+} + L_{\alpha\alpha}^{-} = M_{\alpha\alpha}^{2+} + L_{\alpha\alpha} \tag{2}$$

which indicates a charge-transfer structure for the associated species, $M^{3+}L^- \leftrightarrow M^{2+}L$.

The original discussion of Wells¹ when he considered the MOH^{2+} contact complexes referred in a general way to the modification of the 3d electron distribution by electron delocalization, but concentrated on specific factors possibly affecting individual enthalpy and entropy changes. Because the separation of $\Delta G_{\rm ass}$ values into enthalpy and entropy terms, besides requiring a constancy of medium for comparability, and in optical measurements a problematical temperature independence of extinction coefficient, involves in addition appreciable or unassessable experimental uncertainty (for example, Co^{3+} and V^{3+} data¹, respectively), only the sequence of stabilities given by $\Delta G_{\rm ass}$ will be considered here. $\Delta G_{\rm ass}$ for MOH^{2+} is obtained from -RT log K-18.78 kcal, the K values being the hydrolysis constants given by Wells¹ and +18.78 kcal is ΔG for the ionization of water² at unit ionic strength.

Values of $\Delta G_{\rm ass}$ for fluoride complexes are usually much less negative than those for hydroxylation. For Fe²⁺ complexes Wells⁹ has suggested either greater covalency in the hydroxylated species or a contact configuration for the latter as opposed to outer-sphere structures for other complexes (Cl⁻, CO₄²⁻, etc.); and Magnusson¹⁰ has contemplated the possibility of charge transfer between F-and cation in contact. F- and OH- being isoelectronic and possessed of nearly identical radii and crystal fields, we write for both association processes

$$({\rm H_2O})_8 M^{3+} + L^- = ({\rm H_2O})_5 M L^{2+} + {\rm H_2O}$$

Because spectroscopic evidence indicates close equality of the crystal field stabilization of OH- and the replaced OH₂, and tabulated values of the crystal field splittings for OH₂, OH- and F- are usually very close, the symmetry change $O_h \rightarrow C_{iv}$ can be ignored.

Reaction (3) may then be envisaged as occurring in two stages: the removal of the sixth water ligand, then entry of L^- . Assuming a constancy of the M_{q}^{3} entropies, we can approximate the contribution of the first step to $\Delta G_{\rm ass}$ by equating it to minus one-sixth of the hydration enthalpy ΔH_h of each M^{3+} ion. The residual interaction of M^{3+} with L^- then consists of the crystal field stabilization by L^- which we equate (vide supra) to ΔU_{CF} for water, leaving the purely cation-anion integral-charge interaction ΔG_{ch} plus any covalent interaction ΔG_{CT} . Thus

$$\begin{split} \Delta G_{\rm ass} &= -\frac{1}{6}\Delta H_h - \frac{1}{6}|\Delta U_{CF}| + \Delta G_{\rm ass} + \text{constant} \\ \text{where } \Delta G_{\rm ass} &= \Delta G_{ch} + \Delta G_{CT} \\ &= \Delta G_{\rm ass} + \frac{1}{6} \left(\Delta H_h + |\Delta U_{CF}|\right) - \text{constant} \end{split}$$

Values of $\Delta G_{\rm ass}$ (Table 1) can then be interpreted in terms of variations in ΔG_{ch} or ΔG_{CT} or both. In Fig. 1, $\Delta G_{\rm ass}$ is plotted against $\Delta G_{\rm redox}$ for reaction (2). The oxidation potential E° for $OH_{\rm aq}^{\pm}=OH_{\rm aq}+\epsilon$ is given by Latimer^s, and for $F_{\rm aq}=F_{\rm aq}+\epsilon$ was calculated from the tabulation of Latimer by assuming, as for $OH_{\rm aq}$ (ref. 8),

Table 1. Association, hydration and oxidation-potential data for ML^{2+}

		FOR 1	ML^{x+}		
Ref.	∆G 8° 1 to 4	$\begin{array}{c} \Delta U c F^* \\ (M^{3+}, q) \\ 5, 6 \end{array}$	$\frac{AH_{5}+1,000}{(M^{3+}\text{aq})}^{\dagger}$	△G'ass+ constant	$E^{6}(M^{2+})$ volt 7, 8
TiOH ²⁺ VOH ²⁺ CrOH ²⁺ CrF ²⁺ MnOH ²⁺ FeOH ²⁺ FoF ²⁺ CoOH ²⁺	$ \begin{array}{r} -16.0 \\ -15.1 \\ -13.4 \\ -5.9 \\ -18.7 \\ -15.0 \\ -7.05 \\ -16.2 \end{array} $	23·2 35·7 61·2 61·2 36·0 0 22·0	- 325·4 - 359·5 - 405·5 - 405·5 - 402·6 - 372·7 - 372·7 - 427·4	-66.6 -69.1 -70.8 -63.3 -79.8 -77.1 -69.2 -83.8	1·29 0·255 0·41 0·41 -1·51 -0·77 -0·77 -1·84
$E^{0}(OH^{-}/OH)$:	- 2.83 V	E9 (F-/F)	-4.98 V		

Being standard values for 1 mole 1^{-1} , all \overline{AG} have superscript zero implied; even in keal mole⁻¹.

* From spectroscopy.

† The dH_2 are conventional values for $M_g^{3+} + 3H_{sq}^+ = M_{sq}^{3+} + 3/2H_{2(g)}$; 1,000 added for numerical convenience.

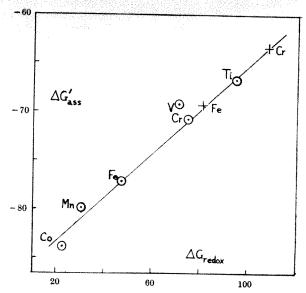


Fig. 1. Correlation between $\Delta G'^{ass}$, the standard free energy of association less water-displacement and crystal-field terms, and $\Delta G_{\rm red}$ for complete electron transfer (in kcal mole⁻¹). \bigcirc , Hydroxyl complexes; +, fluoride complexes.

that the solvation energy of fluorine atoms is small. No attempt has been made to correct $\Delta G_{\rm redox}$ for the juxtaposition neglected in reaction (2).

The approximate linearity in Fig. 1 (slope $\alpha = 0.22$), and especially the collinearity of the MF^{2+} with the MOH^{2+} data, favours a charge transfer contribution ΔG_{CT} as the important variable rather than the simple charge–charge interaction ΔG_{ch} . While current charge-transfer theory¹² does not encompass ΔG values, for structurally similar complexes these values are known often to follow the sequence of spectroscopic energies hv_{CT} which in turn, for M_{kq}^{2+} , bear a 1:1 relation to $-nFE^0$ values^{6,12}. The range of E^0 for Ti to Co represents 72 kcal mole⁻¹, and for the two anions, 33 kcal mole⁻¹.

We emphasize that Fig. 1 represents four essentially independent correlations, as shown in Table 2 where the slope α is calculated separately from each. In particular the last two,

$$\begin{cases} \Delta G_{\rm ass} \ ({\rm FeOH^{2+}}) \ - \ \Delta G_{\rm ass} \ ({\rm FeF^{2+}}) \end{cases} \div \\ \{ nFE^0 \ ({\rm F}) \ - \ nFE^0 \ ({\rm OH}) \} \ {\rm and} \\ \{ \Delta G_{\rm ass} \ ({\rm CrOH^{2+}}) \ - \ \Delta G_{\rm ass} \ ({\rm CrF^{2+}}) \} \div \\ \{ nFE^0 \ ({\rm F}) \ - \ nFE^0 \ ({\rm OH}) \}.$$

are independent of any assumptions about hydration of the cations, requiring only that ΔG_{ch} for F- and OH-, and likewise ΔU_{CF} , be approximately equal for interaction with any one cation. Differences between hydroxylation and fluoride complex formation thus seem entirely attributable to the different ionization energies of OH_{aq}^- and F_{aq}^- .

Table 2. SLOPE a FROM INDEPENDENT CORRELATIONS

Data for	Treatment	Dependence on	Slope a
MOH2+	Corrected for hydration		0.24
CrF ²⁺ and FeF ²⁺ CrOH ² and CrF ²⁺	Hydration "correction	$E^0(M^{2+})$	0.21°
FeOH2+ and FeF2+	cancels out	E'(OH-) and E'(F-)	0.22

This interpretation is not immediately applicable to Cl-, Br- or I- complexes because of differences (compare with L- above) in radii, crystal fields and repulsion

constants. Jorgensen's f and g parameters¹¹ might assist here in assessing the crystal field splittings.

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Chemical Degradation of DNA oxidized by Permanganate

WHEN DNA is treated with potassium permanganate at 37° C and pH 9 for 19 h, the thymine, cytosine and guanine residues are oxidized, but the adenine residues are unaffected^{1,2}. The product (ODNA) is obtained in a high yield as a polymer in which there has been little degradation of the phosphodiester backbone of the DNA. Analytical data and the results of experiments on model compounds2-5 indicated that the thymine, cytosine and guanine residues had been oxidized to ureido residues. In view of the apparent lability of ureido sugars to alkali^{6,7} it seemed that the ODNA should be degraded by alkali to give oligonucleotides containing adenine. Previous work in which ODNA was degraded with alkali or with hydrazine followed by alkali seemed to support this, and from these results values for the distribution of adenine in DNA from a number of sources were obtained8,9. Reinvestigation of these reactions, and examination of other reactions of ODNA, however, have shown that the structure given before and the results quoted for the adenine distributions of the various samples of DNA were not correct.

Thus when ODNA was treated with the diphenylamineformic acid reagent introduced by Burton (2 per cent diphenylamine in 67 per cent formic acid at 30° C for 18 h)10, instead of the complete liberation of phosphorus as inorganic phosphate that was expected, there was only a 50 per cent liberation even after 3 days and then no further increase.

The reaction mixture which had been treated with the Burton reagent at 37° C for 4 days was fractionated on DEAE-cellulose and was shown to contain a number of non-ultraviolet absorbing organic phosphates. These were not obtained pure and were not completely They were, however, derivatives of characterized. 2-deexyribose glycosidically bound to a nitrogenous They did not give the typical yellow colour with Ehrlich's reagent, characteristic of urea derivatives, but they did give urea on strong acid hydrolysis.

These results indicate that ODNA contains both acid labile and acid stable groups. The former would include the adenine residues and probably urea residues; the nature of the latter is unknown.

The action of normal alkali on ODNA at 100° C for 1 h was studied in conditions similar to those used earlier2,8 but on a larger scale (800 mg of ODNA). The reaction mixture was fractionated on a column of DEAE-cellulose using a gradient of ammonium bicarbonate, as previously described2. Fifteen fractions were obtained, ten of which were identified as oligonucleotides containing adenine. Their position of elution from the column indicated that

there was present a series of oligonucleotides of increasing chain length of the general formula (nucleoside), (phos-Four of these ten fractions were further $phorus)_{n+1}$. fractionated by paper chromatography in propan-1-ol: ammonia (d, 0.88): water (11:2:7). (In this solvent the oligonucleotides had much higher mobilities than in the propan-2-ol: ammonia (d, 0.88): water (30:3:15) used previously and much better separations were obtained.) From each fraction several components containing adenine were obtained, but in each case one of the components was present in considerable excess. (In the range 60-67 per cent in the case of the di-, tri- and tetra-nucleotide fractions and 86 per cent in the case of the mononucleotide Measurement of the adenine to phosphorus fraction.) ratios of these components showed, however, that they were less than the theoretical values for components of the type A_nP_{n+1} (0.37, 0.53, 0.52, 0.65 compared with the theoretical values of 0.50, 0.67, 0.75, 0.80 for mono, di-, tri- and tetra-nucleotides, respectively).

The degradation of ODNA with hydrazine followed by treatment with benzaldehyde¹¹ and then alkali was similarly studied and in this case it was also apparent that the degradation was not specific, as shown by the hetero-

geneity of the oligonucleotide fractions.

These results show that ODNA is not specifically degraded to oligonucleotides containing adenine as previous results have implied. This discrepancy can be explained by the fact that the previous work was carried out on a smaller scale and with a different solvent system in which the heterogeneity of the fractions from the DEAE-cellulose column was not detected. The adenine to phosphorus ratios determined in the previous work were too high and this has been traced to a faulty sample of inorganic phosphate used to make standard phosphate solutions. The values given for the amounts of adenine in fractions of 2, 3, 4 and 5 units8,9 in the various samples of DNA are therefore too high.

The inorganic phosphate released by treatment of ODNA with normal alkali at 100° C for 1 h was 28 per cent compared with 54 per cent which would be expected if the degradation gave only oligonucleotides of the type A_nP_{n+1} (assuming a random distribution of nucleotides) and inorganic phosphate. In addition, a non-nucleotide organic phosphate identical with that detected in alkaline hydrolysates of apurinic acid and apyrimidinic acid (ref. 12 and unpublished results of Brammer, Jones, Mian and Walker) was also produced, but amounted to only 7 per cent of the total phosphorus. The fact that the total nonnucleotide phosphorus was only 35 per cent of the ODNA phosphorus also indicated that the alkaline degradation was incomplete.

Attempts have been made to obtain a complete and specific breakdown of ODNA. More drastic treatment with alkali could not be used because of the relative alkali lability of the adenine glycosidic linkage and of the adenine ring in 9-substituted adenines13. In order to try to avoid this, ODNA was treated with N-potassium sulphide (pH 12.5) at 100° C for 5 h. There was more degradation (52 per cent liberation of inorganic phosphate) than with normal alkali for 1 h, but 22 per cent of the adenine of the ODNA was liberated as free base and another 26 per cent was destroyed.

Deoxyinosine has been shown to be stable to alkali¹³, so that it was of interest to examine the action of alkali on deaminated ODNA. The deamination was carried out at pH 4·3 at room temperature for 17 h14. The deaminated product was treated with normal potassium hydroxide at 100° C for 7 h by which time the liberation of inorganic phosphate remained constant at 49 per cent. The products were fractionated on DEAE-cellulose and then by paper chromatography. Although no hypoxanthine and only a trace of deoxyinosine were detected, the oligonucleotides containing hypoxanthine obtained were found to be heterogeneous. This, and the fact that the release of inorganic phosphate was only 49 per cent, showed that in this case also a specific degradation was not

It therefore seems that ODNA contains both acid-stable and alkali-stable residues but it is not known whether the same residues are involved in each case. In this connexion it may be noted that when thymidine-5'-phosphate was oxidized with osmium tetroxide it was necessary to treat it with alkali before the phosphate was liberated as inorganic phosphate on treatment with the Burton reagent and that in the case of deoxycytidine-5'-phosphate there was incomplete liberation of inorganic phosphate even in these conditions15

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BIOLOGY

Odour in Mice

LABORATORY mice sometimes produce a characteristic mousy smell, which on occasion can be obtrusive and unpleasant. This smell comes from the secretion of the preputial glands in the male, and its purpose is to mark territory or establish dominance. Certain conditions will provoke this secretion, among them putting the mice into a clean cage, which has to be promptly marked by the males. Thus, paradoxically, frequent cage changing will result in a smellier mouse room than one in which cages are changed only once in 2, 3 or 4 weeks. is perfectly compatible with health and hygiene if a suitably absorbent bedding material is used.

Recently, some surplus male mice had to be cutled from our colony, and they were put temporarily into an empty. clean polypropylene mouse cage before being taken to the killing chamber. After some minutes, there was a little fighting, but, much more noticeable, a very strong smell was produced. Our colony of some thousands of mice is normally almost free of smell, but these agitated male mice quickly made the room almost intolerable.

It is suggested that if a mouse colony is unpleasantly smelly, the remedy is not to increase the frequency of cage changing, but to examine the husbandry, and correct whatever faults are undoubtedly present.

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Synergistic Effect of Two Stimulants to induce Probing in Stomoxys calcitrans (L.)

INVESTIGATIONS into the efficacy of mammalian skin secretions and their decomposition products, and of blood as stimulants of the probing response in blood sucking Diptera (other than mosquitoes) have produced confusing and often apparently contradictory results1-6. Hopkins4 used hungry, water-sated Stomoxys calcitrans in tests to determine possible chemical stimulants of probing, and obtained a positive response to conditions of high relative humidity and to ammonia evolved from aqueous solutions. This response to ammonia was observed in conditions of high humidity and did not occur when ammonia was evolved from dry ammonium carbonate. Hopkins, however, states that ammonia evolved from aqueous solution retains its stimulatory effect at low humidities. Measurements of relative humidity or of ammonia concentration were not made, although calculations of ammonia concentrations based on partial pressure data were given.

In the course of a detailed study of the physical and chemical factors which control feeding behaviour in Stomoxys calcitrans, I have obtained results which clarify the roles of ammonia and relative humidity in the initiation of probing. Hungry, water-sated flies were subjected to air, the temperature, relative humidity and content of any olfactory stimulant of which were precisely controlled. Dry air which had been filtered through charcoal was conditioned in a large reservoir; the required ammonia concentrations were achieved by injection and evaporation of calculated quantities of 0.880 ammonia solution. The humidity was then adjusted by injection of distilled water and the temperature was stabilized by drawing the air through glass coils which were immersed in a constant temperature water bath.

With this technique I found that ammonia did not induce probing at a series of concentrations from 50 µg/l.-2 mg/l. at 65-70 per cent relative humidity. These concentrations of ammonia also failed to elicit probing at relative humidities ranging from 25-85 per cent when the flies were conditioned to the relative humidity in question. Flies which were, however, conditioned to any level of humidity within this range, and which were then subjected to a sudden increase in relative humidity, probed in the absence of any olfactory stimulant. Adaptation to the new level of humidity was extremely rapid and the response usually disappeared completely within 30 sec.

I carried out an experiment in a constant temperature room at 25° C and 50 per cent relative humidity to determine the effect of the presence of ammonia on this response to an increase in relative humidity. Groups of forty flies which had been starved for 24 h and deprived of water for 1-3 h were subjected individually to increases in relative humidity from the ambient 50 per cent, to 65, 75, 85, 95 and 100 per cent relative humidity, both in clean air and in air containing 1.4 mg/l. of ammonia (shown in preliminary experiments to be the optimal concentration). The flies were observed for 1 min after the rise in humidity and the results are shown in Fig. 1. There is clearly a synergistic effect between the two stimuli in the induction of probing. The nature of the synergy is unknown. Association between ammonia and adsorbed water molecules may result in the stimulation of a single receptor site. Alternatively, two types of receptor may be involved, and the synergy could be a central nervous system phenomenon. Further work is required to resolve this problem.

An insect approaching the skin of a live host would be subjected to an increase in humidity and to a series of olfactory stimuli, including ammonia. The concentrations of ammonia shown to be effective in these experiments are very much higher, however, than could be expected to result from the decomposition of skin secretions on the host's surface. It is thus possible that in the presence of

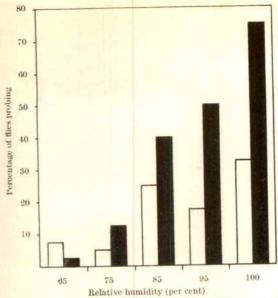


Fig. 1. Percentage probing responses of Stomoxys calcitrans. Flies were conditioned to 50 per cent relative humidity and probing was observed in response to rapid increases in relative humidity to five levels, in the presence (■) and in the absence (□) of 1·4 mg/l. ammonia.

other chemical and physical stimuli on the host's body surface, ammonia influences the probing response at much lower concentrations.

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Identification of the Pokeweed Mitogen in Africa

In 1960, Nowell¹ first described a plant lectin from Phaseolus vulgaris—phytohaemagglutinin, which sessed mitogenic activity for human peripheral blood lymphocytes, that is, the ability to transform the resting lymphocyte into blast like cells. Subsequently, a second phytomitogen was reported in saline extracts of the plant Phytolacca americana—the pokeweed mitogen. In recent studies in our laboratory^{3,4}, we identified and isolated pokewood mitogen in an electrophoretically homogeneous form by preparative multiphase zone electrophoresis on polyacrylamide gel columns and we have shown that it possesses haemagglutinating, leukagglutinating and mito-Further chemical characterization⁵ regenic activity. vealed that pokeweed mitogen is a glycoprotein with a molecular weight of 32,000 containing 3.2 per cent monosaccharide, 1.4 per cent hexosamine and unique aminoacid composition with thirty-three cystine residues. In addition, biochemical, physiochemical and immunochemical studies revealed that pokeweed mitogen differed from p'aytohaemagglutinin in a number of important ways4,5.

The plant species Phytolacca americana—pokeweed a perennial, seems to be homogeneous in the United States and Europe*. In a recent botanical survey of the genus Phytolacca, we have isolated and identified the pokeweed mitogen from plants from various parts of the United States and have demonstrated that it is present in all parts of the plant with the highest concentration in the root. Of special interest immunologically is the effect of pokeweed mitogen on the circulating lymphocyte. Transformation of resting lymphocytes by pokeweed mitogen in vitro produces a spectrum of blast-like cells, predominant among which are cells bearing fine structural resemblance to the phytohaemagglutiniu transformed cell and a distinctive cell type with the cytoarchitectural features of the plasmablast or immunoblast7. In addition, Barker et al. 8 have recently called attention to the specific haematological effects of pokeweed mitogen in a study of twenty-six children who sustained systemic exposure to pokeberries. These children developed a blood picture characterized by an absolute plasmacytosis, eosinophilia and platelet phagocytosis without other evidence of systemic disease.

In the light of the specific role and interrelationship of lymphocytes, plasma cells and precursor blasts in immunological, lymphoproliferative and neoplastic states, the observation that pokeweed mitogen induces distinctive morphological changes of immuno-competent cells both in vivo and in vitro may be of considerable biological importance. Recently, in a comparative electron microscopic analysis of the AL-1 and EB-2 Burkitt lymphoma cell lines, Douglas, Borjeson and Chessin's have reported that in both established cell lines, there is a spectrum of blast-like cells ranging in type from those which display a well developed Golgi apparatus, non-aggregated ribosomes and sparse endoplasmic reticulum to cells containing numerous aggregated ribosomes and an extensively developed rough surface endoplasmic reticulum with dilated cisternae. Furthermore, in both Burkitt cell lines, cell types are present which bear resemblance to the phytomitogen (phytohaemagglutinin, pokeweed mitogen) transformed blast cells. It was therefore of considerable interest to us to investigate the presence of mitogenic activity (pokeweed mitogen) in Phytolacca dodecandra (African pokeweed) especially in the areas in Africa where the

Burkitt lymphoma is found.

One of us identified and gathered material of Phytolacca decandra L. (Fig. 1) in the vicinity of Ibadan. Nigeria. Individual specimens of the berry, stem and roots were finely ground, extracted with isotonic saline, lyophilized and isolated by the previously described methods of Borjeson et al.3. The material was analysed by multiphase zone electrophoresis in 13 per cent and 7.5 per cent polyacrylamide gels without urea according to the procedure described by Reisfeld et al.5. For the isolation and characterization of specific bands, duplicate gels of the American and African pokeweed mitogen varieties, electrophoresed in parallel, were fixed with trichloroacetic acid. The R_F value of each band was determined from the relative position of the buffer front marked with brom-phenol blue to that of the protein bands precipitated with TCA. Having determined the RF value of each band, duplicate gels not fixed with TCA were then out into 1 mm sections and the material from each section eluted in 0.01 molar phosphate buffered saline pH 7.4 and assayed for haemagglutinin and mitogen activity by the methods previously described3. The principal biologically active mitogen band with an RF (0.43) value similar to the American pokeweed mitogen was identified on analytical disc electrophoresis (Fig. 2). Additional bands not present in Phytolacca americana were also seen. Further isolation and characterization of this material revealed that this substance is a glycoprotein and has an ultraviolet absorption spectrum with a maximum at 280 mμ and a secondary peak at 290 mμ similar to the American variety. Haemagglutinating and mitogenic assays performed on the African pokeweed mitogen

isolated at an R_F 0.43 revealed that it possessed similar biological activity to the American variety. Immunodiffusion studies in agar gel revealed that the African pokeweed mitogen (saline extract) displayed numerous antigenic determinants in common with the American

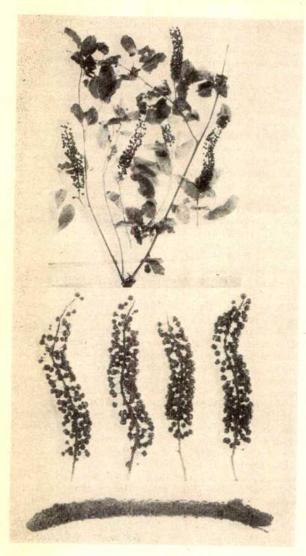


Fig. 1. Specimens of *Phytolacca decandra* L. collected at Ilawo, near Ibadan, western Nigeria. Whole plant seen in upper photograph. Middle photograph is of berries (pokeberry). Lower photograph shows root.

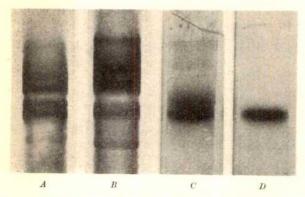


Fig. 2. Analytical disc electrophoresis of American and African pokeweed mitogen stained with Coomasi blue. A, Saline extract of American pokeweed mitogen; B, saline extract of African pokeweed mitogen; B, re-electrophoresis of mitogen band at RF 0-43 (pokeweed mitogen).

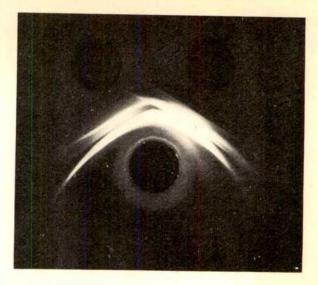


Fig. 3. Double diffusion in agar gel of saline extracts of American (right upper well) and African pokeweed mitogen (left upper well) against rabbit antiserum prepared against American pokeweed mitogen (centre well) displaying numerous common antigenic determinants.

variety using a rabbit antiserum prepared against

American pokeweed mitogen¹ (Fig. 3).

The finding that the Phytolacca dodecandra (African pokeweed) and Phytolacca americana (American pokeweed) possess chemically similar mitogens (pokeweed mitogen) indicates that this plant glycoprotein is common to the genus. Furthermore, the demonstration of a biologically active mitogen in African pokeweed may be of considerable haematological and immunological importance in view of the fact that extracts and preparations of pokeweed have wide medicinal and nutritional applications in Africa10.

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Shell Disease of Ostrea edulis L.

SHELL disease on native oysters in Britain initially became established in Essex¹. It is now well established there and is present in natives in beds at Whitstable and in Cornwall. It has also been found in Clew Bay, Eire (unpublished report by C. Duggan to Irish Fisheries Department). Authors have suggested that oyster shell disease is caused by the presence of a fungus in the shell². No clear descrip-

tion of the organism can be traced.

The pathology of the disease has been frequently described 1.2 and essentially two stages can be recognized. Initially, the disease appears as tiny chalky white specks on the inner surface of the shells. In contrast to the naturally occurring chalky patches, the disease spots have a definite relief, giving the shell in these areas a rough texture. These spots coalesce, and may eventually cover one or both valves. The advanced stage of the disease is recognized as the development of green or brown rubber like spots or warts of conchyolin. Frequently these warts are associated with the attachment of the adductor muscle.

In all oysters examined in this investigation, a fungus has been found present in diseased areas. In preparations of shell in diseased regions the fungus is apparent as a dense network of mycelium. These are of a slight brown tint and quite distinct from the shell boring algae which are present in most shells. The mycelium is 2μ wide, with small ovoid dilations occurring at irregular intervals

(chlamydospores) and measuring $4 \times 6\mu$.

Further detail is only revealed by removing the opaque matrix of shell. The most satisfactory method of decalcification has been found to be the use of the chelate disodium diaminoethylenetetraacetate (EDTA(Na₂))³ which does little structural damage to the shell infesting organisms in question. Diseased shell material decalcified in a 5 per cent solution of sodium EDTA in distilled water leaves only the horny conchyolin warts and a gelatinous substance which is the protein matrix of the shell. Squash preparations of the latter show the presence of fungal hyphae which are hyaline and may be irregularly septate.

It has been suggested^{1,2} that this disease is the same as that known to the French as "maladie du pied". Specimens received from France indicate that shell disease and "maladie du pied" (a variant of which is "Maladie de la charnière") are the same both in the gross nature of pathological effects produced and in the vegetative

structures of the fungus present.

Recently a fungus has been repeatedly isolated from diseased oysters. When grown in liquid media poor in nutrients, the fungus normally remains mycelial, being hyaline, septate in older portions with the characteristic chlamydospores (Fig. 1). These chlamydospores may

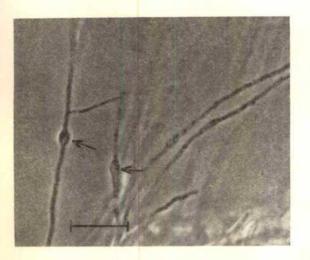


Fig. 1. Fungus from liquid yeast extract peptone medium showing chiamydospores. Phase contrast. Scale equals 10μ .

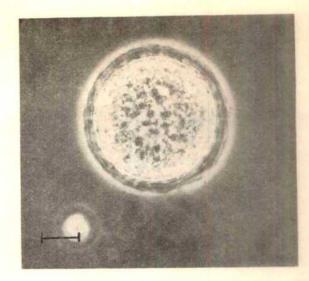


Fig. 2. Mature sporangium showing two-layered wall, the outer layer being thick, gelatinous and laminate. Phase contrast, Scale equals 20μ .

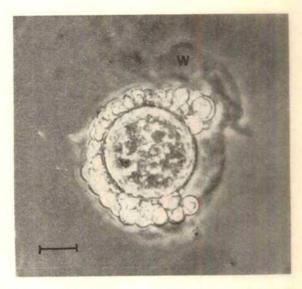


Fig. 3. Zoospore release from sporangium. Ruptured gelatinous layer visible (W). Phase contrast. Scale equals 20μ .

germinate to give rise to spherical sporangia, 30–120µ in diameter, which separate readily from the mycelium, do not have rhizoids and are then free floating. When mature, the sporangial wall is seen to be composed of two layers, the outer layer being gelatinous (Fig. 2). Biflagellate zoospores are formed within the sporangia (Fig. 3) and are diplanetic. The secondary cysts germinate to give rise to sporangia. Once the sporangial state occurs in liquid media (yeast extract peptone) it is then maintained without reversion to the mycelial condition. No sexual stages of this fungus have been seen in the material examined.

This description suggests that this organism should be classified as Phycomycetes, Biflagellatae, Saprolegniales. The eucarpic thallus rules out the families Ectrogellaceae and Haliphthoraceae while the presence of a well developed mycelium and the lack of a rhizoidal system rule out the Thraustochytriaceae. The fungus shares the extensive mycelium characteristic of the Saprolegniaceae, but the zoospores are markedly smaller in size and no sexual stage has been observed.

This seems to be the first time that the fungus which is always found in diseased oysters has been cultured. The epidemic nature of this disease could well be explained by the prolific production of zoospores obtained under suitable conditions.

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Yield Increase from Sorghum Hybrids

In variety trials at thirty sites or seasons in East Africa, the grain yield of the hybrid $H \times 58$ when plotted against the yield of 'Serena', its pollinator parent, showed a linear regression with a coefficient value which did not differ from $b=1\cdot 0$ (ref. 1). This same effect has been obtained from other areas, as shown in Table 1.

The first four entries in the table give comparisons of a good variety with a good hybrid. In each case there is a significant linear regression with a coefficient value which does not differ from b = 1.0. Figure 1 illustrates the results from the United States, and is typical of the graphs obtained from the first three entries of Table 1. Yields obtained from the first three entries of Table 1. of 'Martin' varied widely, from 250 kg/hectare to 7,400 kg/hectare, but in the whole of that range the hybrid gave a constant yield increment: there was no interaction of genotype with environment; the increase from the hybrid was independent of the standard of farming. It would be meaningless to express the increase in yield from the hybrid as a percentage, unless the yield level of the variety were specified. None of the entries in Table 1 gives any support for the idea of slightly increased plant efficiency as a result of heterosis, giving greater expression with good farming than with poor farming.

In the case of the fifth entry in Table 1, the regression indicates a greater benefit from growing the hybrid at high yield levels than at low levels, but the variety 'SB 65' is susceptible to anthracnose, while $H \times 57$ is resistant. In the lush conditions which give high yield the disease is more prevalent and the yield of the 'SB 65' is relatively more depressed. In India the situation seems to be reversed, for the yield advantage of the hybrid becomes less at high varietal yield levels. This can be attributed to differential susceptibility to pests. The hybrid CSH-1 is susceptible to central shoot fly (Atherigona varia) and to the stem borer Chilo4. The local check varieties are resistant, and in the data there are a number of sites where the variety yielded substantially more than the hybrid. This comparison is in any case not as reliable as the others, because the variety is the "local" of the yield tables, differing from place to place. There seems to be no reason to doubt that for hybrids and varieties

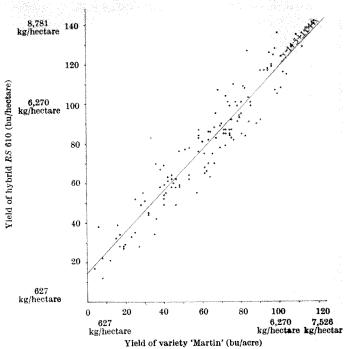


Fig. 1. Regression of yield of hybrid RS 610 on yield of variety 'Martin' in the United States.

with similar levels of resistance to the local pests and diseases, the pattern found in the United States, East Africa and Rhodesia, will also apply to India.

There are obvious practical implications for the relief of hunger: as soon as a hybrid with a yield advantage of 1,000 kg/hectare is available, and it soon will be, and is being grown on 10 million of India's 18 million hectares under sorghum⁵, there will be an additional 10 million tons of grain produced, as long as the fertility is available to produce that amount of grain.

The pattern in the United States supports this view. The mean yield of sorghum for the 3 yr before hybrids were introduced (1954-56) was 1,280 kg/hectare, over 4.6 million hectares. The mean yield for the 3 yr 1962-64 was 2,750 kg/hectare over 4.9 million hectares. About 1,000 kg/hectare of this increase can be ascribed to the hybrids: the remaining 400 kg/hectare or so have probably come from better farming, which would have given the same increase on varieties as on hybrids. Evidently, there must be a lower limit of farming below which the results deduced from the figure cannot apply: all the trials examined have had reasonable treatment; fertility has not been limiting.

The main expression of heterosis for yield in sorghum is in increased grain number, which may be almost doubled relative to the better parent. The small increases in height, stem thickness, leaf area, forage and tiller number could together add up to quite a large increase in photosynthetic area. It seems that in the trials studied, the hybrid floral buds must have produced more florets per hectare than the variety, with a constant increase for a wide range of conditions. The plant factory could

Table 1. RELATIONSHIP BETWEEN HYBRIDS AND VARIETIES

Country	Hybrid	Variety	Mean hybrid yield	Mean variety yield	Increase from hybrid	Regression value	No. of trials
1. East Africa 2. Rhodesia ² 3. 4. United States* 5. East Africa 6. India ³	H × 58 N.K. 300 N.K. 300 RS 610 H × 57 CSH-1	'Serena' 'Framida' 'Red Swazi' 'Martin' 'SB 65' Local check	2,590 5,740 5,740 4,895 2,185 2,460	2,095 8,925 8,560 8,820 1,475 1,835	495 1,815 2,180 1,075 710 625	$\begin{array}{c} 1 \cdot 034 \pm 0 \cdot 086 \\ 1 \cdot 036 \pm 0 \cdot 118 \\ 0 \cdot 926 \pm 0 \cdot 297 \\ 1 \cdot 044 \pm 0 \cdot 034 \\ 1 \cdot 253 \pm 0 \cdot 082 \\ 0 \cdot 663 \pm 0 \cdot 108 \end{array}$	42 32 32 128 81 76

Yields are given in kg/hectare.

* Performance tests were carried out in Kansas, Illinois, Texas, Indiana, Nebraska and Arizona in 1956–65. All reports available at Serere were used.

always fill the extra seeds set in those florets, for a wide range of growing conditions.

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Enzyme Detoxication of 3',4'-Dichloropropionanilide in Rice and Barnyard Grass, a Factor in Herbicide Selectivity

THE herbicide, 3',4'-diehloropropionanilide (DCPA, also known as 'Propanil', 'STAM-F34' and 'DPA'), inhibits photosynthesis and has the remarkable property of selectively destroying barnyard grass (Echinocloa crusgalli) in fields of rice (Oryza sativa L.)2.

DCPA is converted to DCA by soil micro-organisms³ and acylanilide hydrolases of mammalian liver4 and a pseudomonads. We have inferred from these studies, and others less well documented, that similar enzyme destruction might occur in higher plants and that the observed selective toxicity might be attributed to differential rates of destruction in rice and barnyard grass.

We have confirmed our expectations with the discovery that cell free extracts of rice contain a heat labile macromolecule able to catalyse the transformation of DCPA to DCA and other products. Extracts prepared in the same way from barnyard grass were usually devoid of activity or at most had one-twentieth of the activity of rice.

Rice varieties 'Belle Patna', 'Bluebonnet' and 'IR-8' were obtained from the US Department of Agriculture. Barnyard grass seeds, from the same source, were purified to less than 1 per cent contamination by other seeds. All samples of DCPA were given by the Rohm and Haas Company. Seeds were germinated in tap water at 30° C with frequent changes of water for 60 h. Seedlings, grown in 'Vermiculite' in the greenhouse, were collected at the three leaf stage. All aerial parts of these seedlings were collected.

Seeds, germinating seeds and seedlings were frozen in liquid nitrogen and reduced to a fine powder in a Waring The powder was then partially thawed and extracted with 50 mmolar tricine (N-tris-hydroxymethyl methylglycine) buffer at pH 8.5. The extracts were adjusted as required to pH 8.5 with sodium hydroxide, and squeezed through cheesecloth to remove solid debris. Remaining debris and heavy particles were removed by a brief centrifugation at about 500g. Extracts of barnyard grass were concentrated with 'Carbowax 7500' (polyethylene glycol, Union Carbide) in order to obtain detectable levels of activity.

Enzyme assay was performed essentially as described by Kearney⁵ and DCA was determined colorimetrically as described by Pease⁶. Assay mixtures contained 2.5 ml. of buffered extract and 1 µmole of DCPA dissolved in 1 μl. of absolute ethanol. Incubation was at 30° C with slight agitation on a waterbath shaker. For colorimetric

Table 1. FORMATION OF 3,4-DICHLORANILINE IN EXTRACTS OF RICH AND BARNYARD GRASS

Extract		weight g)	μg of l	DCA/h	U/i	nl.	Total fresh	U/g of weight
	Rice	Byg	Rice	Byg	Rice	Byg	Rice	Byg
Seeds* Three leaf	200	200	$52 \cdot 1$	0.95	20.84	0.38	2.5	0.12
plants	283	723	24.5	Low	4.9	*****	18-0	0.51

Byg. Barnyard grass.

* Dry seeds and germinating seeds gave nearly identical values.

† Estimated value obtained from concentrated extract.

readings, samples were partitioned with chloroform and separated by centrifugation. The unit of activity is defined as the amount of enzyme activity producing 6.2 mumoles/h in the conditions described.

A comparison of the activities of extracts of rice and barnyard grass appears in Table 1. We estimate that dry rice seed is able to produce at least 2.5 µg of DCA/h/g of fresh weight and that the three leaf seedling is sufficiently active to produce 10 to 25 µg/h/g of fresh weight.

Our rice preparations contained no intact chloroplasts. but membrane fractions rich in chlorophyll, loosely termed "grana", were prepared by differential centrifugation and proved to be very active. The data presented in Table 2 show most of the activity sediments with the grana. Attempts to solubilize the enzyme with butanol resulted in total inactivation, and sonication at 20,000 c/s for 30 min was without effect. DCA formation by the particulate enzyme was linear with time (Fig. 1).

Table 2. Separation of 3',4'-dopa hydrolase activity from bice by differential centrifugation

Supernatant	Colour	v/ml.	Per cent of initia
1,000g, 5 min	Dark green	4.9	100
2,500g, 5 min	Dark green	5-2	100
10,000g, 15 min	Light green	1.5	30
$10,000g, 60 \min$	Light green	1.6	30*

* The remaining 30 per cent could be reduced to 10 per cent by dialysis followed by centrifugation. This step also sedimented the remaining chlorophyll. The activity removed from the extract was accounted for by the activity recovered from the sedimented fractions.

The products of the reaction were determined by thinlayer chromatography after 2 h incubation of a dialysed. soluble portion of the rice enzyme with ring-labelled ¹⁴C-DCPA. An ether extract of the reaction mixture was transferred to methanol, applied to a silica gel thin-layer plate, and developed in chloroform: methanol: pyridine (100:5:1). The results (Fig. 2) show that DCPA was transformed into six products, numbered on the figure in order of decreasing radioactivity. Quantitation of the radioactivity showed that 23 per cent of the substrate was transformed. DCA accounted for about 13 per cent of the transformed radioactivity and nearly all of the rest remained at the origin.

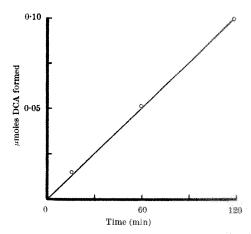


Fig. 1. Formation of 3,4-dichloroaniline in rice extracts. The enzyme was obtained from the particulate fraction. Each point represents the amount of DCA formed from 1 μ mole of DCPA added at zero time.

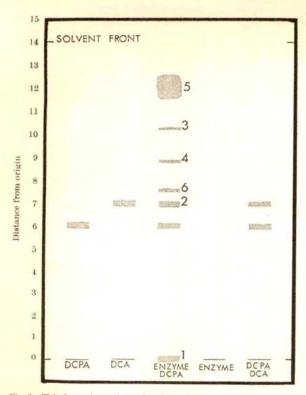


Fig. 2. Thin-layer chromatography of transformation products of DCPA formed in rice extracts. The enzyme was obtained from the scluble fraction, concentrated four-fold by carbowax and dialysed against 5 mmolar buffer at pH 8-5. Spots were located under ultraviolet illumistion and quantitated by liquid scintillometry. The enzyme reaction mixture contained 40 μ moles of DCPA at a specific activity of 0-25 μ c./ μ mole. Products 3-6 represent about 0-4 per cent of the total radioactivity.

These results suggest that acylanilide hydrolase is one of several enzymes acting on DCPA or at the end of a catenated set of enzymes; therefore we cannot equate detoxication of the herbicide simply with the formation of DCA. But it is equally clear that acylanilide hydrolase activity is strongly correlated with the resistance of the rice plants to herbicide action.

Detailed investigation of this enzyme will be important not only because of the importance of rice in world food production but also for an elucidation of biochemical mechanisms in a form of selective toxicity.

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Effect of Gibberellic Acid on Genetically Controlled Tumour Formation and Vascularization in Tomato

SEVERAL effects of gibberellic acid on plant growth and differentiation have been recognized 1-4, but there have been no previous reports of any effect on tumorous genotypes in vivo. This communication reports the effect of gibberellic acid on a genetically controlled condition resembling a tumour and on vascular development in a tomato hybrid. The tumour condition appears on the hybrid plants carrying the Frosty spot (Frs) gene from Lycopersicon chilense on the background of L. esculentum variety 'Tiny Tim', and is transmitted as a dominant trait5. Tumours consisting of groups of undifferentiated parenchymatous cells develop along the veins of the leaves, after which the affected tissues become chlorotic and necrotic5,6. Plants with a tumour trait appear dwarfed and have poorly developed vascular tissue, which results in weak stems. The non-tumorous plants, however, are normal and show good vascular development. In the tumorous plants, differentiation of vascular tissue stops after three to six layers of secondary xylem have been formed (Fig. 1A). In contrast to this, the derivatives of cambium in the normal genotype are many and show relatively more lignification in the secondary xylem (Fig. 1B).

We tested for several plant hormones (kinetin, auxin and gibberellic acid), but only the specific effects of

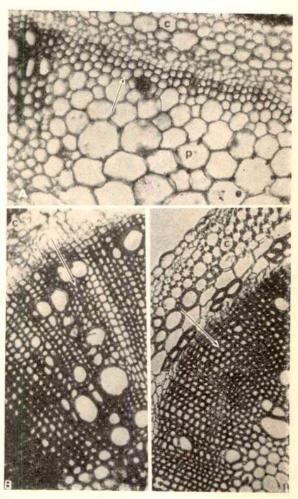


Fig. 1. Transverse sections of tomato stems, $\times 125$. A, Stem from a tumorous plant; B, stem from a non-tumorous plant; C, stem from a tumorous plant sprayed with gibberellic acid. Secondary xylem is indicated by arrows, pith by the letter p and the cortex by c.

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gibberellic acid will be described here. Young plants (2-3 months old) of tumour and non-tumour tomato genotypes were sprayed with gibberellic acid dissolved in distilled water. No wetting agents were used in these experiments. Control plants were sprayed with distilled water. One dose of 2 mg of gibberellic acid in 2 ml. of water was sprayed all over the plant, effectively inducing secondary xylem differentiation, and doses of 10 mg in 10 ml. of water sprayed over a period of 5 days did not recognizably harm the plants. Plants were kept in the greenhouse which was maintained at about 23° C. For anatomical studies, transverse sections were cut with a hand razor at different distances along the stem, stained with safranin and mounted in 50 per cent glycerine.

The tumorous plants which had been sprayed with gibberellic acid elongated rapidly, and during the elongation period the new leaves which emerged after the treatment did not develop tumours. When further elongation ceased, these plants developed tumours on the leaves and continued to do so until they died. The stems of the treated tumour plants were stiffer and sturdier than the untreated tumour plants. The observed effects of gibberellic acid on the non-tumour tomato plants included rapid elongation of the stem (Sachs7 and Paleg8) and earlier stiffening of treated stem compared with untreated non-tumour plants.

Table 1. EFFECT OF GIBBERELLIC ACID ON THE DEVELOPMENT OF SECOND-ARY VASCULAR TISSUE

Genotype and treatment	Diameter of stem in mm serial sections	No. of layers of secondary $xylem \pm S.E.$
Tomour untreated	2·0 2·5 4·0 6·0	$\begin{array}{c} 0 \\ 3\cdot1 & \pm 0\cdot27 \\ 3\cdot8 & \pm 0\cdot19 \\ 6\cdot7 & \pm 0\cdot87 \end{array}$
Tumour sprayed with gibberellic 2 mg (21 days)	acid 2:0 1:8 2:5 3:0	$\begin{array}{c} 6 \cdot 25 \pm 1 \cdot 04 \\ 9 \cdot 6 \ \pm 0 \cdot 27 \\ 16 \cdot 5 \ \pm 0 \cdot 56 \\ 16 \cdot 4 \ \pm 0 \cdot 74 \end{array}$
Non-tumour control	2·0 2·5 3·0 4·0 4·5	$\begin{array}{c} 0\\ 8\cdot 9 & \pm 0\cdot 30\\ 7\cdot 25 \pm 0\cdot 30\\ 15\cdot 7 & \pm 0\cdot 76\\ 26\cdot 2 & \pm 1\cdot 97 \end{array}$

The anatomical analysis of the treated and control tumour and non-tumour plants is presented in Table 1. It is clear that development of secondary xylem proceeds at different rates in the untreated tumour and non-tumour stems. For example, in stems about 4 mm thick, the average number of secondary xylem layers is 3.8 in the tumour genotype (and consists mostly of tracheids or narrow diameter xylem elements), while in the non-tumour stem the number of secondary xylem layers averages 15.7 (and consists of numerous enlarged xylem vessels). The tumour stem was sectioned 21 days after treatment with 2 mg of gibberellic acid, and showed greater vascularization and lignification of vessels than did the untreated stem (Fig. 1C). More than 2 weeks are required before the effects of gibberellic acid are noticeable on vascularization.

The evidence presented here indicates that plants carrying the Frs gene are unable to form an adequate vascular system for self support, and show a growth which resembles a tumour on the leaves. This implies an association between tumorigenesis and vascularization. It was suggested recently that a tumorous condition on tomato leaves probably results from an abnormal auxin metabolism. These abnormalities, associated with the presence of the Frs gene, can be partly overcome by an exogenous supply of gibberellic acid. It seems reasonable to suggest that the Frs gene interferes with, among other things, the metabolism of substances which are similar to gibberellin and which are essential to normal plant growth. There is considerable evidence that auxin (indolyl-3-acetic acid) plays an important part in differentiation of vascular tissue in plants⁹⁻¹¹. Whether exogenous gibberellic acid is involved in the mobilization of the auxin from the leaves to the stem for vascularization, or induces the synthesis of endogenous auxin in the stem, or whether it is directly involved in the induction of secondary xylem differentia-

tion and recovery to normal state, remains to be investigated.

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Antagonism of Tryptamine Stimulated Growth in Apices of Avena Coleoptile by Xylamidine Tosylate

XYLAMIDINE tosylate has been shown to antagonize peripheral actions of 5-hydroxytryptamine in isolated rat uterus and in a variety of in vivo tests with rats1. In studies of the role of tryptamine and other indole compounds in promoting the growth of excised Avena coleoptile apices I have found that xylamidine tosylate is a powerful antagonist of tryptamine stimulated growth in this plant tissue.

The procedure for growing Avena coleoptiles and the methods of experimentation were described earliers Excised apical sections were 10 mm long. Concentrations of xylamidine tosylate of 10-4 molar, and more, markedly inhibit the extension growth of the coleoptile sections. After a 22 h growth period at 25° C coleoptile sections treated with 10^{-4} molar xylamidine to sylate grew 1.7 mm compared with 5.1 mm of growth in the controls. Concentrations of xylamidine tosylate of 10-5 molar and less do not affect linear growth. Apical sections of Avena coleoptiles respond to exogenous indolyl-3-acetic acid (IAA); at 10-3 and 10-6 molar the response is an increase in linear growth. Treatment with xylamidine tosylate at concentrations of 10-5 molar and less does not alter this response in any way (Table 1). The inhibition of growth by xylamidine tosylate at 10⁻⁴ molar is partially relieved by IAA.

The growth of Avena coleoptile apices is also promoted by tryptamines, which, when incubated with Avena colcoptile sections in the presence of xylamidine tosylate, does not promote growth (Table 2).

Table 1. EFFECT OF XYLAMIDINE TOSYLATE ON THE GROWTH RESPONSE OF EXCISED Avena COLEOPTILE APICES IN THE PRESENCE AND ABSENCE OF EXOGENOUS IAA

IAA conc. (moles/l.)	10-4	Xylamidine	tosylate cor 10-*	nc. (moles/l.) 10 ⁻⁷	()
10 ⁻⁶	$3 \cdot 2 \pm 0 \cdot 2$	9·8 ± 0·7	9.8 ± 0.9	9.2 ± 0.6	9·7±0·7
10 ⁻⁶	$3 \cdot 1 \pm 0 \cdot 2$	6·7 ± 0·4	6.9 ± 0.3	6.4 ± 0.4	6·6±0·4
0	$1 \cdot 7 \pm 0 \cdot 1$	5·0 ± 0·4	4.7 ± 0.2	4.7 ± 0.4	4·8±0·3

The increase in growth increment after 22 h of growth at 25° C is expressed in fmm $\,\pm\,$ the standard error of the mean of ten replicates.

Table 2. EFFECT OF XYLAMIDINE TOSYLATE ON TRYPTAMINE STIMULATED GROWTH IN Avena COLEOPTILE APICES

(moles/l,) 10 ⁻¹	10-5 10-6	10-4	
10-6 1.9 ± 0.2 4.9	2 ± 0.4 5.2 ± 0.5 9 ± 0.4 5.4 ± 0.3 0 ± 0.4 5.1 ± 0.4	5.0±04	9-3 ± 0-8 7-1 ± 0-6 76-8 ± 0-4

Growth increment was measured as in Table 1.

Xylamidine tosylate antagonizes the action of tryptamine in plant tissue in a manner analogous to its antagonism of the action of 5-hydroxytryptamine in animal tissues1. Furthermore, the concentrations of xylamidine tosylate which are effective in antagonizing the effect of the indole amines in both plant and animal tissue are smaller than toxic concentrations. In plant tissues, enzymes able to convert tryptamine to IAA have been extensively studied2-4,7, and it is well established that tryptamine is converted to IAA by plant tissues. Inhibitors of amine oxidases have been shown to prevent the formation of IAA from tryptamine^{5,6}, and to inhibit growth stimulated by tryptamine in excised Avena coleoptile apices. The effect of xylamidine tosylate in the experiments with Avena coleoptiles reported here is similar to that of amine oxidase inhibitors; in view of this it is tempting to suggest that, in the coleoptile tissue at least, xylamidine tosylate acts in an analogous if not an identical manner to the amine oxidase inhibitors, by blocking the conversion of tryptamine to IAA. This conclusion is supported by the data of Table 1, where xylamidine tosylate does not affect IAA induced growth.

Whether xylamidine tosylate antagonizes the action of 5-hydroxytryptamine by a similar mechanism is not known. It must be remembered that indoleacetaldehyde is an intermediate in the conversion of tryptamine to IAA² and that xylamidine tosylate could exert its effect either before or after the formation of the aldehyde.

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IMMUNOLOGY

Cell Viability in Leucocyte Cultures stimulated with Phytohaemagglutinin

While investigating the effect of various tissue extracts on the division of cells stimulated with phytohaemagglutinin (PHA) we noticed that in control cultures with PHA there seemed to be a significant increase in the number of non-viable cells. We recorded the number of viable cells and the total number of cells in thirty-eight subsequent experiments. The viability of cells grown with PHA is important in view of previous observations on the cytotoxic action of lymphocytes after exposure to PHA1,2, and the use of PHA in patients with aplastic anaemias3,4. The changes also affect the quantitation of cell responses in culture.

Defibrinated or heparinized venous blood from healthy blood donors was used and leucocyte cultures were prepared from leucocyte rich plasma. The method of culture was essentially that of Moorhead, Nowell, Mellman, Battips and Hungerford⁵ except that 0.3 ml. of PHA was only added to one of a paired culture. Phytohaemag-glutinin was obtained from Burroughs Wellcome and three different batches were used. The amount of nutrient (TC 199) and autologous plasma was the same in both cultures. Lymphocytes were obtained from defibrinated blood by the technique of Coulson and Chalmers using carbonyl iron particles and methyl cellulose. Lymphocytes were also obtained by the method of Rabinowitz7, by passing plasma rich in leucocytes through a column of glass beads; both methods gave 90 per cent or more lymphocytes.

The lymphocytes were cultured in a manner similar to the leucocytes. Cultures were examined at intervals of 3 and 6 days. On each occasion the number of white cells, cell viability, blastic transformation and uptake of tritiated thymidine were assessed in each of the paired Viable cells were assessed as the cells which cultures. excluded dye after exposure to 1 per cent trypan blue for 30 min at 37° C. The number of transformed cells in each culture was recorded while examining 500 cells, and expressed in absolute numbers per cm³ of culture. cells incorporating DNA before division were estimated These were prepared from each by autoradiography. culture; samples of 1 ml. were exposed to 5 µc. of tritiated thymidine (specific activity 4.2 c./mmole) for 1 h. The cells were fixed and spread on glass slides, coated with nuclear gel emulsion (Ilford G 5), exposed in the dark at 4° C for 6 days and then developed. The cells beneath the emulsion were stained and the number of labelled cells was recorded in each cm3 of culture.

In the control cultures of leucocytes or lymphocytes grown alone the degree of blastic transformation was never more than 2 per cent and the uptake of tritiated thymidine was less than 0.5 per cent.

In five out of sixteen 3 day leucocyte cultures there were more white cells when the cultures were stimulated with PHA. The number of non-viable cells was markedly increased in the cultures stimulated with PHA (P < 0.01); in most cultures there were twice as many dead cells as in the corresponding control cultures. The absolute number of transformed cells was correlated with the number of viable cells in the cultures, as was the number taking up tritiated thymidine. There was a considerable scatter of results (Fig. 1) and they are not statistically significant. For blastic transformations $r_{xy} = 0.37$, P > 0.1, n = 12; for uptake of tritiated thymidine $r_{xy} = 0.19, P > 0.1, n = 14.$

In five out of twenty-two 3 day lymphocyte cultures the white cells were increased in the cultures containing PHA, and the number of viable cells was increased in four of these cultures. The number of non-viable cells was significantly increased in the cultures stimulated with PHA, P < 0.001. There was good correlation between the number of viable cells in the stimulated cultures and the degree of blastic transformation (Fig. 1) and uptake of tritiated thymidine, and the results are highly significant. For blastic transformation $r_{xy} = 0.96$, n = 21; for tritiated thymidine $r_{xy} = 0.91$, n = 17; and in both cases P < 0.001.

In half the 6 day leucocyte cultures there were more cells in the cultures treated with PHA than in the controls.

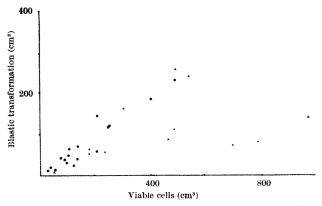


Fig. 1. Relationship of viable cell count to blastic transformation in cultures 3 days after stimulation with PHA. A. Leucocytes; •, lymphocytes.

In seven out of eighteen lymphocyte cultures the number of viable cells was greater in the treated culture. The number of non-viable cells was still significantly more in the PHA cultures. The degree of correlation of uptake of tritiated thymidine with the viable cell count is not as good in the 3 day cultures, but is still statistically significant. For 3 day cultures $r_{xy} = 0.91$, P < 0.001, n = 17; for 6 day cultures $r_{xy} = 0.88$, P < 0.001, n = 15.

The difficulties of evaluating changes occurring in cell cultures especially in the presence of PHA are well known. Nevertheless, with care in sampling the findings reflect the happenings in the culture. The results we obtained repeatedly and significantly indicate that the PHA used in our cultures had a marked cytotoxic effect on the growing cells. This effect was observed in both leucocyte and lymphocyte cultures, and the numbers of non-viable cells were greater in the leucocyte cultures. The non-viable cells were of varying sizes although large round cells seemed to be the most prominent cell in our 3 day cultures; very few granular cells remained.

We do not know yet whether PHA is equally toxic to cells from all parts of the body or whether this effect occurs only in vitro. Sarkanys has demonstrated a toxic effect on human embryo liver cells in vitro, and we have observed increased cell death in splenic cell cultures. The cytotoxic effect of lymphocytes found after exposure to PHA² may be a product of lymphocyte death rather than an immune reaction. How these findings relate to the use of PHA in vivo is hard to see. Experimental evidence suggests that PHA administered intravenously to mice after irradiation produces a reduction in the number of peripheral blood and marrow cells.

The close correlation between the viable cell count and the degree of activity in the lymphocyte cultures suggests that the changes induced by PHA are independent of the number of white cells present in culture for a wide range of numbers. This does not hold true for the leucocyte cultures, but the differences in these cultures are probably related to the varying numbers of granular cells.

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Response of Lymphocytes to Penicillin: Comparison with Skin Tests and Circulating Antibodies in Man

The peripheral blood lymphocytes of man respond to a variety of stimulants in tissue culture, and undergo changes in structure including enlargement and mitosis. Non-specific stimuli such as phytohaemagglutinin, as well as specific antigens such as tuberculin, to which the cell donor has been sensitized, may evoke these changes1,2.

Although the lymphocyte stimulation test has been described as a measure of sensitization to penicillin2, this test has not been correlated with any other immunological parameters of penicillin sensitivity. An extremely sensitive haemagglutination technique has been developed which is

capable of detecting as little as $10^{-4} \ \mu g/ml$. of IgG penionloyl specific antibody in human sera3. Using this test, antibodies of 19S (IgM) and 7S (IgG) molecular classes have been detected in all of fifty-four patients completing recent therapy with penicillin, although the significance of these antibodies is not known³. On the other hand, antibodies which sensitize skin and are detected by direct skin tests and passive transfer tests have been implicated in the pathogenesis of anaphylactic reactions to penicillin. In more than twenty patients with anaphylactic reactions to penicillin, skin-sensitizing antibody was detected when tests with penicillin or its degradation products were carried out shortly after the reaction had subsided4-4. In contrast to the significance of immediate skin test reactions in anaphylaxis, the significance of delayed skin test reactions to penicillin which reach a maximum at 48 h is unknown. Positive delayed skin tests to penicillin have been reported in 5.5 per cent of patients who have not had allergic reactions to the drug7

With such techniques and skin test materials available, we considered that correlation of the in vitro lymphocyte response to penicillin with the presence of measurable anti-penicillin antibodies should be investigated. Twentysix persons were tested for immediate and delayed skin reactivity to penicillin. Blood was drawn simultaneously for haemagglutinating antibody titres and lymphocyte cultures. The subjects formed three groups: (a) fourteen patients with a history of an episode clinically compatible with an allergic reaction following penicillin therapy; (b) nine persons with no history of allergic reactions following penicillin therapy; and (c) three persons who claimed never to have been exposed to therapeutic doses of the drug. Two subjects in the first group were studied on

two occasions at least 3 weeks apart. Skin tests were performed with 0.02 molar penicillin @ (approximately 10,000 U/ml.) and penicilloyl-polylysine, a multivalent hapten in which the carrier molecule is a polylysine of about twenty lysine residues in a molecule

and which carries approximately thirteen penicilloyl The concentration used was 6×10^{-8} molar calculated as penicilloyl. The skin tests were carried out as described earliers, except that commercial solutions of penicilloyl-polylysine were used. Reactions were read after 15 min and 48 h. The haemagglutination test was also performed as described earlier3. Cultures (4 ml.) containing 3×10⁶ peripheral blood lymphocytes were incubated for 5 days in the presence of 100 U of penicillin G. The cellular response was compared with that in negative control cultures containing no penicillin and positive control cultures containing phytohaemagglutinin.

The results are summarized in Tables 1 and 2. Table 1 shows that the ten patients with immediate weal and flare skin test reactions to either penicillin or penicilloylpolylysine all showed a positive lymphocyte response. This was indicated by an increase of at least 5 per cent of reacting cells (large cells plus dividing cells) compared with the percentage of such cells in negative control cultures. No subject with negative immediate and negative delayed skin tests gave a positive lymphocyte response. The cells of one subject (Table 1, patient 10) who had never experienced an adverse reaction to penicillin, but who had a positive immediate skin test, reacted to penicillin in vitro. Only ten of the fifteen subjects listed in Tables 1 and 2 with a history of a reaction following penicillin therapy had positive immediate skin tests and lymphocyte responses. Five of the fifteen gave no objective evidence of penicillin allergy by any of the three tests. The cells of all subjects responded normally to phytohaemagglutinin.

Three of the twenty-six subjects had a delayed skin test reaction to penicillin. In these patients, the delayed skin tests did not show a constant correlation with the in vitro lymphocyte response, in contrast to the usual positive response to tuberculin of cells cultured from subjects demonstrating delayed hypersensitivity to tuber-

culin. Two of the three delayed reactors had negative immediate skin tests and negative lymphocyte responses at the time of the initial tests (Table 2). When re-tested 3 months later one of these two patients showed a marked decrease in the intensity of the delayed skin reaction, and had also developed a positive immediate skin test to penicilloyl-polylysine. Lymphocyte cultures performed at this time showed a stimulatory effect with penicillin. The third patient with delayed sensitivity had a positive lymphocyte response when first seen despite a negative immediate skin test. Five weeks later, after receiving $1\cdot2\times10^6$ U of penicillin, he had developed a strongly positive immediate weal and flare reaction to penicilloyl-polylysine. This patient had had local swelling 48 h after injection of penicillin 1 yr before the initial tests.

One explanation for the association between weal and flare skin test reactions and positive in vitro lymphocyte responses is that peripheral blood lymphocytes may produce skin-sensitizing antibodies in certain patients allergic to penicillin. The one case in which there was a temporal discrepancy between lymphocyte stimulation and skin sensitizing antibody (Table 2, patient 1) may simply represent a greater sensitivity of the culture technique compared with the skin test in detecting the presence of small numbers of antibody-producing cells. It may also be merely a fortuitous association, the development of lymphocyte responsiveness slightly preceding the appearance of skin-sensitizing antibody, but representing an entirely separate immune mechanism.

In preliminary studies, using fluorescent techniques, we have been able to demonstrate IgG, IgM and IgA within lymphocytes from patients with positive immediate skin tests when cells were cultured in the presence of penicillin. These studies also indicate that such cells show specific binding for penicillin. Others⁸ have already demonstrated the production of specific penicilloyl-antibodies by lym-

Table 1. Correlation between in vitro responses of peripheral Lymphocytes, from patients treated with penicillin, to circulating haemagglutinating antihodies and to immediate weal and flare skin tests to penicillin G and penicilloyl-polylysine

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Patient	Type of clinical reaction	Per cent lympho- cyte response*	Skin test positive to	HA Ab	1./titre
1	Serum sickness	21	PPL	198	32
		21	PPL	198	0.2
2	Serum sickness	15	PPL	78	1,024
		8	PPL	7S	1,024
3 4 5 6 7 8 9	Serum sickness	24	PG	198	32
4	Serum sickness	8	PPL	$7\overset{\circ}{S}$	2.048
5	Urticaria	8 7	PPL and PG	78	1,024
6	Urticaria	15	PPL	$\widetilde{7S}$	256
7	Urticaria	î	PPL	198	4
8	Anaphylactic	9	PPL and PG	$7\tilde{S}$	1.024
	Anaphylactic	14	PG	$19\tilde{S}$	64
10	None	14	PPL and PG	7S and 19S	128
11	Urticaria	Õ	Tests neg.	198	32
12	Urticaria	ŏ	Tests neg.	0	64
13	Urticaria	ŏ	Tests neg.	198	256
14	Erythema	ŏ	Tests neg.	198	128
15	Erythema	ŏ	Tests neg.	78	512
	nodosum		Loss neg.	1.5	012
16	None	0	Tests neg.	7S	512
17	None	ě	Tests neg.	7S and $19S$	256
18	None	ŏ	Tests neg.	198	4
19	None	ŏ	Tests neg.	198	64
20	None	ŏ	Tests neg.	198	32
21	None	ŏ	Tests neg.	198	256
22	Normal control	ŏ	Tests neg.	198	64
23	Normal control	ŏ	Tests neg.	198	64
en S		•	T 0000 +10 60+	200	04

Two figures are given when tests were repeated on two separate occasions at least 3 weeks apart. HA Ab, haemagglutinating antibodies; PG, penicillin G (10,000 U/ml.); PPL, penicilloyl-polylysine (6×10-5 molar).

*Increase over controls in percentage of cells showing enlargement or mitosis in the cultures to which penicillin had been added. 0, No response or response less than control value.

Table 2. LYMPHOCYTE RESPONSE AND DELAYED SKIN TESTS TO PENICILLIN

Patient	Date	Clinical reaction	Per cent lymphocyte response	Delayed skin test	Immediate skin test
1	9-1-64	Normal control	0	+	0
2	12-1-64	None	6	+	+
3	1-26-66	Local swelling	Not done	+	ő
	$\begin{array}{c} 2-9-66 \\ 3-1-66 \end{array}$		16	Not done	Not done

phocytes cultured in vitro by use of similar haemagglutination and other techniques.

Although our studies in man indicate a correlation between lymphocyte transformation and immediate hypersensitivity, reference should be made to the recent work of Oppenheim. Wolstencroft and Gell⁸, who showed a relationship between delayed hypersensitivity and lymphocyte transformation in the guinea-pig, in the absence of detectable antibody.

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MICROBIOLOGY

Virus-like Particles in Blood Lymphocytes in Acute Marek's Disease

During a preliminary survey by electron microscopy of blood from eases of acute Marek's disease in chickens, virus-like particles were observed within cytoplasmic vesicles of lymphocytes. The experimental disease was induced by the intraperitoneal inoculation into day old chicks of a commercial broiler breed of heparinized whole blood obtained from cases of acute Marek's disease from a field outbreak in November 1965. Blood was withdrawn from the wing vein of eight of these experimentally injected birds which showed the paralysis or tumours typical of the disease and from one bird which had been similarly injected but showed no signs of involvement. The birds were killed and the diagnosis was confirmed by macroscopic and histological examinations. As control material, blood was obtained from six uninjected and apparently healthy birds of the same breed which had been reared apart from the treated group. Blood was also obtained in a similar manner from two broilers from the Lebanon which were affected with spontaneous acute Marek's disease and from three spontaneous cases of classical Marek's disease occurring in Brown Leghorns from the stock at the Poultry Research Centre. Leucocytes were prepared for electron microscopy by a method based on that of Anderson¹.

Virus-like particles were observed within cytoplasmic vesicles in a small proportion of lymphocytes from five of the eight birds affected with experimental acute Marek's disease and in both the spontaneous cases from the Lebanon. Similar particles have been found in lymphocytes from one of the six uninoculated control birds and in the asymptomatic chicken which had been inoculated with whole blood which subsequently proved to be infective. In the latter two cases, however, not more than one virus particle in each vesicle section was seen,

whereas vesicle sections containing clusters of up to twelve particles were present in lymphocytes from some cases of animals with the experimental disease (Fig. 1). Particles were not found in blood lymphocytes from spontaneous classical Marek's disease. The particles were morphologically similar to the RNA viruses associated with the avian leucosis/sarcoma group of diseases. They were oval, averaged 88 mu in the longest axis and consisted of a dense central nucleoid of about 40 mu diameter surrounded by a double coat of protein. They seemed to be formed by budding from cytoplasmic membranes. have been detected only in lymphocytes; they have not so far been found in a more restricted survey of infiltrated nerves, gonadal and renal tumours.

Acute Marek's disease is highly infectious so that without more elaborate isolation facilities than those available in this experiment, accidental infection of the controls cannot be excluded. The presence of particles in the greater proportion of cases of acute Marek's disease and the greater number of these particles in diseased cases as compared with the controls, however, together with the known infectivity of whole blood in the disease, suggest that the virus particles may be in the condition. On the other hand, the presence of virus particles does not necessarily mean that they are the causal agents; there are several reports in the literature of particles of similar morphology to the avian tumour viruses occurring in tissues from apparently healthy, normal chickens, chicken embryos or tissue cultures which originate from apparently normal embryos2-6. They are usually taken to signify latent infections with avian tumour viruses or related non-pathogenic viruses. Particles of similar architecture have been seen, in the nerve of Remak of an embryo which was negative in the resistance inducing factor (RIF) test. This suggests that they could have been the RIF negative⁸ Marek's disease agent. Considerable evidence has recently been presented indicating the association of a virus resembling herpes with acute Marek's disease. The exact role of this DNA virus and the possible involvement of other viruses in the disease are not yet certain, so that the observation of another virus-like particle in the cytoplasm of intra-vascular lymphocytes from cases of acute Marek's disease is of interest.

Classical Marek's disease has always been regarded as difficult to transmit. Confusion between this condition and lymphoid leucosis undoubtedly invalidates some earlier claims of success, while other positive results may have been with the acute form, which in our experience is certainly not a new manifestation of the disease.

Fig. 1. Electron micrographs showing intracytoplasmic vesicles containing virus-like particles in blood lymphocytes from cases of experimentally transmitted acute Marek's disease.

Transmission of the classical form using material containing intact cells has recently been reported10, but an associated infectious agent has not yet been characterized. It may be that classical and acute Marek's disease are aetiologically distinct.

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Emetine and Blocking Excystment of Schizopyrenus russelli Cysts

Yorke and Adams1 have found that 5 per cent emetine fails to kill the cysts of Entamoeba histolytica in 30 min This communication deals with the effect of emetine in preventing excystment of Schizopyrenus russelli and the cysticidal action of the drug in conjunction with sodium lauryl sulphate.

Cysts of S. russelli produced in monobacterial culture with Escherichia coli were used. Viable sterile cysts, free from bacteria, were obtained by the method of Singh et

al.2. Aqueous extract of E. coli and 2 per cent L-glutamic acid (pH 6.0) were used as excystment agents³

Cysts of S. russelli, treated with emetine (1,000 µg/ml.) together with one of the excystment agents, failed to excyst in 24-48 h. When emetine was removed by washing the cysts in double distilled water, there was nearly 100 per cent excystment in the presence of E. coli extract. There was a progressive increase in the percentage of excystment when concentration of the emetine in the excystment agent was reduced. With 62.5 µg/ml. of emetine, a concentration not amoebicidal for S. russelli4, excystment resembled that of control untreated cysts. Cysts, treated with emetine (1,000 µg/ml.) together with excystment agents, were morphologically normal. These results indicate that emetine blocks the action of excystment agents on the cysts, without affecting their viability. Even the ninhydrin reaction of glutamic acid was inhibited by emetine (1,000 µg/ml.). As Dhar pointed out⁵, there seems to be a weak interaction between emetine and

glutamic acid when they are in solution, even though

they can be separated chromatographically.

Cysts treated with 15.6 µg/ml. of aqueous solution of sodium lauryl sulphate for 48 h readily excysted in extract of E. coli after the removal of sodium lauryl sulphate. When they were treated with the same concentration of sodium lauryl sulphate plus emetine (1,000 µg/ml.) for 48 h and washed with distilled water, there was no excystment in the presence of E. coli extract. Staining with eosin showed the cysts to be dead. The protoplasm of these cysts together with the inner cyst wall was very much shrunken. Thus sodium lauryl sulphate seems to make the cyst wall permeable to emetine. This approach to the curing of dysentery by making the cysts permeable to amoebicides by the use of surfactants has not yet been attempted, but it may be applicable to the elimination of cysts of E. histolytica in human carriers.

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Leukin, a Bactericidal Agent from Rabbit Polymorphonuclear Leucocytes

The term leukin was proposed almost 60 years ago to denote a class of granulocytic antibacterial agents selectively active against Gram positive bacteria1. During the past decade, sufficient progress has been made to indicate the potential significance in host defence of such bactericidal agents. Knowledge of the chemical nature of a potent leukin present in rabbit neutrophiles first emerged in 1956. On the basis of high arginine (17 per cent) and low lysine (4.5 per cent) content, this leukin was considered to be a protamine derivative². It was subsequently shown that bacteria undergoing dissolution within developing inflammatory lesions become progressively coated with a highly basic arginine-positive substance³, which is probably delivered to the bacterial surface during phagocytosis. Leucocytic granules, which contain the bulk of the bactericidal agent4, appear to fuse with phagocytic pouches containing bacteria and to release their contents into these pouches during degranulation^{5,6}. highly localized intracellular delivery system is probably most important because highly basic proteins or polypeptides would be neutralized and rendered less effective in an extracellular milieu. It is interesting that granulocytes from patients with an X-linked granulomatous disease seem to phagocytose normally but do not kill ingested bacteria7. The leucocytic defect is apparently related to improper degranulation rather than to a deficiency in the bactericidal agents.

This communication describes a further purification and characterization of the leukin obtained from rabbit granulocytes. Adult rabbits received intraperitoneally 200 ml. of physiological saline containing 10 µg of endotoxin. Eight hours later, each animal received by the same route 150 ml. of saline containing heparin. The granulocytes, of which 95 per cent were polymorphonuclear, were washed, pooled and subjected to the extraction and purification procedure described earlier2.

Some of the final product (6 mg) was dissolved in 6 ml. of 0.03 molar acetate buffer, pH 4.0, and fractionated on

'Sephadex G-25'. The leukin preparation and the 'Sephadex' fractions of these were adjusted to pH 6.0 or 7.6 before antibacterial assay. Graded amounts of test fractions were added to tubes containing 0.1 ml. of 5 per cent dextrose and 0.4 ml. of a 10-2 dilution of an 18 h broth culture of the desired bacterium (about 10⁶ bacteria). Phosphate buffered saline at pH 6.0 or 7.6 was added to a final volume of 1.5 ml. After 1 h at 37° C. 5 ml. of nutrient broth was added and incubation continued for 10 h. Antibacterial potency was measured photometrically by the capacity of test fractions to inhibit outgrowth of inoculae2.

An agar slant-tube method was also used to assay bactericidal activity. Graded amounts of test fractions were added to 18×150 mm tubes (in triplicate) containing 0.1 ml. each of 5 per cent dextrose and nutrient broth and 0.3 ml. of 2×10^{-6} dilutions from 5 h broth cultures (about 100 bacteria). Buffered saline at pH 6.0 or 7.6 was added to a final volume of 1.0 ml. and the tubes incubated for 1 h at 37° C. A sample of 7 ml. of molten nutrient agar (at 47°-48° C) was pipetted rapidly into each tube. Tubes were immediately positioned at a shallow angle, cooled and incubated at an inclined angle for 16-20 h at 37° C. Colonies were counted over opaque white light to determine the percentage kill as compared with controls.

The bactericidal potency of the leukin at alkaline pH was quite different for the bacterial strains tested (Fig. 1). The concentration of leukin which killed 50 per cent of the Micrococcus aureus inoculum was only one-tenth of that required to kill a similar number of cells of Escherichia coli at pH 7.6. This difference in susceptibility was not obtained at pH 6.0, in which case the susceptibility of

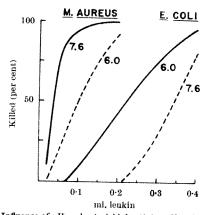


Fig. 1. Influence of pH on bactericidal activity of leukin (0.01 per cent) as observed against M. aureus and E. coli. The agar slant-tube method was used. ——, Optimum pH for activity against each strain.

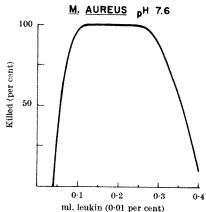


Fig. 2. Reversal of bactericidal activity in excess of leukin as observed in the slant-tube assay.

E. coli approached that of the Gram positive strain. This pH effect was also noted with the 0901 strain of Salmonella typhosa which likewise proved to be more

susceptible to leukin at acid reaction.

In the agar slant tube assay, a type of "prozone" was observed at higher concentrations of leukin (Fig. 2). This inoculum-sparing effect seemingly is related to the particular methodology employed. Because the highly basic leukin is probably fixed onto the bacterial surface, as demonstrated with other basic proteins and polypeptides, the subsequent addition of acidic agar to incubated mixtures may cause a rapid displacement and neutralization of the basic polypeptides. This would permit multiplication of those bacteria not damaged during the previous incubation period.

Fractionation of the leukin preparation on 'Sephadex G-25' resulted in the separation of several peaks (Fig. 3). Each fraction was tested for bactericidal activity and only the mid-region fractions II and III were active (Figs. 4 and 5). Although much inactive material was separated (fraction I_A and I_B), the heterogeneity of the bactericidal fractions is apparent and complements the earlier finding

of electrophoretic heterogeneity2.

The arginine content of fraction II was 26 per cent, determined by the Sakaguchi method¹⁰; this represents a significant enrichment in basicity. The importance of the basic amino-acids, arginine or lysine, as determinants in the antibacterial activity of basic proteins and polypeptides is recognized.

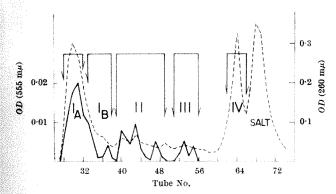


Fig. 3. 'Sephadex' fractionation of leukin. The 86×2.5 cm column contained 95 g of 'Sephadex G-25' equilibrated with saline-acetate buffer, 0-03 molar, pH 4. Elution with buffer at 75 ml/h with a tube volume of 6 ml. (---) Optical density, 260 m μ ; (----) optical density, 555 m μ . Biuret readings were taken on 1 ml. samples.

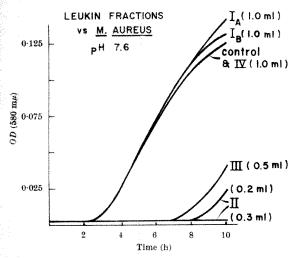


Fig. 4. Antistaphylococcal activity of 'Sephadex' fractions determined by broth outgrowth method. Values in parentheses represent amounts of designated fractions tested separately in each experiment.

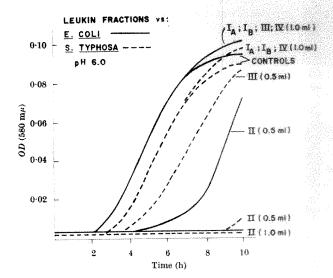


Fig. 5. 'Sephadex' fractions tested against two Gram negative species as measured by inhibition of outgrowth in broth. The values in perentheses represent amounts of designated fractions tested separately in each experiment.

The difference of susceptibility between Gram positive and Gram negative bacteria at alkaline pH suggests that the leukin preparation contains more than one kind of antibacterial agent. The other known antibacterial agents present in polymorphonuclear leucocytes are histone and lysozyme. The low lysine content of leukin and the absence of antibacterial activity in the excluded 'Sephadex' fraction I_A-I_B, argue against the presence of histone. Lysozyme was not detected by standard assay. Thus the leukin preparation appears to contain one type of bactericidal agent, namely the protamine-like proteins or polypeptides originally described².

The relationship of leukin to the bactericidal agent phagocytin¹¹ cannot be ascertained in the absence of chemical data about the latter. The many common descriptive properties of leukin and phagocytin, however, indicate a strong resemblance; they seem to be the same substance. The observation that phagocytin resists pepsin digestion is in keeping with the known resistance of protamines to hydrolysis by many proteolytic enzymes, including

pepsin 12,13

It was suggested initially that leukin derived from the cell nucleus, but the subsequent studies of Cohn and Hirsch' demonstrated that the bactericidal activity originated in the "lysosomal" granules. They found that little or no activity was released from granules at pHs greater than 5. This dependency on acid pH for release of potent bactericidal activity from whole granulocytes was observed in the earlier leukin study.

Seegers and Janoff isolated from granules of the rabbit neutrophil an arginine-rich fraction (MCF) which caused the rupture of rat mast cells¹⁴. The leukin preparation was subjected to 'Sephadex' fractionation as used by these authors and bactericidal activity was eluted in the same region as MCF. A comparison of the known characteristics of leukin and MCF indicates that they are closely related, if not identical. Both agents have high arginine and low lysine contents, behave similarly in gel filtration, show cationic heterogeneity in electrophoresis and are heat stable.

Zeya and Spitznagel have shown that the bactericidal proteins extracted from granules of the guinea-pig neutrophil contain large amounts of lysine as well as arginine¹³. Apparently this antibacterial agent is different from rabbit leukin and more closely related to the arginine rich histones^{13,16}. Studies are in progress to elucidate the structure and mode of action of the bactericidal leukins from rabbit and human granulocytes.

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Atypical Menaquinone Pattern in a Strain of Staphylococcus aureus

KORMAN¹ described the production of coagulase positive and pleiotropic, coagulase negative mutants from a strain of S. aureus, NCTC 8511 (propagating strain 53 of the International Phage Typing series), by ultraviolet irradiation and selection on tellurite glycine agar. Amino-acid, amino sugar and teichoic acid compositions of the cell walls of the Korman parental strain (NCTC 8511) and of the mutants have also been published^{2,3}. We have now determined the menaquinone content of these strains.

Studies on the distribution of menaquinones (MK) in Micrococcaceae4 have revealed characteristic patterns associated with each of the accepted species Staphylococcusaureus, Micrococcus luteus and M. roseus, and the classification of certain strains of Micrococcus proposed by Rosypal et al.5 has also been substantiated by MK patterns. Earlier work has also shown that the parental Korman strain has an MK pattern different from that of any other staphylococcus or micrococcus so far examined. It contains, in addition to other isoprenologues, MK-4 and MK-5, neither of which had previously been extracted from bacteria.

This communication describes a comparison of the MK pattern shown by twenty strains of S. aureus with those of four cultures of S. aureus, NCTC 8511. from different sources, including the Korman parental strain, and four of the strains described as mutants by Korman¹. All strains were grown on nutrient agar plates (blood-agar base: Oxoid, Ltd.) and quantities of between 2 and 5 g wet weight of cells were collected; the MKs were extracted and the isoprenologues separated and identified as described by Jeffries et al.4.

Table I shows the MK patterns (percentage of individual isoprenologues) of the strains studied and, for comparison, the mean and range values obtained with twenty other strains of S. aureus varying in antibiotic sensitivity patterns and bacteriophage types. The MK patterns of the twenty different strains of S. aureus and of three out of four cultures of NCTC 8511 are similar, with MK-8 as the chief and MKs-9 and -7 as the minor isoprenologues. A different and distinct MK pattern was shown by both the Korman parental strain of this organism and the coagulase positive mutant (HS 635) derived from it; on bacteriophage type, however, both these strains were indistinguishable from the three strains of NCTC 8511 from other sources. In a personal communication Dr Korman has stated that she received her culture of NCTC 8511 from Dr M. L. Morse in 1958 and that the mutant HS 635 was produced early in 1959. Both the Korman parental NCTC 8511 and HS 635 now have a common MK pattern, possibly supporting their relationship; this pattern is, however, different from that of the culture NCTC 8511 which we received from Dr Morse in 1967. This suggests that either the MK pattern of the Korman parental strain changed before the mutant HS 635 was produced from it, or that the MK pattern of both strains altered subsequently. The Korman strains were received as agar stab cultures, but we are unaware of either the composition of the media used for their maintenance or the frequency with which they were subcultured before we received them. In our laboratory, the MK pattern of the Korman parental strain has, like those of other staphylococci and micrococci, remained constant after several transfers on Lemco agar.

The coagulase negative strains (HS 968 and HS 1160) contained only MK-8 and MK-7. The isoprenologue ratios were, however, different in the two strains and the MK patterns resembled those previously found in other coagulase negative staphylococci. From HS 968, described as a ccagulase negative mutant derived from the coagulase positive mutant (HS 635), Korman¹ claims to have isolated a single colony of a revertant mutant (HS 1159), which she regarded as identical with the parental strain (NCTC 8511) in all respects apart from pigmentation. Table 1 shows that HS 1159 produced an MK pattern similar to

Table 1. MENAQUINONE PATTERNS OF Staphylococcus STRAINS

Strain and source		No. of	f Total (μg/g s wet wt.)	MK-9	Per cent MK-8		uinones* ual isoprenc MK-6	ologues MK-5	MK-4
S. aureus (20 strains)†	Various bacteriophage types and antibiotic sensitivity patterns	1	243 (93-433)	-	-	13 (5-21)	ND	ND	ND
NCTC 8511 (Asheshov) NCTC 8511 (Baird-Parker) NCTC 8511 (Morse)	Coagulase + ; phage type 53/54/75/77/84/85	$\frac{3}{3}$	$257 \cdot 3 \pm 139 \cdot 2$ $232 \cdot 7 \pm 147 \cdot 7$ $312 \cdot 0 \pm 108$	87 (7 81·0 84·0 73·0	± 7	18·0 ± 4·5 15·0 ± 7 27·0 ± 7	1.0 ± 1.7 1.0 ± 1 ND	ND ND ND	ND ND ND
NCTC 8511 (Korman parental) HS 635 (Korman)	Coagulase +; lactose +, mannitol +, sucrose +, fructose +, galactose +; phage type 53/54/75/77/84/85 Coagulase +; lactose -, mannitol -, suc-	5	98·1 ± 23·7	ND	16·4 ± 1·5	47·4 ± 8·6	11·4 ± 1·5	15·4 ± 5·2	9·4 ± 2·4
<i>HS</i> 968 (Korman)	rose -, fructose -, galactose -; phage type 53/54/75/77/84/85 Coagulase -; lactose +, mannitol +, suc- rose +, fructose +, galactose +; phage	. 2	78·4 ± 37·3	ND	5·0 ± 4·2	$58 \cdot 5 \pm 2 \cdot 8$	22·5 ± 2·1	13·0 ± 5·6	1·0 ± 0
HS 1160 (Korman)	resistant Coagulase - ; mannitol + , sucrose - , fructose + , galactose - ; phage resistant	3	102.7 ± 62.0 17.7 ± 4.5	ND ND	56.0 ± 10 31.0 ± 13	44.0 ± 10 69.0 ± 13	ND ND	ND ND	ND
HS 1159 (Korman revertant)	Coagulase+; lactose+, mannitol+, sucrose+, fructose+, galactose+; phage type 6/47/53/54/75/83A/81		191·0 ± 88·8	62-0	± 4	38·0 ± 4	ND	ND	ND

^{*}MK totals and percentages for the 20 strains of S. aureus are given as means, with ranges in parentheses. The MK totals and percentages for the other strains are the means of replicate assays, with standard deviations in parentheses.

† A further ten strains of S. aureus examined after alkaline digestion gave a similar MK pattern.

MK-8 was the principal isoprenologue in all assays but, because MK-9 and MK-8 were inseparable in 3 out of 13 assays, a single value is given for the combined isoprenologue fraction. In assays where MK-9 was separated it formed a mean 6 per cent of the total MK.
MK, Menaquinone; ND, not detected; NCTC, National Collection of Type Cultures, London, England.

that of most S. aureus strains and therefore different from that of the Korman parental strain. Bacteriophage typing patterns revealed six differences in reactions between the alleged revertant (HS 1159) and the parental strain. Further evidence that the "revertant" is unrelated to, and was therefore probably not derived from, NCTC 8511 is provided by studies in which NCTC 8511 was found to be lysogenic for two phages, both of which lyse HS 1159. Furthermore, HS 1159 carries a phage with a different host range from either of the phages carried by NCTC 8511.

Menaquinone patterns have thus revealed a difference, not detectable by bacteriophage typing, between a strain of S. aureus used in genetical studies and three other cultures of the same strain. The atypical MK pattern of the Korman parental strain, in addition to being different from that of thirty other strains of S. aureus, contained MKs not previously encountered in forty-nine strains of staphylococci and forty-one strains of micrococci. We do not know why the parental strain has shown a changed MK pattern. The MK pattern of the same strain from other sources and those of the other strains of S. aureus have remained stable on subculture, and also after freezedrying. The MK patterns of a wide variety of micrococci have shown similar stability.

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Lack of Resistance of Adult Mice to Erythrocyte-borne **Bittner Virus**

BITTNER virus, the classical mouse mammary tumour virus (MTV), can be detected in bioassays of various organs and tissues of infected mice including mammary tissues and erythrocytes1-3. We have shown that the final result of the administration of MTV-carrying RBC or of mammary tissue extracts to young hosts is identical: namely, the production in test mice which are free from MTV of mammary nodules and tumours containing electron microscopically visible B particles. The MTV in these two tissues differ, however, in several respects^{2,3}. MTV activity in mammary tissues (M-MTV) is itself associated with B particles, but such particles do not seem to be associated with RBC which bear MTV activity (R-MTV). Resistance to tumorigenesis of adult animals following oncogenic viral administration is well known⁴. Several investigators have demonstrated that adult mice are more resistant than young ones to mammary tumour development after administration of MTV^{5,6}. We have recently suggested that this resistance in adult mice of the BALB/c strain is systemically mediated. Dux and Mühlbocks,

however, have proposed that both host age and tissue age are involved in the resistance of older mice to MTV. R-MTV has been shown to precede the appearance of M-MTV in naturally infected mice, and so it was thought possible that a failure in R-MTV production which followed administration of M-MTV to adult mice might account for the low incidence of mammary nodules and tumours. We have now compared the effects of administration of the two forms of MTV to mice of different ages.

In all experiments BALB/cfC3H/Crgl (C+)served as MTV donors and syngeneic MTV free BALB/ cCrgl (C−) female mice of different ages were used to Whole blood and test the effect of R-MTV or M-MTV. washed intact or haemolysed erythrocytes from C+ mice served as the source of R-MTV. The source of M-MTV was cell free extracts of lactating mammary tissues from C+mice. The methods for preparation of R-MTV and M-MTV are similar to those reported previously7,9 except that the diluent contained 0.005 molar magnesium chloride; the latter seems to have a stabilizing effect on MTV. Both R-MTV and M-MTV preparations were administered to C - mice intraperitoneally. The effect of MTV on hosts was determined by the noduligenic assay for the detection of MTV1,10. Data are reported both in terms of percentage hosts with nodules, and incidence of nodules in each nodule-bearing animal (unpublished data indicate that only the former is correlated quantitatively with the dose of virus).

Experiments 1 and 2, summarized in Table 1, show the effect of R-MTV and M-MTV in mammary noduligenesis in immature and mature (8 week and older) C-mice. Two sources of R-MTV were employed in these experiments, and both induced mammary nodules in mice of all ages, the incidence varying between 86 and 100 per cent. Many hosts in most of these groups developed mammary tumours as well. No statistically significant differences were observed in the percentage of test mice with nodules among different age groups. In contrast, M-MTV, which produced a high incidence of nodules in the 3 week old group, was much less effective in the 18 week old group. The percentage of mice with nodules in the older group treated with M-MTV was significantly lower $(P \leq 0.001)$ than that in all other groups in experiments 1 and 2.

Table 1. MAMMARY NODULES IN FEMALE C- MICE INJECTED WITH REC OR LACTATING MAMMARY TISSUE EXTRACTS FROM C+ MICE

		Host*									
Exp No.	Age and sex of tissue donor	Tissue injected	Age in weeks (No. of mice in parentheses)	% with nodules	No. nodules/ host+						
1 <i>a</i>	10 week old female	Intact or haemo- lysed RBC (0·1 ml, RBC/mouse)	3 (12) 8 (7) 25 (5)	92 86 100	44-4± 5:8 43-2± 6:6 48-2± 6:9						
16	20 week old male	Intact RBC (0·1 ml, RBC/mouse)	$\begin{array}{ccc} 8 & (8) \\ 20-25 & (6) \\ 52 & (6) \end{array}$	88 100 100	$\begin{array}{c} 93.4 \pm 17.4 \\ 87.0 \pm 11.1 \\ 87.5 \pm 15.7 \end{array}$						
2	8 month old parous lacta- ting female	Mammary gland extract (25 mg equiv./mouse)	3 (16) 18 (16)	94 19	15-9± 3-6 5-7± 2-8						

* With the exception of one group of 52 week old parous females (in exp. 1b), all hosts were virgin females.
 † Mean ± S.E.M. number of nodules in nodule-bearing bosts.

The experiments reported in Table 2 were designed to determine the presence or absence of R-MTV in mice inoculated with M-MTV. Whole blood from individual donors was collected immediately before and at the end of the assay period and was administered to C- mice. The results (Table 2) clearly show that R-MTV formation results from M-MTV administration in mice of both age groups and that the animals carry infectious virus throughout the assay period.

These results substantiate our previous suggestion 2,3 that R-MTV differs from M-MTV in its biological behaviour. R-MTV can induce mammary nodules in mice of all ages whereas nodules and tumours induced by M-MTV are primarily restricted to mice which are immature, that is, relatively immunologically incompetent11 at the time of virus administration. These results also show that tissue

Table 2. MTV ACTIVITY IN BLOOD OF MICE FROM EXPERIMENT 2 (TABLE 1)

Age of blood		4 weeks after	MTV injection	od from same de	mors was drawn a		MTV injection		
donor at MTV inoculation (weeks)	No. donors* with BBA/No. donors used	No. hosts surviving	% Hosts with nodules	No. of nodules/ host†	No. donors* with BBA/No. donors used	No. hosts surviving	% Hosts with nodules	No. of nodules/ host†	
3 18	15/16 14/15	22 16	91 94	39.7 ± 5.9 50.9 ± 7.1	15/15 15/15	$\frac{21}{22}$	100 86	46·5 ± 5·4 47·5 + 5·9	

*BBA, blood-borne MTV activity; blood from each donor was injected into two hosts at each time period, although not all hosts survived noduligenic treatment. Each host received 0·1 ml. of blood.

† As in Table 1.

Whole blood from individual donors was injected into female C - hosts 3 weeks of age.

age is unimportant in the development of nodules and

tumours resulting from R-MTV administration. Although exogenous R-MTV administration results in nodule and tumour development in adult mice (supposedly immunologically competent), endogenous R-MTV appearing after M-MTV administration was incapable of producing this effect in adult mice. That R-MTV induces nodules in hosts of all ages is perhaps not surprising in view of the recent evidence that the viral nucleic acid of R-MTV could be associated with host H-2 antigens and not with B particle antigens³. The possibility that B particle antigens are lacking in R-MTV is also suggested by the fact that no precipitation reaction with rabbit anti-B particle antiserum is observable when RBC lysates from C + mice are used as the antigen¹². It is thus unlikely that the host immunological defence mechanisms will be operative against R-MTV (coated or associated with host H-2 proteins) which is administered to syngeneic mice of various ages. This explanation, however, cannot account for the low incidence of noduligenesis in M-MTV inoculated adult mice which produce R-MTV soon after the virus administration. A hypothesis can be suggested to explain this enigmatic situation. Because the M-MTV preparations were made from relatively old mice, they are likely to carry both viral (B particles) and associated tumour antigens, and the injection of such extracts has been shown to induce transplantation resistance against C+ mammary tumours in adult C- mice¹¹. Thus it is possible that adult mice injected with mammary tissue extracts are immunized against both these antigens, and their immunity against either or both is involved in the rejection of preneoplastic or neoplastic clones, induced by endogenous R-MTV before these clones have an opportunity to establish themselves. Tumour-specific immunity has been suggested as the reason for resistance to tumour development after infection with oncogenic DNA viruses such as the polyoma virus in adult mice13, and this immunity has also been suggested for one RNA virus-induced tumour system¹⁴. In the mammary tumour system¹⁵, like most other RNA virus systems, it is difficult to determine whether tumour specific or virus specific antigens are involved in resistance, because the virus persists in the tumours. Earlier reports have shown that although adult mice injected with a single dose of MTV extract usually fail to develop tumours, they are nevertheless able to transmit the virus to their progeny in their milk^{5,6}. R-MTV is destroyed by oral administration^{2,3} and these results therefore suggest that the older mice might be producing M-MTV (B particles). Thus antiviral antibodies may not play any significant part in these mice although some adult C- mice injected with repeated doses of MTV extracts are known to produce such antibodies¹⁶. If so, then the relative lack of noduligenesis and tumorigenesis in adult mice (with persistent viraemia) following administration of M-MTV might be the result of immunity against the tumour-specific antigens which were present in the original MTV-carrying mammary tissue extracts. This could explain the presence of viraemia along with immunity against the colonization of preneoplastic or neoplastic foci. Further electron microscopic and transmission experiments to determine the presence or absence of B particles in the mammary tissue of adult mice inoculated with M-MTV are necessary to elucidate the mechanism of resistance of adult mice to MTV-induced noduligenesis and tumorigenesis.

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PATHOLOGY

Establishment of Cell Lines from Peripheral Leucocytes in Infectious Mononucleosis

Cell lines have been established in vitro from peripheral leucocytes taken from patients with leukaemia1-4, lymphomas¹ and malignant melanoma with leukaemoid reaction5. There are also two reports of cell lines derived from pre-sumably normal leucocytes^{6,7}. We have found that in infectious mononucleosis cell lines can readily be established from peripheral leucocytes.

Patients with a provisional diagnosis of infectious mononucleosis on clinical and haematological findings were referred to us from hospitals in Brisbane. Six cases with a positive Paul-Bunnell test⁸ were accepted as infectious mononucleosis (see Table 1); each had fever, sore throat and lymphadenitis and atypical mononuclear cells in the blood.

Heparinized blood was allowed to stand in a vertical tube at room temperature until sufficient plasma separated. Leucocytes from the plasma were washed twice in Eagle's basal medium containing foetal calf serum and other additives and cultivated at an initial concentration of $1.5-5.0 \times 10^6$ /ml. in 5 ml. of medium in either 25 ml. conical flasks¹⁰ or 60 mm plastic tissue culture dishes (Falcon Plastics). Cultures were incubated at 36° C in a humidified atmosphere of 5 per cent carbon dioxide in air. Partial changes were made in the fluid medium twice weekly. The cultures were examined in the living state and were maintained until proliferation was evident or

Table 1. DETAILS OF PATIENTS AND LEUCOCYTE OULTURES

			Date of				Diffe	erential	WBC per	centages Atypical		Date	Initial	Days to onset of	Cell line
Patient	Sex	Age	onset of illness	Spieno- megaly	WBC/ mm³	Neutro- phils	Lympho- cytes	Mono- cytes	Eosino- phils		Other	culture	No. of	definite proliferation	designation
1 2	F M	18 6	12.12.66 25.1.67	- +	8,200 25,000	47 20	50 80	2	0	25 Many	Basophil 1	21.12.66 6.2.67	5×10 ⁴ 5×10 ⁴	33 (Negative at	QIMR-GOE
		-			-				-	-				57 days)	
8	M	12	3.2.67	-	10,500	25	43	18	5	40	Plasma cells	15.2.67	2 × 10 ^s	(Negative at 23 days)	
4	F	23	1.2.67	+	10,300	28	63	5	3	62	Basophil 1	15.2.87	5×10^{6}	(Negative at 48 days)	
5	F	20	1.2.67	-	11,000	33	67	0	0	Many		17.2.67	5×10^4	18 (dish) 27 (flask)	QIMR-STE
0	F	28	17.2.67	+	6,200	33	55	11	0	15	Myelocyte 1	10.3.67	1.5 × 10		QIMR-DAR
W	BC, w	hite b	lood cells.												

until all remaining cells were dead. As soon as the first cell line became established it was clear that in further studies the possibility of contamination with other cell lines already present in the laboratory should be rigorously excluded. Consequently, later cultural procedures were isolated as completely as possible from any other cell culture studies.

The cultures from the six patients were similar in the early stages. Within the first 2 or 3 days many of the cells in suspension died, but some successfully attached and included fibroblastic cells, large undifferentiated cells and smaller irregular cells which sometimes formed small The attached cells usually remained in good condition but were not seen to multiply. Usually after 2-3 weeks of culture, but varying with individual cultures, most smaller attached cells became slightly less irregular and detached from the vessel. In unsuccessful cultures the detached cells degenerated, but in successful cultures some remained viable as shown by very clear cytoplasm and prominent spiky extensions. Such cells were usually present in small groups; definite proliferation in a culture was indicated by unequivocal enlargement of these small aggregates. The period of culture at which this stage was reached is listed in Table 1. Once initiated, proliferation continued actively and the small aggregates of irregular cells increased in number and in size up to about 1 mm.

The three lines established (Table 1) behaved similarly in culture and resembled each other in the appearance of the viable cells; these properties so far appear to be stable. In each line two types of mononuclear cells could be identified in smears stained with Giemsa-primitive cells varying in size from 14 to 23µ with plentiful cytoplasm, and less frequent larger cells (30 to 45µ) with a more reticular nucleus. The cultured cells did not resemble characteristic atypical mononuclear cells of infectious mononucleosis and appeared to be undifferentiated haemopoietic cells.

In the conditions of culture, cell lines were established with relative ease from the blood in infectious mononucleosis; the proportion of cultures successful (3/6) was apparently higher than that obtained with leukaemic blood (2/10) or normal blood (0/13), although the number tested is small. The recent demonstration that the proportion of peripheral mononuclear cells synthesizing DNA is greatly increased in infectious mononucleosis is relevant to the tissue culture results reported here¹¹. It is probably significant that in another laboratory the proportion of successful lymphoblastoid fibroblast cultures from bone marrow was also higher in infectious mononucleosis (13/22) than in leukaemia (6/77, ref. 12). not clear, however, why the lymphoid cells grown from bone marrow in that study were dependent on the fibroblasts in the culture, while our cultures from blood grew freely in suspension.

It is most interesting, particularly because certain basic similarities between infectious mononucleosis and leukaemia have been pointed out13,14, that the lines of cells grown from infectious mononucleosis should bear a general resemblance to those from leukaemia. These results could be interpreted as suggesting similar actiology in infectious mononucleosis and leukaemia. Another possibility is that the two are actiologically distinct yet have in common the presence of cultivable cells in blood. In view of the establishment of cell lines from leucocytes in various neoplastic conditions1 and from apparently normal leucocytes^{6,7}, further studies are necessary to determine the significance of cultivable cells in blood.

Thorough virological examination of the new cell lines is essential, in view of the long suspected viral aetiology of infectious mononucleosis and of the presence of particles resembling herpes virus in several strains of cells derived from cases of leukaemia1,15 and Burkitt lymphoma¹⁶⁻¹⁸ and from non-malignant leucocytes^{6,7}. In tests made so far, no virus has been recovered from the new cell lines by inoculation of various tissue cultures. I thank Dr A. F. Knyvett, Dr A. K. Patel and Dr T. A.

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Tumours induced by Chicken Sarcoma in Monkeys conditioned with Progesterone

REPORTS of skin and organ transplantation, as well as of tumour induction and serial passage in laboratory animals, have casually mentioned that the duration of graft survival is enhanced by pregnancy1-3; more tumours can

be induced in pregnant and adult female than in adult male rodents³. There are examples of experimental animals, unexpectedly found to be pregnant, which have been discarded from the experiment⁴ with this explanation. Furthermore, several workers have shown newborn mammals to be more susceptible to virus infection, and less resistant to skin or organ transplantation, than mature animals of the same species.

We have used monkeys to test the hypothesis that progesterone is directly or indirectly involved in the increased susceptibility of the pregnant, as well as of the newborn mammal. Our first results were encouraging. Eleven out of eleven otherwise resistant juvenile and adult monkeys, conditioned with progesterone and with demonstrated elevated concentrations of progesterone in the blood, developed large fast-growing tumours 1–3 weeks after inoculation of a chicken sarcoma (Carr–Zilber) preparation.

Data on eighteen normal monkeys which failed to develop tumours after inoculation of chicken sarcoma material^{5,6} are summarized in Table 1. Sixty-two out of sixty-five control monkeys conditioned with corticosteroid developed tumours; details have already been published^{5,6}. There was invasion of the rib cage in four monkeys, as well as metastases to the lungs in two others and to the liver in three others. In two of these monkeys, the metastatic lesions caused typical tumours when they were back passaged to chickens.

Table 1. TUMOUR INCIDENCE IN JUVENILE AND ADULT MONKEYS

	No.	Tumour incidence
Normal Adult controls Pregnant	18 2	0 2
Treated Hydrocortisone (single dose) 'Imuran' (5 days)	3 8	0 7
X-ray (155 r) 'Celestone' (daily I.M.)	3 65	3 62
Progesterone (aqueous I.M.) 'Provera' (oral)	8 I	8 1

Recently, eight juveniles (two of which were male) and two adults (five M. mulatta, four M. speciosa and one C. aethiops) were conditioned with daily intramuscular injections of 5 mg/kg of progesterone and then inoculated with the chicken sarcoma material containing 1.5×10^8 ... 12×10^8 infective viral units. All developed tumours at the site of inoculation in 8-15 days and these tumours were histologically fibrosarcomas, with, in addition to the typical fusiform cells characteristic of the tumours in newborn and corticosteroid-conditioned monkeys, large rounded cells with acentric nuclei scattered throughout. Virus was demonstrable in all these tumours by back passage to chickens (Table 2).

Table 2. CHICKEN SARCOMA-INDUCED TUMOURS IN PROGESTERONE CONDITIONED MONKEYS

Preparation	No.	Treatment	Tumours	Latency
Pregnant mulatta Normal adult mulatta	2	None 'Provera' (oral)	8 3	7–15 days 18 days
Speciosa mulatta	3) 5;	Progesterone (I.M.) 5 mg/kg/day	24	7-21 days

Two females of M. mulatta in the third trimester of pregnancy were inoculated with a chicken sarcoma mince containing 20×10^8 and 25×10^8 infective viral units as described before^{5,6}. Each developed three large fast-growing tumours at the sites of inoculation. In the first animal, the three tumours regressed and disappeared 3-4 weeks after biopsy and parturition; one tumour of the second animal was removed 7 days after parturition for histology and for viral recovery examination; at that time, the other tumours had ceased to grow and daily intramuscular injections of progesterone were started. In 14 days the tumours had attained proportions much larger than previously, and a larger tumour recurred at the site of excision (Table 2).

An adult M. mulatta female was given five 10 mg tablets of 'Provera' on each of 2 days before the inoculation of 20×10^8 infective viral units into three sites. Tumours developed at these sites in 5 days after inoculation (Table 2).

In control monkeys receiving daily corticosteroids, the body weight at first reflected a progressive loss, and several months later reached a plateau at a low level, but animals receiving daily progesterone gained weight and maintained these weights. Incisions made for the removal of tumours or for biopsy in the monkeys treated with progesterone healed in half the time required in corticosteroid treated animals.

In ten animals injected or fed large doses of progesterone, there were no respiratory infections, diarrhoea or febrile illnesses. This contrasts with the monkeys conditioned with corticosteroid, of which 25-40 per cent developed one or the other of these conditions. The decrease in the number of lymphocytes in the circulation, seen in the monkeys conditioned with corticosteroid, was also observed in the monkeys conditioned with progesterone.

The concentrations of progesterone and 4-pregnane-20α-ol-3-one in the plasma were measured in some of these conditioned animals by the double isotope dilution technique (personal communication from Gandy) (Table 3). Concentrations of progesterone were 80–230 times greater than in normal male and female monkeys. Values in two pregnant monkeys and an 11 day old infant were considerably less than in treated animals, and were less than those seen at mideycle of non-pregnant adult females.

Monkeys injected or fed daily with progesterone, as well as pregnant monkeys, developed tumour's at the site of inoculation of the Carr-Zilber strain of the Rous chicken sarcoma material. Concentrations of progesterone and one of its isomers were found to be generally greater in the susceptible monkeys than in the resistant monkeys. Progesterone is known to be a precursor of the adrenocorticosteroids, but it is not yet certain whether progesterone acts as an immunodepressant indirectly through the stimulation of adrenocorticoids or directly on some phase of antibody production, release, transport or specific activity. The absence of side effects in these monkeys at the doses used in this experiment, while healing was faster and all the monkeys gained in weight, suggests that progesterone can be preferable to corticosteroids for certain clinical conditions. This substance may control the immune response, for example, in pregnancy (from the maternal as well as from the foetal point of view).

Table 3. PLASMA PROGESTERONE CONCENTRATION IN MONKBYS

.	No.	Progesterone	20a-Hydroxy- progesterone μ g/100 ml.	Dose
Normal		0.15 0.01	0.04.0.08	
Male Female	3	0.17-0.21	0.04-0.07	
Preovulatory	3	0.10-4.0		
Midcycle	$\frac{2}{2}$	7.0-13	0.49-0.63	
Premenses	2	$1 \cdot 23 - 1 \cdot 32$	0.07-0.09	
Infant (11 days)	1	0.30	0.07	
Pregnant Third trimester	2	0.44-0.62	0-21-0-23	
Treated Female	4 1	14·8-26·42 26·94	3·08-10·5 5·63	5 mg/kg
Male	T	20'94	9.09	5 mg/kg

The inadequately explained susceptibility of the pregnant and newborn mammal to pathogens and the weakened resistance to skin grafts, organ transplants and tumour passage now seem to embrace the concept of elevated progesterone concentration in these animals.

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PHYSIOLOGY

Ammonia and the Regulation of Acidity in Human Eccrine Sweat

THE acidity of human eccrine sweat has been reported to be regulated by the lactate system^{1,2}, by amino-acids³ and by the bicarbonate system⁴. We have examined correlations between acidity and electrolytes of eccrine sweat.

Sweat was collected from nine men and two women from January to May 1966, with intervals of one or more weeks between collections from each subject.

On the day before each experiment, the subject cut his fingernails, shaved both arms with an electric razor and washed each arm for 3 min with a surgical brush using tap water and a solid detergent ('Vel', Colgate-Palmolive; this material is neutral in reaction and non-allergenic for our subjects). On the day of the experiment, the subject washed the arms for 3 min with warm tap water and detergent and then rinsed the arms with running, warm tap water.

The hot room was kept at DBT 40° C, WBT 21° C. After walking on a level treadmill for 15 min at 5.6 km/h (men) or 4.8 km/h (women), the subject rinsed both arms with demineralized water. They were dried gently with towels previously boiled in distilled water and oven dried. The subject donned elbow length bags (vinyl plastic wet dressing Bunyan bag, National Carbon Co.) secured with elastic bands 5 cm wide. Evaporative loss of water from inside was 1 per cent/h. The subject again walked on the treadmill for 80 min, stopping every 15 min for 5 min for the drainage of sweat from the bags. Considerable care was taken to obtain "pure sweat", that is, fluid free of particulate matter when viewed at a magnification of 430.

The sweat was centrifuged at 2,980g for 15 min at 25° C. If the supernatant was still turbid, it was frozen, thawed and recentrifuged; if "pure", it was analysed immediately or frozen at -20° C. Repeated freezing and

thawing had no effect on pH or solute concentrations.

Analyses were: sodium and potassium by flame photometry^{5,6}, chloride with Cotlove titrator⁷ (American Instrument Co., Silver Spring, Maryland), lactate by colorimetry⁶, total nitrogen, ammonia and urea by nesslerization after appropriate acid digestion, aeration or treatment with urease^{9,10} and amino-acids by the method of Spackman, Stein and Moore¹¹ (Beckman Co., Pasadena, California). Hydrogen ion concentration at 25° C was measured with a glass electrode^{12,13} (Expandomatic, Beckman) calibrated at pH 4·01 with phthalate buffer and at pH 6·86 with phosphate buffer.

Values for pH ranged from 4.70 to 7.99, with a mean of 6.40 (Table 1). Variation was much less within individuals than between them.

Table 2. Correlations between pH of human ecorine sweat and concentrations of electrolytes (n=33)

Item	Units	Correlation coefficient with pH
NH ₃	m.equiv./l.	0·56
Lactate	mmoles/l.	0·25
K	m.equiv./l.	0·30
Cl	m.equiv./l.	0·40
Na	m.equiv./l.	0·48

The highest coefficient of correlation (-0.56) was found between pH and ammonia (Table 2). Positive correlations were found between pH and sodium, chloride, potassium and lactate. As the acidity increased, the concentration of ammonia increased and those of sodium and chloride decreased.

Other correlations with pH and ammonia were probably autocorrelations. With increasing rates of sweating ammonia concentration decreased, the sweat became more alkaline, and osmolality increased. Thus there was a good correlation among these three measurements.

Although there are substantial differences between individuals, factors controlling the acid-base balance in sweat should appear in all subjects. By analogy with urine, several possible buffer systems might be considered for sweat. These include phosphate¹⁴, lactate¹⁴, bicarbonate⁴, amino-acids³ and ammonia¹⁵.

Inorganic phosphate cannot contribute. One remarkable feature of sweat is that specimens collected as described contain virtually no phosphorus, inorganic, acid hydrolysable or bound.

Lactate is unlikely to be important in the acid-base balance of sweat. Its pK is 3.9, and so its effective buffer range is beyond the range of pH seen in our specimens. Furthermore, lactate showed only a poor correlation with pH.

The bicarbonate system might be operative in the alkaline range, as suggested by Kawata⁴. Seven of thirty-three specimens were alkaline, thus agreeing with Kawata⁴ but not with Foster¹⁵, who stated that human sweat is always acid, cat's paw sweat alkaline. Dissociation curves tor carbon dioxide in the P_{CO₂} range of 20-130 mm of mercury were studied in six specimens. The plots of P_{CO₂} against dissolved CO₂ were linear, not curvilinear as they would be for a bicarbonate system. They ran

Table 1. ELECTROLYTE CONCENTRATIONS AND $p{
m H}$ OF HUMAN ECCRINE SWEAT

					-			
Subjects	No. of specimens	pΉ	Sodium (m.equiv./l.)	Potasslum (m.equiv./l.)	Ammonia (m.equiv./l.)	Chloride (m.equiv./l.)	Lactate (m.equiv/.i.)	Urea (mmoles/l.)
Men E.B. I* E.B. II* J.B. L.D. W.K. J.M. P.Ma. P.Ma. P.Mo. J.N. M.S.	2 2 5 5 1 1 1 2 4 7	6-02 6-88 4-88 ± 0-13 7-36 ± 0-35 6-98 7-71 6-89 6-39 6-61 ± 0-45 5-86 ± 0-67	$ 76 \cdot 0 \\ 46 \cdot 0 \\ 32 \cdot 2 \pm 1 \cdot 7 \\ 61 \cdot 2 \pm 6 \cdot 2 \\ 55 \cdot 0 \\ 99 \cdot 0 \\ 57 \cdot 0 \\ 75 \cdot 0 \\ 113 \cdot 8 \pm 8 \cdot 9 \\ 53 \cdot 6 \pm 9 \cdot 0 $	5·8 5·6 6·1 ± 0·7 8·5 ± 1·4 7·8 6·9 7·7 9·9 9·9 ± 0·7 5·6 ± 0·4	$\begin{array}{c} 2 \cdot 9 \\ 2 \cdot 1 \\ 3 \cdot 8 \pm 0 \cdot 5 \\ 3 \cdot 0 \pm 0 \cdot 3 \\ 2 \cdot 4 \\ 1 \cdot 8 \\ 2 \cdot 7 \\ 5 \cdot 0 \\ 3 \cdot 4 \pm 0 \cdot 8 \\ 3 \cdot 2 \pm 0 \cdot 2 \end{array}$	72·1 43·3 27·2±2·2 52·3±4·4 43·6 91·1 46·1 67·5 111·6±10·0 47·4±7·9	$\begin{array}{c} 12 \cdot 3 \\ 9 \cdot 7 \\ 15 \cdot 1 \pm 1 \cdot 8 \\ 20 \cdot 2 \pm 2 \cdot 1 \\ 19 \cdot 1 \\ 14 \cdot 8 \\ 24 \cdot 3 \\ 23 \cdot 7 \\ 19 \cdot 4 \pm 1 \cdot 7 \\ 14 \cdot 6 \pm 1 \cdot 0 \end{array}$	$12 \cdot 1$ $19 \cdot 1$ $9 \cdot 1 \pm 1 \cdot 7$ $10 \cdot 6 \pm 2 \cdot 4$ $7 \cdot 9$ $4 \cdot 7$ $8 \cdot 6$ $9 \cdot 5$ $8 \cdot 6 \pm 2 \cdot 3$ $12 \cdot 3 \pm 1 \cdot 9$
Women M.B. C.T. Mean	2 1 (n 33)	7·75 6·91 6·40	70·0 63·0 63·8	6·2 5·3 7·1	1·6 2·9 3·1	59·1 62·0 57·6	16·6 10·6 16·6	6·2 7·8 10·4

Means for each subject are given, and standard deviations for four or more specimens. * Series II, before acclimatization. Series II, after acclimatization.

Table 3. AMINO-ACIDS IN SWEAT REPORTED IN THREE DIFFERENT PAPERS RANKED IN ORDER OF HIGHEST CONCENTRATION

Amino-acid	Morimoto and Johnson*	Hadorn, Hanimann Anders, Curtius and Halverson†	Coltman, Rowe and Atwell ‡
Citrulline	*****	16	1
Serine	1	1	2
Glutamic acid	- man	7	3
Aspartic acid	6	waterna	4
Arginine	11	17	5
Threonine	4	5	G
Alanine	2	4	1 2 3 4 5 6 7 8 9
Leucine	8	10	8
Glycine	4 2 8 3 5	2	9
Histidine	5	2 6 3	10
Ornithine		3	11
Lysine	-	14	12
Valine	7	9	13
Phenylalanine	12	15	14
Tyrosine	10	18	15
Proline	13	11	16
Tryptophan		19	17
Taurine	********	when any	18
Hydroxyproline	Minustern.	house.	19
Methionine	14	18	20
Isoleucine	9	12	-
Asparagine		8	
Cystine	*****	20	*******

*Stimulus: exercise and heat.
Collection: impermeable bag on arm.
Analysis: column chromatography. † Stimulus: none, subject at rest.
Collection: impermeable bag on hand.
Analysis: column chromatography.
† Stimulus: iontophoresis of pilocarpine.
Collection: methylcellulose sponge.
Analysis: high voltage paper electrophoresis.

parallel with but about 2 mmoles of $\rm CO_2/l.$ higher than similar points for water. Carbamino compounds could account for this difference.

We confirm reports by others that human sweat contains free amino-acids¹⁶, 18. Twenty-three different aminoacids have been detected (Table 3). In order of highest concentration Coltman, Rowe and Atwell¹⁷ listed citrulline, serine and glycine. Hadorn et al.18 found the order to be serine, glycine, ornithine and alanine. We found three amino-acids above 0.4 mmoles/l.: serine, alanine and glycine. Titration curves of sweat concentrated ten-fold by freeze drying in vacuo18 had inflexion points at pH 2.6, 3.2, 3.4, 3.7, 3.8, 4.2, 4.5, 9.4, 10.4 and 11.5. Some of these might be associated with amino-acids. human sweat regularly contains amino-acids in concentrations great enough to affect the acid-base balance.

The correlation between ammonia and acidity was good in thirty-six specimens as shown by the regression equation for Fig. 1. With a pK of 4.8 the ammonium-ammonia buffer system has an effective range of about pH 3.8-5.8. Our highest concentrations of ammonia were from 5 to 8 m.equiv./l., occurring at pH values of less than 6. At larger pHs the concentration of ammonia was usually 3 m.equiv./l. or less. We accept the view20 that, like the kidney, the sweat gland can synthesize ammonia.

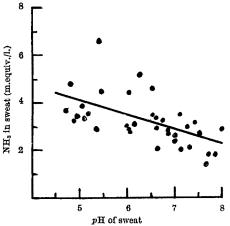


Fig. 1. The total ammonia concentration of thirty-six specimens of human eccrine sweat compared with the pH at 25° C. The regression line, calculated by the method of least squares, is for the equation: Y = -0.59X + 7.1 where Y is ammonia concentration in m.equiv./1. and X is pH of the specimen at 25° C.

We conclude that several buffer systems may be at work in eccrine sweat depending on the acidity. When the sweat is acid the ammonia-ammonium system pre-Phosphate is absent. When sweat is dominates. alkaline the carbonic acid-bicarbonate system is prominent. Amino-acids may affect the acid-base balance at any acidity.

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Stimulation of Egg Laying: Possible Neuroendocrine Function of Bag Cells of Abdominal Ganglion of Aplysia californica

Two groups of identified cells in the abdominal ganglion of Aplysia californica have been classified as neurosecretory on the basis of several morphological features1,2. These are the two clusters of bag cells and the white cells R3-R14 (Fig. 1). To investigate the possible neurosecretory function of this ganglion I homogenized four ganglia, and injected the material into the haemocoel of a small animal. Twelve hours later I found a string of gelatinous material resembling the eggs which are occasionally laid by larger animals. This suggested that the ganglion contains a substance which stimulates egg laying. examine this possibility further four ganglia from large animals (500-700 g) were homogenized in 0.25 ml. of sea water in a small glass tissue grinder fitted with a glass pestle. The resulting suspension was injected into the haemocoel of a large animal (500 g) which had been isolated for a week, during which time it laid no eggs. Within 1.5 h of the injection the animal laid a string of eggs weighing approximately 2 g. In two other experiments, this animal again laid eggs after similar injections.

On the basis of their previously described electro-physiological properties¹⁻³, the bag cells seemed the more

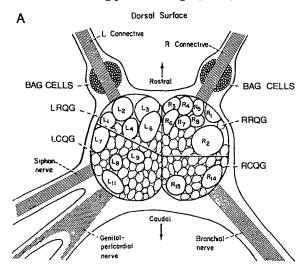
Table 1. PRESENCE OR ABSENCE OF EGG LAYING FOLLOWING INJECTION OF BAG CELL EXTRACT AND CONTROL EXTRACT

Experimental animals	Weight	No. of experiments	No. of donor ganglia	Eggs
\boldsymbol{A}	502	2	3	Present
В	476	2	3 3	Present Present Present
C D	228 148	1 1	1	Present Present
Control animal	В			
\boldsymbol{A}	502	2	3	Absent
В	476	2	3 3	Absent Absent
$_{r}^{E}$	240 152	1 1	1	Absent Absent Absent

Control extract contained abdominal ganglion with the bag cells removed.

likely of the two neurosecretory clusters to be responsible for egg laying. The white cells are tonically active, and received only a very weak synaptic input. On the other hand, the bag cells are usually silent, but can be set into prolonged synchronous activity after a train of strong stimuli to the connective.

To test the hypothesis that the bag cells contained a substance responsible for egg laying, I injected animals with a preparation made from bag cells that had been removed from the rest of the ganglion (bag cell extract). Control animals were injected with a preparation made from the remaining parts of the ganglion (control extract).



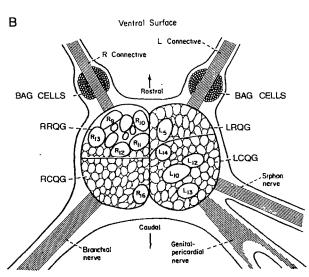


Fig. 1. Schematic representation of the abdominal ganglion of Aplysia californica showing the position of the two neurosecretory clusters: the bag cells and the white cells, R3-R14.

Bag cell extracts (Table 1) were injected into four animals and all of these laid eggs within 1 h. Two of the animals were injected with bag cell extract a second time, I or 2 days after the first injection, and they again laid eggs. Injections of control extract in these two animals and in two other animals did not induce egg laying (Table 1).

These results support the hypothesis that the bag cells. a distinct neurosecretory cluster, secrete a substance that stimulates egg laying in Aplysia.

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Inhibition of Strontium and Calcium Uptake by Rat Duodenal Slices: Comparison of Polyuronides and Related Substances

It has been established that intestinal absorption o' strontium is inhibited by sodium alginate taken orally. both in the rati-3 and in man4,5, while calcium absorption is not significantly reduced. Alginic acid is a polyuronide composed of L-guluronic and D-mannuronic acid residues. both acids can occur in the same uronide chain. Polygalacturonic acid was also found to inhibit the absorption of strontium more than that of calcium, when given to rats by stomach tubes. The effect of polyuronides other than alginate and polygalacturonate on the intestinal absorption of strontium has not been ascertained.

In this study, the uptake of strontium-85 and calcium-45 by slices of rat duodenum incubated in vitro was measured in the presence of polyuronides and related substances. The method, based on that of Schachter et al... has been described elsewhere 10. Six duodenal slices from 6-8 week old male rats were incubated for 1 h at 37° C in 10 ml. of medium, the composition/ml. of which was 118 umoles of NaCl, 4·8 μmoles of KCl, 25 μmoles of NaHCO₃. 27·8 μmoles of glucose, 0·025 μmoles of CaCl₂, 0·025 μmoles of SrCl2, calcium-45 and strontium-85 as tracers, and 500 µg of test substance. At the end of the incubation the slices were assayed for calcium-45 and strontium-85. This method provided a convenient means of comparing several substances, and has been shown to give results in good qualitative agreement with absorption experiments in vivo10.

The materials investigated included sodium alginate (Manucol SS/LD/1, Alginate Industries Ltd.) containing 69 per cent guluronic acid, polygalacturonic acid (Koch-Light) and pectin (BDH). These were compared with derivatives of cellulose and amylose obtained by oxidation Two commercial oxycelluloses with nitrogen dioxide. were tested: 'Oxycel' (Parke-Davis) and 'Surgicel' (Ethicon). Oxycelluloses so formed contain high proportions of uronic acid groups but also some reducing groups indicated by high copper numbers¹¹. Amylose (Sigma) was oxidized at room temperature by NO₂ in CCl₁ (50:50 by volume) for periods from 21 to 213 h¹²; five preparations were tested. All the polyuronides were added to the incubation medium as sodium salts. The carboxyl contents of oxidized cellulose and amylose were estimated

Table 1. UPTAKE OF STRONTIUM-85 AND CALCIUM-45 BY DUODENAL SLICES IN THE PRESENCE OF POLYURONIDES AND RELATED SUBSTANCES

Experi- ment No.	Test substance	Carboxyl content (m.equiv./ 100 g)	Copper No.	Mean uptak (% of cont Strontium-85	rol value)
1	Alginate		3	65	90
	Polygalacturonate	_	7	86	84
	'Oxycel'	369	52	103	101
	'Surgicel'	354	37	93	93
2	Alginate		3	71	90
	Pectin	_	34	88	85
3	Alginate		3	68	91
	Amylose	1	0.5	99	108
	Oxidized amylose	(a) 624	55	87	83
	-	(b) 717	66	77	78
		(c) 720	60	97	87
		(d) 758	68	90	85
		(e) 806	66	82	80

In each experiment the uptake of calcium-45 and strontium-85/g wet tissue is expressed relative to controls incubated without test substances, and is the mean of between two and six flasks of silces.

according to Lüdtke¹³, and copper numbers were determined by Braidy's method14.

The results are given in Table 1. Alginate was included in each experiment for comparison, and in each case inhibited the uptake of strontium more than that of calcium. In contrast, strontium uptake was not inhibited more than calcium uptake by the addition of either polygalacturonic acid or pectin. Of the two oxycelluloses tested, 'Oxycel' did not inhibit the uptake of either cation, whereas uptake of both calcium and strontium was reduced by 7 per cent in the presence of 'Surgicel'. These products contained approximately the same proportion of carboxyl groups, but 'Oxycel' had a higher copper number. The oxidized amyloses had higher carboxyl contents and copper numbers than either

 β -1,4-poly-D-mannuronic acid

 β -1,4-poly-D-galacturonic acid

 β -1,4-poly-D-glucuronic acid

Fig. 1. Structures of polyuronides referred to in the text.

oxycellulose; all of them inhibited strontium and calcium uptake to a limited extent, but did not reduce the uptake of strontium more than that of calcium.

The selective inhibition of strontium uptake by sodium alginate confirms previous work¹⁰, in which it was also shown that the inhibition of strontium uptake relative to calcium was increased when the guluronic acid content of the alginate was increased. From this and the Sr/Ca selectivity coefficients for various alginates given by Haug and Smidsrød¹⁵, it can be concluded that the selective inhibition of strontium uptake by alginates is produced by the guluronic acid residues they contain. Polygalacturonate reduced the uptake of calcium and strontium about equally, in contrast with the greater inhibition of strontium absorption observed in vivo8. Of all the substances investigated, only sodium alginate inhibited strontium more than calcium uptake in vitro. The explanation for this is presumed to lie in the configurations of the various polyuronides (Fig. 1). It is not known whether the L-guluronic acid residues of alginate are α-linked or βlinked; the D-mannuronic acid residues are \$-1,4-linked (ref. 16), and so are the p-galacturonic acid units of polygalacturonic acid and pectin. 'Oxycel' and 'Surgicel' can be regarded as β -1,4-polyglucuronic acid, in which are also some reducing groups and in which not all the C₆ groups are oxidized to carboxyl: the theoretical carboxyl content of a polyuronic acid is 568 m.equiv./100 g. The various oxidized amyloses were presumed to be α-1,4polyglucuronic acid, but containing additional carboxyl groups other than those at C6, as well as some reducing groups.

I suggest that the ability of sodium alginate to inhibit uptake of strontium more than that of calcium arises from the particular disposition of functional groups, such as carboxyl, in polyguluronic acid; that this configuration favours the preferential binding of strontium ions; and that it is not reproduced in the other polyuronides considered in this study.

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Sperm Maturation in Rabbit Epididymis

In mammals, spermatozoa mature while passing through the epididymis. Whether or not the factors governing the maturation process are intrinsic to the spermatozoa1 or whether they reside in the epididymal secretions is not

In the rabbit, there are virtually no fertile spermatozoa in the caput or proximal corpus epididymidis, and fertilizing ability is suddenly acquired when the spermatozoa pass through the distal region of the corpus epididymidis3-6. This, and the fact that, in the rabbit, the normal time which spermatozoa spend in each epididymal segment is known, makes this species especially suitable for investigating sperm maturation.

To determine whether the development of fertilizing ability depends on passage through the distal region of the corpus epididymidis, spermatozoa were retained for different times by double ligature in the proximal caput, distal caput, or the proximal region of the corpus epididymidis. Testes were reached by means of either an abdominal or a scrotal incision, and after the operation they were gently pulled back into the scrotum by a vacuum cleaner applied to the exterior of the scrotum. Ligatures were applied so as to avoid damage to the vascular system of the epididymis. When the proximal and distal caput were ligatured, the whole caput was detached from the testis, the fat was trimmed around the flexure region and one ligature was made on the flexure, and another at the junction of the caput and the corpus. At autopsy, testes and epididymides were removed. The ligatured epididymal regions from both sides were cut up in 1 ml. of 9 per cent sodium chloride. Sperm suspensions were injected through the uterine wall of both uteri of a doe injected 75 min before with 50 IU of human chorionic gonadotrophin (HCG). In each case another doe, serving as a control, was injected with the same number of spermatozoa from the cauda epididymidis. Females were killed 25 h after injection of HCG. The oviducts were flushed and the eggs recovered were examined with the ordinary and phase contrast microscope for evidence of fertilization. The criterion for fertilization was the presence of both pronuclei or of cleavage. After fertilization had been demonstrated, the coverslip was pressed down to squash the ova, and spermatozoa in each ovum were counted. When spermatozoa were trapped in a layer of mucin, their number, determined before squashing, was subtracted from the total.

In this way, it has been possible to establish the following. (a) Spermatozoa from the caput and the proximal corpus epididymidis have almost no fertilizing ability (Tables 1 and 2). Below the middle corpus spermatozoa are able to fertilize; 57 per cent of 142 ova exposed to spermatozoa from the distal corpus were fertilized. (b) When retained in the proximal corpus epididymidis for 12 h (time usually required for passage from the proximal to the distal corpus region, a small fraction of the sperm population becomes fertile: 95 per cent of the ova fertilized by these spermatozoa were recovered from does inseminated with more than 9×10^6 spermatozoa (Table 1). After 24 or 72 h in the proximal corpus, more spermatozoa are fertile, for in does inseminated with only $2 \times 10^{\circ}$ spermatozoa, all ova were fertilized. The sperm population as a whole, however, is not yet fully mature, for four out of five does inseminated with 0.5×10^6 spermatozoa kept for 24 h in the proximal corpus did not become pregnant, whereas all the does inseminated with the same small number of spermatozoa from the distal cauda did become pregnant. (c) When spermatozoa were retained by ligature in the distal region of the caput epididymidis for 1 day, 3 days (normal transit time for spermatozoa from this region to reach the distal corpus region7), 8 days or 12 days, very few spermatozoa become fertile. The only fertilization was in a doe inseminated with 40×10^6 spermatozoa. (d) Spermatozoa never become fertile in the proximal caput, even if kept there for a week, although this represents approximately the time usually needed for the spermatozoa to pass through the entire epididymal duct. (e) When testes are accidentally retained in the abdominal cavity, spermatozoa from the distal cauda epididymidis lose their fertilizing ability.

These results indicate that sperm maturation is complex. Only part of the spermatozoa in the lower corpus epididymidis are fertile, for only 57 per cent of eggs are When retained artificially in the proximal corpus region, spermatozoa can mature, although they need a longer time. Most spermatozoa from the proximal and distal caput region cannot so do. It is realized that ligature is a rather unphysiological procedure and that the secretory function of the ligatured portion of the epididymis may thereby be impaired. This does not prevent spermatozoa from the ligatured proximal corpus from becoming fertile, nor does it prevent the spermatozon from the proximal and distal caput region from developing their capacity for motility. After ligature for 8 or 12 days, spermatozoa are much more motile than usual in the epididymis. The ductuli efferentes were severed, and so most of the testes were swollen and showed patches of white seminiferous tubules full of sperm. When released in saline, these spermatozoa were motile, which is unusual for rabbit testicular spermatozoa. This confirms Young's observation1, in similar circumstances, in the guinea-pig testis. He also observed that, after isolation of the whole epididymis for 20-25 days, 44-49 per cent of the females inseminated with spermatozoa from the proximal cauda region became pregnant, whereas 33 per cent became pregnant when inseminated with proximal cauda spermatozoa from unligatured animals. Young concluded that sperm maturation is a process inherent to the spermatozoa, which begins before they leave the testis and continues after they have been carried into the epididymis but independently of any specific action of its secretion1. In the light of our results, it seems that spermatozoa need

Table 1. FERTILIZING ABILITY OF EPIDIDYMAL SPERMATOZOA RETAINED BY LIGATIONS IN THE PROXIMAL REGION OF THE CORPUS EPIDIDYMIDIS

		Interval between operation and autopsy of the male								
	No operation 6 h			12	12 h		24 h		72 h	
	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
Spermatozoa from	corpus	cauda	corpus	cauda*	corpus	cauda*	corpus	cauda*	corpus	cauda*
Number sperm/inseminate (× 10°)	2.9 ± 0.8	2.9 ± 0.8	3.9 ± 1.0	3·9 ± 1·0	7.5 ± 2.2	2.7 ± 0.4	2·7 ± 0·4	2.7 ± 0.4	5.7 ± 1.5	5.7 ± 1.5
Total No. of ova	46	30	34	38	100	51	51	31	64	56
No. of ova fertilized	0	24	1	38	22	47	38	30	56	53
Percentage fertilization	0	80.0	2.9	100.0	22	92.1	74.5	96.7	87.5	94-6
Total No. of sperm/fertilized ovum		5.5 ± 1.3	5	16.9 ± 1.3	4·6 ± 1·1	22.8 ± 3.4	11.0 ± 1.5	29.7 ± 3.6	$11 \cdot 1 \pm 1 \cdot 2$	16.4 ± 2.0

^{*} Insemination with distal cauda spermatozoa from the same ligated epididymis.

Table 2. Fertilizing ability of epididymal spermatozoa retained by ligations in the proximal and distal regions of the caput epididymidis Interval between operation and autopsy of the male

							_					12 da	iys†		
	No ope	ration	1 6	iay		3 days			8 days		Test			orchid	
		Distal	Distal	Distal*	Proximal	Distai	Distal*	Proximal	Distal	Distai*	scro Distal	Distal*	Distal	stis Distal*	
Spermatozoa from	Caput	cauda	caput	cauda	caput	caput	cauda	caput	caput	cauda	caput	cauda	caput	cauda	
No. of sperm/inseminate	15.1	15.1	21.1	21.1	23.1	5.8	9.5	15.8	19-3	19.3	36-0	36-0	16.0	16.0	
(×10°)	± 1·7	±1.7	± 7·9	±7·9	± 5·2	± 3.5	± 4·6	± 3⋅0	± 7·5	±7·5	± 9.3	± 9.3	± 6.6	± 6.6	
Total No. of ova	47	33	20	13	26	57	42	34	42	38	32	29	28	19	
No. of ova fertilized	1	29	1	13	0	0	33	0	3	34	0	24	0	0	
Percentage fertilization	2·1	87.9	5.0	100	0	0	78-5	0	7.1	89-5	0	82-7	0	0	
Total No. of sperm/fer-	1	28.0	2	20.0	-		11.9		0	11.9		8.0		_	
tilized ova		± 2.3		± 2·1			±1.7			±1.3		±1.0			

Insemination with distal cauda spermatozoa from the same ligated epididymis.
 Operated males were checked every day after the operation and those found with testis in the abdominal cavity are listed separately.

the special environment created by the epithelium of the corpus epididymidis.

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BIOCHEMISTRY

Microsomal Protein Synthesis in the Isolated Perfused Liver during Actinomycin Blockage of RNA Synthesis

In mammalian tissues the mRNA template for protein synthesis has been found to be fairly stable in contrast to that in the microbes¹⁻³. An average half life of 2 h was demonstrated for the liver mRNA in steady state conditions4. Study of the cessation of protein synthesis after treatment with actinomycin, however, showed variable results. Even a very large dose of actinomycin could not affect the protein synthesis of the liver slices in contrast to that of the cell free system. In some cases, the in vitro microsomal protein synthesis was also unaltered6 although the actinomycin gradually decreased the in vivo protein synthesis7. A correlation has been demonstrated between the decay of mRNA and the cessation of the in vitro protein synthesis while RNA synthesis was blocked by actinomycin showing 8-12 h (ref. 8) and 1 h (ref. 9) to be an average half life for the liver messenger. Our own work was stimulated by conflicting reports of the effect of actinomycin on protein synthesis in the liver. We have measured the protein synthesis and changes in the messenger receptor sites in a cell free system obtained from isolated perfused liver to which actinomycin had been added.

Livers from female *CFE* pithed rats (200 g) were perfused through the portal vein^{10,11} and also through the hepatic artery. The perfusion fluid in most experiments was defibrinated whole rat blood, dialysed for 24 h at 4° C against Krebs solution containing an amino-acid mixture of the composition of normal rat blood or against washed red blood cells in Krebs solution containing 2.5 g/100 ml. of bovine albumin. An amino-acid mixture (50 mg/ 100 ml.) and glucose (50 mg) were infused during the experiment. The portal oxygen tension, the pressure and rate of flow of blood and the surface temperature of the liver were continuously recorded. The amount of haemoglobin, the haemolysis, the packed cell volume and the concentration of glucose and urea of the perfusion fluid were regularly examined. At the termination of the perfusions the concentration of different microsomal enzymes. the lysosomal activity and the morphology of the liver were carefully measured. No significant difference from the control livers was seen.

Both the control and the treated livers perfused simultaneously with the same fluid and in the same conditions

were homogenized in a medium of 0.15 molar sucrose, 0.025 molar potassium chloride, 0.01 molar magnesium chloride and 0.035 molar tris buffer, pH 7.8. Microsomal pellets were obtained by centrifuging the 15,000g postmitochondrial supernatant at 105,000g for 1 h. washed pellets of both the control and treated livers containing equal amounts of microsomal proteins were incubated for 5-30 min at 37° C in conditions such that protein synthesis takes place^{12,13} with ¹⁴C-phenylalanine (U) or a mixture of ¹⁴C-amino-acids from chlorella hydrolysate in the presence of "pH 5·0 enzyme" fractions obtained from the control animals unless otherwise stated. In some experiments, an increased amount of polyU was added to the reaction mixture and the components were adjusted for the optimal attachment of the ribosomes to the increased amount of polyU (unpublished work of The reaction was stopped with Nievel and Golberg). 10 per cent trichloroacetic acid containing cold amino-acids and the protein was purified by carefully extracting the acid soluble, lipid and nucleic acid fractions. The dry precipitates were dissolved in NCS solubilizer and transferred to vials, mixed with a toluene containing scintillator and counted in a Nuclear Chicago model 720 liquid scintillation spectrometer, using the channel ratio method for the quenching correction.

An increasing amount of actinomycin D, up to 1.5 mg added to the perfusion medium for 5 h, produced only a variable decrease in the protein synthesis (Table 1). The effect was seen only in the microsomes and the results were not affected whether the "pH 5.0 enzymes" were derived from the control or treated liver. No correlation was observed between the effect on the microsomes and the period of exposure of the liver to perfusion with actinomycin. The nuclear RNA synthesis of the isolated liver is greatly reduced by doses much smaller than those which we used14. It is therefore possible that the observed reduction in the rate of incorporation was a result of non-The reduction in the incorporation, specific effects. however, was accompanied by different rates of polyU directed phenylalanine incorporation (Table 2). longer the liver was exposed to perfusion with actinomycin, the faster was polyphenylalanine formed. polyU dependent increment of the treated liver was greater than that of the control perfused for from 3.5 to 6.5 h.

Table 1. Beffect of actinomycin D on the microsomes and "pH 5-0 enzyme" preparations

		d.p.m./mg of microsomal protein*				
No.	Actinomycin D treatment (mg/h)	Microsomes control+	Microsomes control + "pH 5 enz" treated	Microsomes treated + "pH 5 enz" control	Microsomes treated + "pH 5 enz" treated	
1 2 3 4 5	1·5/5 0·5/5 0·25/5 0·25/3·5 0·25/2	2,179 (100)† 2,586 (100) 1,261 (100) 1,837 (100) 3,024 (100)	2,781 (128)† 3,219 (124) 1,501 (119) 1,630 (89) 2,826 (93)	1,547 (71)† 1,904 (74) 1,031 (82) 1,597 (87) 2,524 (83)	1,598 (73)† 1,921 (74) 793 (63) 1,731 (94) 2,299 (76)	
6	0.25/0.5	3,954 (100)	3,411 (86)	2,618 (66)	2,426 (61)	

^{*} In vitro incubation for 30 min.
† Percentage of the untreated samples.

Table 2. EFFECT OF ACTINOMYCIN D ON THE ENDOGENOUS AND POLYU DIRECTED MICROSOMAL PROTEIN SYNTHESIS OF THE PERFUSED LIVER

	Acuno-							
	mycin D		d.p.m./m	g of micros	omal protein			
	reatment		PolyU (μg)					
110.			50	75	150	250		
	(mg/h)		90	10	700	200		
1		483 (100)*	577 (199)†	756 (157)†	973 (201)†	1,058 (219) †		
-	0.25/0.5	391 (81)	462 (118)	452 (116)	776 (198)	766 (196)		
2		532 (100)	574 (108)	745 (140)	968 (182)	1,014 (191)		
	0.25/2	315 (59)	324 (103)	313 (99)	601 (191)	588 (187)		
3 1		452 (100)	669 (148)	775 (171)	933 (206)	950 (210)		
	0.25/2	297 (66)	393 (132)	407 (137)	577 (194)	573 (193)		
4‡		348 (100)	348 (100)	466 (134)	641 (184)	679 (195)		
- +	0.25/3.5	265 (76)	314 (118)	399 (151)	692 (261)	766 (289)		
5		195 (100)	357 (183)	315 (162)	440 (226)	446 (229)		
-	0.25/5	234 (120)	510 (218)	605 (259)	752 (321)	815 (348)		
6‡		587 (100)	605 (113)	685 (128)	1.063 (198)	971 (181)		
- +	0.25/5	400 (74)	485 (121)	876 (219)	1,452 (363)	1,559 (390)		
7‡		494 (100)	465 (94)	617 (125)	1,116 (226)	1,239 (251)		
	0.25/5	359 (73)	344 (96)	425 (118)	952 (265)	1.155 (322)		

^{*} Percentage of the control endogenous protein synthesis.
† PolyU dependent increment: percentage of the corresponding endogenous incorporation.
† Experiments with the perfusion of washed red blood cells.

These changes were not affected if whole blood or a semisynthetic medium with washed red blood cells was perfused. Similar results were obtained in vivo after up to 2 days of pretreatment with a corresponding amount of actinomycin (unpublished work of Nievel and Golberg). Assuming that there is a common receptor site for the mRNA and for the external coding agents, the polyU dependent increment reflects loss of endogenous mRNA bound to the ribosomes¹⁵. No concomitant decrease in the ability of the isolated microsomes to synthesize protein was observed. Similar results were obtained for the in vitro protein synthesis in different experimental conditions.

The different reactions of the treated and control microsomes to stimulation by polyU, in comparison with unstimulated endogenous incorporation on the one hand and the absence of any effect of actinomycin on the microsomes on the other, point to the complexity of the interpretation of the messenger decay obtained either from the gradually decreasing protein synthesis or from the increased stimulation by polyU. If, however, the template for protein synthesis in the liver is very stable, the increase in the messenger receptor sites reflected by the increased incorporation dependent on polyU possibly does not demonstrate the decay of that messenger coding for most of the protein synthesis of the perfused liver.

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In vitro "Lipid" Metabolism in the **Developing Human Foetus**

Ir can be concluded from animal data that the human foetus synthesizes lipids, chiefly from carbohydrates1-3. We have measured the synthesis of fatty acids and sterols from acetate and citrate in human foetal tissue taken at various periods of gestation. The total lipid content of the organs and the ability of the 8 week placenta to take up palmitate and incorporate it into lipids was also determined.

Tissues were obtained from foetuses after therapeutic interruption of gestation at 8, 16, 23, 24, 26 and 28 weeks. The mammary glands of two postpartum rats 10 days old were used as controls of enzyme activity. The various tissues were washed in cold isotonic saline and homogenized in 0.25 molar sucrose. The protein contents of the microsomal and supernatant fluid fractions of the homogenates were measured after high speed centrifuga-Acetate-1-14C (10 μmoles) or citrate-1-5-14C

 $(3 \times 10^6$ d.p.m. and $2 \cdot 0 \times 10^5$ d.p.m., respectively, New England Nuclear, Inc.) was added to 1.0 ml. of medium in each incubation vessel⁵. The optimal reaction was started by the addition of supernatant fraction (5 mg of protein/1.0 ml.) and microsomes (1.25 mg of protein in 0.2 ml. of 0.25 molar sucrose). The incubation vessels were maintained at 38° C for 45 min. After incubation, lipids were extracted, dried under a vacuum and dissolved in chloroform. Samples of 0.5-0.8 ml. of lipid extract were mixed with 0.5 mg of a mixture of standard lipids (Applied Physiology Laboratory). The extracts were fractionated by thin-layer chromatography?. Phospho-The extracts were lipids, free fatty acids and triglycerides were scraped from the plates, pooled and saponified in the presence of 4 per cent potassium hydroxide in 95 per cent ethanol at 100° C for 1 h. After acidification and extraction with hexane, the fatty acid residue was dissolved and counted in a scintillation counter. Sterol fractions were scraped directly into the scintillation vial containing a mixture of toluene with 4 per cent 'Cab-o-Sil' and 4 g/l. of BBOT (Packard Inst.) and were counted in the same way. Duplicate determinations of total lipid and protein content were made of two tissue samples for each period of gestation4,8. The ability of placental tissue to incorporate fatty acids was tested by incubating slices from two 8 week human placentas8 in a medium containing free fatty acids. Disappearance of these acids from the medium and incorporation of palmitate-I-14C into tissue triglycerides and phospholipids were determined7,8.

The data indicate that until the twenty-eighth week of gestation the human foetus is unable to form measurable amounts of fatty acids from citrate or acetate (Table 1). At 28 weeks, the foetal liver is as active as the adipose tissue of the rat mammary gland. The foetus then becomes active in fatty acid production from carbohydrate metabolites. Because citrate-1-5-14C is a better fatty acid precursor than acetate-1-11C, the foetal liver probably contains the citrate cleavage enzymes which can be demonstrated in rat mammary gland. The concentrations of liver and brain lipids increase 2.5 and 3 times, respectively, between the eighth and the sixteenth weeks of gestation but tend to remain constant thereafter except for samples of brain tissue which have a high value of 72 mg/g at 28 weeks. Placental lipids tend to decrease after the twenty-sixth week of gestation. Lung lipids show little change with age (Table 2). Because all foctal tissuecontain measurable amounts of lipids at each period of gestation studied, the foetus must obtain the necessary precursors from the placenta and/or maternal blood. Placental slices utilized 1.05 pequiv. of free fatty acids/g when incubated with 1.65 µequiv./ml. of fatty acid mixture. When 3.30 µequiv./ml. of fatty acid mixture was added, I g of placental slices utilized 2.45 µequiv. of free fatty acids. Placental slices took up 14.6 and 10.1 per cent labelled palmitate/g with two different levels of free fatty acids. Equal percentages (2.3 per cent and 2.4 per cent) of labelled palmitate were incorporated into placental

Table 1. Fatty acid and sterol synthesis by foetal tissuc at 28 weeks' ${\tt GESTATION}$

	Acetate-	-1-14C	Citrate-1-5-110		
Tissue	Fatty acids	Sterol	Fatty acids	Sterol	
Liver	1.0	0	167.0	5-0	
Lung	0	0	0	υ	
Brain	3.3	5	0	Ų	
Placenta	0	O	ø	Û	
Control	1.9	1.5	121.3	23-2	

The results are the average of two experiments carried out in duplicate and are expressed in $m\mu m$ of substrate converted to fatty acids or sterolsing

Control: 10 day post partum rat mammary gland.

Table 2. LIPID CONTENT (mg/g) PROTEIN OF FOETAL TISSUES

Weeks of gestation	Liver	Brain	Lung	Placenta
8	80	10	_	300
16	200	30	_	260
23	220	_	190	270
26	250	34	200	230
28	250	72	160	170

phospholipids and triglycerides. Although the human placenta cannot significantly synthesize lipids from acetate or citrate, free fatty acids can be transferred into the 8 week placenta, where they can be converted into placental lipids in vitro. Circulating maternal fatty acids could thus be precursors of foetal lipids until the twenty-eighth week of gestation. This infers that foetal lipids are not synthesized de novo from carbohydrates before the twenty-eighth week of gestation. Hirsch's data demonstrate that dissimilarities in fatty acid composition can be detected between mother and foetus?. Partial or total de novo synthesis from carbohydrate is possible in term foetal tissue, and our results show that this takes place as early as the twenty-eighth week of gestation. Additional evidence for the possible maternal origin of fatty acids in young foetuses is that the human placenta transfers fatty acids in vitro (our data) and in vivo to the foetus10.

Lipoprotein lipase is also present in the syncytium¹¹. In contrast, Villee has published data which show a small conversion of acetate 2-¹⁴C to lipids by foetal liver and placental slices as early as the twentieth week of gestation3. We suggest that most of the measured radioactivity could be in the non-fatty acid fraction of the phospholipids and triglycerides.

The synthesis of fatty acids and sterols from acetate-1-14C or citrate-1-5-14C by brain tissue starts as early as the sixteenth week of gestation. This may well be related to the development of independent brain myelinization.

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Inhibition by Cortisol of Deiodination of Thyroxine by Rat Liver in vitro

GLUCOCORTICOIDS have been shown to flatten the slope of peripheral disappearance of 121 I-thyroxine in man1 and decrease its hepatic content in man² and rat³. Galton and Ingbar4 have shown that deiodination of thyroxine by rat liver is mediated by two components, one of which is heat resistant and lost by leaching of liver slices in buffer and another which is heat labile and possibly an

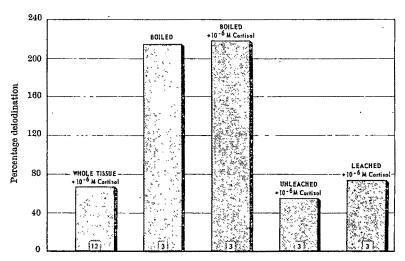


Fig. 1. Shows mean values for percentage deiodination. The number of experiments is indicated at the bottom of each column.

This latter component is probably similar to enzyme. the heat labile, microsomal system for degrading thyroxine

We used male Sprague-Dawley rats (200 g), and pieces of liver of known weight were homogenized at 4° nine volumes of Krebs Ringer phosphate buffer pH 7.4. In leaching experiments, weighed liver slices were kept in 50 ml. of this buffer for 30 min and then homogenized in fresh buffer. In some experiments, liver homogenates were boiled for 15 min, cooled and rehomogenized before use. All incubation vessels contained I ml. of homogenate and 1×10^{-7} to 4×10^{-7} molar ¹³¹I-thyroxine in 1 per cent human serum albumin. Cortisol was added in the final concentration of 10-6 molar. In control vessels the volume was made up with buffer. After incubation for 1 h at 37° C in a metabolic shaker, 1 ml. of plasma was added to each vessel to stop further reaction. Chromatographic quantification of deiodination was achieved by the method of Galton and Ingbar⁶. Both original material and iodide were combined for calculating the deiodination. Deiodination of ¹³¹I-thyroxine by the rat liver microsomal system was investigated by the method of Wynn et al.5. All results are expressed in terms of deiodination by the control liver homogenate or microsomal system taken as 100 per cent.

Figure 1 shows the inhibition of deiodination by whole liver homogenates by 10-8 molar cortisol. In paired experiments, leached homogenized slices showed only slightly less inhibition than unleached controls. Cortisol, however, had no effect on deiodination by boiled homogenates (increase in deiodination after boiling has been noted previously4). Cortisol (10-6 molar) had no effect on the percentage of iodide in the buffer or Krebs Ringer phosphate buffer in which liver slices had been leached. Cortisol produced inhibition of deiodination by the rat liver microsomal system which requires 7×10^{-4} molar ferrous sulphate for its optimal activity. Percentage deiodination in the presence of varying concentrations of cortisol was as shown in Table 1 (average of duplicate or more observations in six experiments).

Our results therefore indicate that the inhibitory effect of cortisol is probably on the enzymatic deiodination of thyroxine. The decrease in deiodination of thyroxine would contribute to the flattening of peripheral degrada-

Table 1. PERGENTAGE DESORDINATION IN THE PRESENCE OF CORTISOL

Cortisol	Percentage deiodination
10 ⁻⁴ molar	76-8
10 ⁻⁵ molar	74-5
10 ⁻⁶ molar	72-8
10 ⁻⁷ molar	71-8
10 ⁻⁸ molar	86-5

tion slope of 131I-thyroxine seen after treatment with cortisone. The importance of this effect on deiodination is. however, in doubt, for recent observations indicate that deiodination of thyroxine is not related to the mechanism of action of thyroid hormone?.

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Competitive Inhibition of Histochemical Substrates for Glycosidases

HISTOCHEMICAL reactions are dependent on enzyme specificity. The reaction should be enhanced by conditions that are optimal for the enzyme and should be diminished in the presence of inhibitors. Inhibition of the substrate reaction may be effected either by enzyme inactivation or by competition with analogous compounds. methods have been used to define the specificity of numerous in vivo and in vitro biochemical systems.

Halogen-substituted indoxyl substrates have been synthesized recently for $\beta\text{-D-galactosidase}^1$, $\beta\text{-D-glucosidase}^2$, $\beta\text{-D-glucuronidase}^3$ and $\beta\text{-D-fucosidase}^4$, and enzyme specificity has been demonstrated for each. The substrates contain an identical indolyl moiety (5-bromo-4chlorindol-3-yl) and glycosides which differ from each other only in the configuration or substitution of C4 or C8 (Fig. 1). We observed the comparative effect of analogue inhibitors on the reactions of these structurally similar

Specimens of jejunum were obtained from freshly killed, adult Sprague Dawley rats, frozen in a dry ice and acetone bath and sectioned in a cryostat at -15° C. The sections were incubated for 3 h at 37° C in a solution of the substrate and inhibitor. The pH range and the

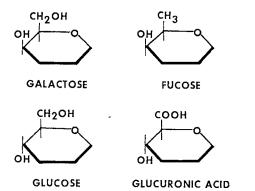


Fig. 1. Comparison of glycon moleties illustrating the structural similarity of the indoxyl substrates. In each case the compound is a β -isomer and the glycoside is in the D-configuration.

optimum pH for reactions in the jejunum were determined for each substrate. The substrate concentration was adjusted so that reactions of similar intensity were obtained for each by the end of 3 h. Sections in each experiment were prepared from a single block of tissue and were mounted at the tip of coverslips in order to conserve substrate (final incubation volume was 1 ml.). To each substrate solution, inhibitors were added so that the final concentration was 0.01 per cent to 2 per cent. After incubation, the sections were dehydrated, cleared and mounted in balsam. Inhibition was graded (0 to ++++) by the degree of diminished staining and the inhibitor concentration which was required for this effect. The staining reaction occurred over a broad range of pH values. The pH optima were: galactosidase, 5.4; fucosidase, 6.2; glucuronidase, 4.8; and glucosidase,

In general, inhibition of the reaction was most marked when both the substrate and inhibitor were derived from the same sugar. Results for the four substrates are listed in Table 1. A variable degree of inhibition resulted from addition of the sugar in the substrate to the incubating solution. Staining was diminished in the glucuronidase reaction by the presence of 0.1 per cent glucuronic acid (+++). In contrast, 2 per cent glucose had no effect on the glucosidase reaction. Aldonolactones were the strongest inhibitors, but overlapping occurred between galactosidase and fucosidase, and between glucosidase and fucosidase. Glycoside derivatives showed the specificity of carboxyl group location. Gluconic acid (C1 carboxyglucose) inhibited glucosidase activity, but had no effect on glucuronidase activity. Saccharic, galacturonic and glucuronic acid (C, carboxyl groups) all inhibited the glucuronidase reaction.

Disaccharides were weak inhibitors. Lactose (glucosido- β -galactose) was a consistent inhibitor of galactosidase only at a concentration of 2 per cent. Cellobiose (4glucosido-β-glucose) had no effect on glucosidase but weakly inhibited the fucosidase reaction. In addition to the compounds listed in Table 1, no inhibition was noted with the following commercially available sugars and sugar derivatives: L-fucose, 2-deoxyglucose, mannose, rhamnose (6-deoxymannose), sorbose, ribose, L-arabinose, lyxose, fructose, inulin, stachyose (Lupeose), sucrose (4-fructosido- $\alpha\text{-glucose}),$ maltose (4 glucosido- $\alpha\text{-glucose}),$ trehalose (1-glucosido- $\alpha\text{-glucose}),$ raffinose (melitriose), ducitol (galactitol), sorbitol (glucitol), inositol, adonitol, N-acetylglucosamine and phospho-glucuronic acid. The substrate hydrolysis was identical in these four indolyl glycosidase methods, and the rate and intensity of the staining reactions were similar. The overlapping inhibition between galactosidase and fucosidase activities in this study has

Table 1. INHIBITION OF INTESTINAL GLYCOSIDASES

	sidase	Fucosidase	dase	Glucosidase
Galactose	+_+	0	0	o o
Fucose	Ō	+	0	Ō
Glucuronic acid	Ō.	Ō	+++	Ō
Glucose	0	0	0	0
Galactonolactone	+++	+	0	0
Fuconolactone	+	+++	0	0
Glucuronolactone	0	0	++	0
Saccharolactone	0	0	++++	0
Gluconolactone	0	+	0	+++
Gluconic acid	0	0	0	+++
Saccharic acid	0	Ō	++++	Ó
Mucic (galactaric) acid	0	0	++	+
Galacturonic acid	0	0	++	0
Polygalacturonic acid	0	0	++	+++
Xylose	0	0	0	+
Lactose	+	Ò	0	0
Celloblose	0	+	0	0
Melibiose	0	0	0	+

0, No effect on tissue staining.
+, Diminished staining, 2 per cent; inconsistent or no effect at lower concentrations.
+, Complete inhibition, 2 per cent; diminished staining, 1 per cent.
+++, Complete inhibition, 1 per cent; diminished staining, 0·1 per cent.
++++, Complete inhibition, 0·1 per cent; diminished staining, 0·01 per cent. cent. These sugars and sugar derivatives were all in the D form.

been noted previously in biochemical assays. Similarly the inhibition of glucosidase activity by xylose is probably the result of similarities between glucosidase and xylosidase and/or the similar configuration of glucose and xylose6.

These data confirm enzyme specificity for glycoside configuration and linkage7. Neither L-fucose nor αlinked glycosides had the inhibitory effect of D-fucose and β-linked glycosides. Carboxyl substituents seemed to be more important than either other end groups or the configuration of the pyranoside ring. Rhamnose (6deoxymannose) did not inhibit fucosidase, but all compounds with C₆ carboxyl groups were strong inhibitors of glucuronidase. In contrast, glucose and galactose derivatives did not necessarily inhibit glucosidase and galactosidase. There is no obvious explanation for the absence of glucosidase inhibition by glucose. The reason for the weak inhibition of fucosidase activity by gluconolactone and cellobiose or the similar effect of melibiose (6-galactosido-α-glucose) on glucosidase is also unknown.

Dannenberg and co-workers8 have used histochemical methods to study the conditions of activation and inhibition of several hydrolytic enzymes in macrophages (unpublished data) and Fishman et al.9 have studied the inhibition of glucuronidase. These data confirm their findings with compounds analogous to the galactoside and glucuronide. These studies demonstrate that analogue and end-product inhibition are useful techniques for defining the specificity of histochemical methods for enzyme detection. I thank Arthur M. Dannenberg, jun., and Bjarne Pearson for their advice and assistance.

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Imidazole and Sequestration of Calcium Ions by Sarcoplasmic Reticulum

SARCOTUBULAR fragments prepared from homogenates of skeletal muscle are able to sequester vigorously calcium ions if ATP is available, and they show the activity of Mg-ATPase and Ca-ATPase which are thought to serve in the energy requiring sequestration of calcium ions. Although these functions have been the subject of much experimentation, little is known about their molecular bases. The uptake and release of calcium ions by the sarcotubular system are believed to be an integral part of excitation-contraction coupling and relaxation of muscle, and so it is important to understand the molecular mechanisms which underlie these processes. Hasselbach and Seraydarian¹ have reported that certain sulphydryl groups of the sarcotubular membranes play an important part in both the uptake of calcium ions and the related ATPase activities. Experiments carried out in our laboratory, however, indicate that a functional group other than the sulphydryl is also involved in these activities.

A purified sarcotubular fraction was prepared from rat skeletal muscle by a modification of the method of Seraydarian and Mommaerts² and this fraction had two to three times as much Mg-ATPase and calcium ion sequestering activity as the unpurified microsomes.

The effect of ageing the purified sarcotubular fragments on their calcium ion sequestering and Mg-ATPase activities was examined by suspending them in 0.25 molar sucrose (about 0.7 mg of sarcotubular protein/ml. of suspension). This suspension was stored at 2° C and samples were taken periodically to follow the course of ageing on calcium ion sequestration and Mg-ATPase activities. Various chemical agents were added to test their ability to modify the effect of ageing; unless otherwise stated, these agents were added at a concentration of 5 mmolar at zero time.

Mg-ATPase was assayed as follows: a 2 ml. system containing 20 mmolar tris-hydrochloric acid (pH 7·2), 3 mmolar magnesium chloride, 3 mmolar ATP and 50 μg of sarcotubular protein was incubated for 20 min at 37° C; the reaction was stopped by adding 0.2 ml. of 50 per cent trichloroacetic acid. The inorganic phosphate liberated was measured colorimetrically by the method of Fiske and Subarrow³

The uptake of calcium ions by the sarcotubular fragments was measured in the incubation system of Carsten and Mommaerts⁴. Samples were removed from the system at various intervals, and the amount of calcium-45 bound by the sarcotubular fragments was measured by the 'Millipore' filtration method of Martonosi and Ferretos.

After solubilizing the protein of the sarcotubular fragments with 0.16 per cent deoxycholate and heating on a steam bath, the protein concentration was measured colorimetrically by the method of Lowry et al.6.

The effect of ageing on the capacity to sequester calcium ions is shown in Fig. 1. The sarcotubular fragments lose their capacity to bind calcium ions when they are stored in 0-25 molar sucrose, but not when stored at higher sucrose concentrations (for example, 0.5 molar and 1 molar). Compounds containing pyrophosphate, such as ATP, ADP and inorganic pyrophosphate, prevent loss of the ability to take up calcium ions during ageing, while cyclic AMP and creatine phosphate are far less effective. Albumin (1 per cent) also prevents loss of sarcotubular calcium ion sequestering activity during ageing. Albumin contains sulphydryl groups and so we felt that these functional groups might be responsible for its protective effect, but none of the tested sulphydryl reagents (Cleland's reagent, glutathione, cysteine) prevented the diminution of activity during ageing. EDTA (2.5 x 10-5 molar) also failed to protect. Imidazole compounds markedly protect

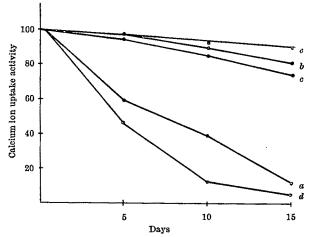


Fig. 1. Effect of various reagents on ageing of calcium sequestration occurring in fragments of sarcotubular membrane. Concentration of sucrose (a), 0-25 molar; of ATP (b), histidine (c) and glutathione (d), 5 mmolar; of albumin (e), 1 per cent. Temperature for storage was 2° C. Concentration of microsomes, 0-7 mg of protein/ml. Activity is expressed in relation to zero time value which is arbitrarily set at 100.

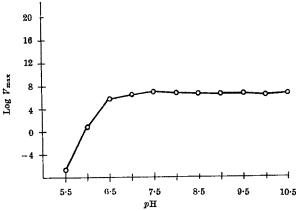


Fig. 2. 11 cct of pH on the log V_{max} of Mg-ATPase. V_{max} was obtained from the cuble reciprocal plot of ATPase activity against ATP concentration at different pHs. V_{max} was determined in optimal conditions with regard to the ratio of [Mg++] to [ATP] and total [ATP] at each pH

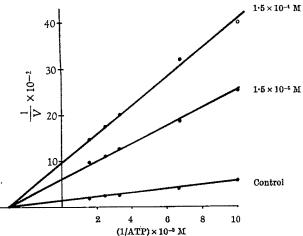


Fig. 3. Effect of photo-oxidation with rose bengal on calcium uptake by sarcotubular fragments. (The control experiment involved the same concentration of rose bengal but no illumination.)

the activity during ageing, histidine and urocanic acid being considerably more effective than imidazole and imidazole acetic acid. The amino-acids arginine, serine, cystine, methionine and tryptophan also protect the calcium ion uptake activity during ageing but are much less effective in this respect than the imidazole compounds. The activity of Mg-ATPase in the sarcotubular fragments is also lost during ageing but can be protected from this effect by the same agents which protect the calcium ion sequestration system. There is one exception: Cleland's reagent does not protect the sequestration of calcium ions but does protect the Mg-ATPase system.

These data suggested that the imidazole group plays a part in the biochemistry of the sequestration of calcium ions by sarcotubular fragments. Because the sequestration of calcium ions and the activities of ATPase are thought to be related, we investigated the molecular basis of the sarcotubular Mg-ATPase activity. In Fig. 2, the log $V_{\rm max}$ of Mg-ATPase is plotted on the y axis and the $p{\rm H}$ on the x axis. The activity of Mg-ATPase seems to be independent of $p{\rm H}$ when the $p{\rm H}$ is greater than 6.5, but when the $p{\rm H}$ is less than 6.5 the activity of Mg-ATPase declines rapidly as the $p{\rm H}$ decreases. These data imply that the Mg-ATPase involves a group with a pK near 6.5. Of the amino-acid residues in the protein of the sarcotubular membranes, the histidine residues are the ones most likely to have a pK near 6.5 (ref. 7).

Imidazole groups are photo-oxidized in the presence of methylene blue⁸ or rose bengal⁹. Sarcotubular fragments were therefore subjected to the following treatment. They

were suspended in 35 per cent sucrose solution (approximately 100-150 mg of sarcotubular protein/ml. of suspension) containing either 5×10^{-7} molar rose bengal at pH 7.4 or 5×10^{-6} molar methylene blue at pH 6.8 and were illuminated with a 100 W tungsten lamp, without shaking at 2° C. At the time intervals of Fig. 3, samples were assayed for evidence of calcium ion sequestration. In the case of both rose bengal (Fig. 3) and methylene blue (data not recorded) photo-oxidation caused a marked loss of ability to sequester calcium ions. The effect of similar treatment on Mg-ATPase activity was examined; illumination was carried out while ATPase activity was being assayed (incubation for 15 min at 37° C). Rose bengal (not shown) and methylene blue (Fig. 4) competitively inhibit the activity of Mg-ATPase. Although these photooxidation experiments are consistent with the concept that imidazole groups function in both the sequestration of calcium ions and the activity of Mg-ATPase in the sarcoplasmic reticulum they do not provide unequivocal proof. for methionine and tryptophan residues are also attacked by the photo-oxidation procedures.

The effects of sulphydryl inhibitors on the ATPase activity of the sarcoplasmic reticulum were also investigated (Fig. 5). In contrast to the effects of photo-oxidation, salyrgan functions as a non-competitive inhibitor of the ATPase as does p-chloro-mercuri-benzoate (PCMB), while N-ethylmaleimide (NEM) is an uncompetitive inhibitor of the sarcotubular Mg-ATPase.

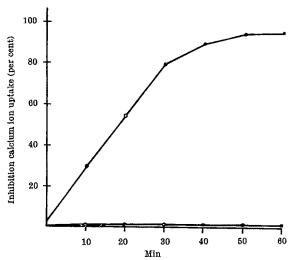


Fig. 4. Competitive inhibition of Mg-ATPase by methylene blue. Plot of reciprocal velocity 1/V (where V is μ g of inorganic phosphate formed in 20 min) against 1/[ATP] (where [ATP] is in mmolar). The control experiment involved the same concentration of methylene blue but no illumination.

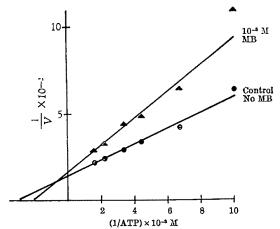


Fig. 5. Non-competitive inhibition of Mg-ATPase by salyrgan, plotted as in Fig. 4.

The photo-oxidation data are consistent with the presence of some group other than thiol (such as histidine, tryptophan or methionine) at a site concerned with the sequestration of calcium ions by sarcotubular membranes and of such a group in sarcotubular Mg-ATPase activities, these groups perhaps being involved in the binding of the relevant substrates. The pH dependence of the sarcotubular Mg-ATPase and the marked protective action of imidazole compounds against loss of Mg-ATPase activity and the ability to take up calcium ions during ageing of the sarcotubular fragments strongly suggest but do not rigorously prove that it is the imidazole group which is

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Role of Intestinal Lymphatics in Copper Absorption

THE immediate appearance of copper-64 in the peripheral blood of rats, rabbits, sheep and men after oral ingestion of the isotope indicates that the upper gastrointestinal tract is an important site for the absorption of copper¹⁻³. The role, if any, of the intestinal lymphatics in the absorption mechanism is unknown and has not been reported. In order to elucidate this role, we have compared the relative proportions of absorbed copper which are transported from the intestine by portal blood and by lymph.

Three mongrel dogs, weighing 12-15 kg, were anaesthetized with intravenous pentobarbital. After laparotomy, an 'Intramedic PE 120' polyethylene catheter was passed into the portal vein through a mesenteric vein and was kept patent during the intervals between sampling by means of a slowly dripping solution of 5 per cent dextrose in normal saline containing 10 U of heparin/ml. A second catheter was inserted into the thoracic duct at its junction with the subclavian vein to collect lymph during the period of the experiment. As soon as lymph was flowing freely, 0.5 mc. of copper-64 nitrate containing 600 µg of ionic copper was administered in milk to each animal either through a peroral rubber tube into the stomach or by a duodenostomy. In one experiment, the milk mixture was placed just beyond the ligament of Treitz into a 12.5 cm loop of jejunum isolated by ligatures at both ends. The concentration of copper-64 in plasma and lymph was assayed in a Nuclear-Chicago well-type scintillation detector DS 202 (V) and a model 8725 analyser/ scaler. One millilitre of portal blood was drawn at intervals of 5-15 min for about 90 min. All the lymph flowing from the thoracic duct was collected during the same intervals. The mean concentrations of copper-64 in plasma and lymph, determined in three experiments, were plotted against time. Figure 1 shows that the copper concentration of lymph rises more slowly than that of portal blood

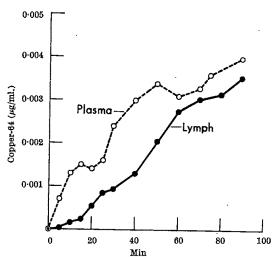


Fig. 1. Mean concentrations of copper-64 in plasma and lymph at various times following administration of copper-64 nitrate to three dogs.

and remains much lower for approximately 1 h, after which concentrations of copper in both fluids tend to become equal. Of the 600 µg of copper placed in the loop of jejunum which was tied off, 310 µg remained in the lumen and intestinal wall after 90 min. Of the 290 µg of copper absorbed, only 0·12 μg was recovered from the total of 38 ml. of lymph collected in this period. The remainder—little less than 290 µg—clearly had been absorbed through the blood which could not be collected quantitatively.

The quantitative insignificance of lymphatic absorption of copper has also been noted in a study with a man aged 28 suffering from Laënnec's cirrhosis and portal hypertension. Following therapeutic catheterization of the thoracic duct (for decompression of ascites4) and umbilical vein, the concentration of copper-64 given intragastrically in a dose of 1,000 μg of copper reached 0.005 μg in 192 ml. of lymph, collected over 110 min. The concentration of absorbed copper in lymph is lower than that in portal blood (Fig. 1) and the volume of lymph produced in a given time is considerably less than that of portal blood flow4, and so the amount of copper absorbed through the lymph is negligible compared with that transported through the blood. Albumin binds almost all copper immediately after absorption and is present in roughly equal concentrations in lymph and blood4, and is therefore unlikely to account for the difference in copper absorption observed. Amino-acids which are passed into the portal circulation⁶ also bind copper⁷. This suggests a possible role for these substances in the transport of the metal across the mucosa.

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Antilipaemic Effect of Nicotinamide

THE antilipaemic effect of nicotinic acid is well known¹ and the clinical value of this compound as an agent for lowering the content of cholesterol has been demonstrated^{2,3}. Although many explanations have been offered, the mechanism by which nicotinic acid exerts its antilipaemic effect is not known. It is generally agreed, however, that the effect of decreasing cholesterol is unrelated to this compound's known vitamin role as a precursor of pyridine nucleotides, because nicotinamide, which is more readily incorporated into pyridine nucleotides, has little effect on serum cholesterol concentrations in man^{2,3}.

I have shown that nicotinamide is as effective as nicotinic acid in decreasing the concentration of cholesterol. triglycerides and free fatty acids in the serum of the rat. I have also shown that both agents are very effective in decreasing the content of serum triglycerides and free fatty acids in the dog. Animals were fasted 16 h before they were given the drug (2 mmoles/kg); blood was campled at various times. Blood lipid was assayed by standard analytical methods. In the rat, all lipid classes measured were considerably reduced by both agents (Table 1). Similarly, free fatty acids and triglycerides in the serum were reduced in the dog but in these conditions the content of cholesterol was unchanged (Table 2). The time interval between the administration of the drug and the maximal effect on the different lipids varied greatly, and so, for the purpose of this discussion, only the maximal effects and the time at which they occurred are included in Tables 1 and 2.

A maximal decrease in free fatty acids occurred much earlier than the decrease in serum triglycerides which in turn was much earlier than the decrease in serum choles-This temporal relationship has been observed before with nicotinic acid4,5. So pronounced is the effect of nicotinic acid on concentration of free fatty acids in serum that it has been suggested that the primary activity of this acid is to inhibit the release of free fatty acids from adipose tissue depots, while the effect on other blood lipid constituents is an indirect consequence of this activity4,5.

A reduction in cholesterol content caused by nicotinamide and nicotinic acid in rabbits fed cholesterol has been reported by Fontenot⁶. The reduction was paralleled by a corresponding increase in nicotinamide adenine dinucleotide (NAD) in packed erythrocytes. It was suggested that the antilipaemic action of nicotinamide is unique to the rabbit. The experiments reported here indicate that in two other species nicotinamide is as effective as nicotinic acid as an antilipaemic gent.

Ricci and Pallini7 found an increase in free nicotinic acid in the liver of rats injected with nicotinamide, thus

Table 1. EFFECT OF NICOTINIC ACID AND NICOTINAMIDE ON SERUM LIPID LEVELS IN THE RAT

Triglycerides Total cholesterol (mg/100 ml.) \pm S.E.* (mg/100 ml.) \pm S.E.* FFA (μequiv./l.) ± S.E.* Compound Control Treated Control Treated Control Treated Nicotinic acid 432 ± 15 $103 \pm 10 † 66 \pm 8$ Nicotinamide 371 ± 14 $138 \pm 17 † 83 \pm 9$ 8 ± 1 † 26 ± 1 † 85±7 47±4† 79±4 48±2†

Nicotinic acid and nicotinamide were administered as a single subcutaneous dose of 2 mmoles/kg. There were eight rates in each group. *Serum free fatty acids (FFA), triglycerides and cholesterol were measured 15 min, 4 h and 24 h, respectively, after the drug had been given. †Significance of difference from control, < 0.001.

Table 2. Effect of nicotinic acid and nicotinamide on serum lipid levels in the dog

Triglycerides (mg/100 ml.) \pm S.E.* Total cholesterol (mg/100 ml.) \pm S.E. FFA (μequiv./l.) ± S.E.* Compound Control Treated Control Treated Control Treated Nicotinic acid 300 ± 52 188 ± 49 113 ± 4 Nicotinamide 344 ± 36 $211 \pm 21 + 163 \pm 4$ 75 ± 6† 105 ± 13 114 ± 9 76 ± 6‡ 116 ± 16 114 ± 12

Nicotinic acid and nicotinamide were administered as a single subcutaneous dose of 2 mmoles/kg to three dogs.

* Serum FFA, triglycerides and cholesterol were measured 1 h, 6 h and 24 h, respectively, after the drug had been given.

† Significance of difference from control, < 0.05.

† Significance of difference from control, < 0.001.

demonstrating the presence of a pathway for conversion of nicotinamide to nicotinic acid. This pathway may also exist in the dog and rabbit, which would be consistent with the in vitro data which indicate that the hypolipacmic effect of these substances is a consequence of the presence of free acids. The lack of antilipaemic activity of nicotinamide in man may mean that the conversion pathway does not exist. The demonstration of an antilipaemic action of nicotinamide in some species must be taken into consideration when explaining the mechanisms by which nicotinic acid and related compounds decrease concentrations of cholesterol. A vitamin effect of these substances cannot be disregarded yet.

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CYTOLOGY

Structural Basis of Quantitative Variation in Nuclear DNA

RECENT surveys show that variation in the amount of nuclear DNA, quite independently of change in chromosome number, is frequently associated with the divergence and evolution of both plant and animal species 1-10. The extent of such variation is often very great, particularly among the anglosperms. One of the many problems posed by these findings is the nature of the chromosome structural changes which give rise to the DNA variation. Two proposals have been put forward. The first is a differential polynemy, that is, a difference in the lateral multiplicity of DNA strands between chromosomes of different species4,9. While this proposal has many theoretical attractions there is, to date, no direct evidence in its favour. Indeed, Callan¹¹ has adduced evidence which argues strongly against this view. The second possibility is that the nuclear DNA variation is caused by lengthwise incorporation or loss of chromosome segments such as result from duplication or deletion. Observations in Lolium, and in Chironomus, hybrids at pachytene of meiosis and in polytene nuclei, respectively, show that the chromosomes of species with different nuclear DNA contents differ in respect of segmental duplications. There are therefore good grounds for attributing at least part of the DNA variation to such duplications. The following work in Allium provides further evidence to support this view. What is more, it shows that segmental duplications can account entirely for the DNA charges observed.

The nuclear DNA content of Allium cepa is about 27 per cent greater than that of A. fistulosum (Table 1). The A. cepa chromosomes, as would be expected, are correspond-

Table 1. AMOUNTS OF NUCLEAR DNA IN 2C ROOT TIP INTERPHASE NUCLEAR OF Allium cepa AND A. fistulosum

	1	NA (arbitrary unit	s)
	Replicate 1	Replicate 2	Mean
Allium cepa	33-5	33-5	33.5
Allium fistulosum	27.2	25.6	26-4
forguroments by Replace	nhotometru	from ton calle in on	ch ranlicate



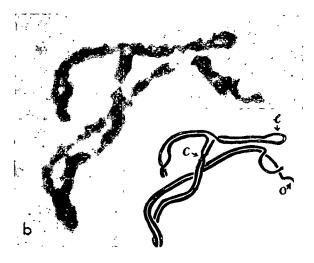


Fig. 1. a, First metaphase of meiosis in a pollen mother cell of A. $cepa \times A$. fistulosum. Note the asymmetry of bivalents. b, An isolated chromosome pair with an interstitial loop (l) and terminal overlap (a) resulting from duplication (or loss) of chromosome segments. The duplicated segments comprise about 35 per cent of the total length. c, Probable position of the centromere.

ingly larger than those of A. fistulosum. It can easily be shown that structural differences which account for the chromosomal DNA variation are spread throughout all members of the chromosome complement. This follows from the fact that in the species hybrid all bivalents at first metaphase of moiosis are asymmetrical (Fig. 1a) and all univalents of a pair are unequal. Measurements of chromosome lengths in the bivalents and among univalents show that the size and hence the DNA differences between homolog ous chromosomes range from about 10 per cent to 60 per cent. If these differences are completely accounted for by segmental duplications it follows that in each pair of chromosomes at pachytene we should expect extensive loops or overlaps comprising from 10 per cent to 60 per cent of the total length. Allium pachytenes do not lend themselves readily to a complete analysis. We have, however, isolated a number of different chromosome All the isolated pairs show loops and overlaps caused by duplications. An example is shown in Fig. 1b. Moreover, the duplicated seg nents make up the expected 10 to 60 per cent of the total length—sufficient to account entirely for the DNA variation without having to invoke a differential polynemy.

One of us (R. N. J.) received a Ministry of Agriculture postgraduate studentship.

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APPLIED SCIENCE

Tensile Strength of Wet Granular Materials

CARR¹ has explained the decrease in the tensile strength of wet granular materials with decreasing moisture content by assuming that the decrease in the number of liquid bridges, spanning the gaps between particles, outweighs the gain in strength of the remaining bridges as their volume is reduced. We have analysed this argument for a packing of equal spheres using our results for the properties of liquid bridges between equal spheres2 and for the spatial distribution of spheres in a random pack-These have been combined with the theoretical results of Fisher4.

The attractive force caused by a liquid bridge between equal spheres shows an approximate linear decrease with sphere separation from a maximum F_{max} , near contact, to zero, at the rupture separation x_r . There is a linear relation between x_r and the bridge neck diameter at contact, so that x_r may be obtained by extrapolation, knowing the properties of the bridge at contact² (Fig. 1). Fisher4 has calculated the attractive force and neck diameter for bridges at contact (Table 1). These results make it possible to construct force against separation diagrams for bridges of different volumes. These follow an approximate law.

$$F_x = F_{\text{max}} \left(1 - \frac{x}{x_r} \right) \tag{1}$$

where F_x is the force at separation x, x being expressed in sphere diameters. For convenience, the normalizing factors used by Fisher are used where appropriate.

The results on the random packing of spheres yield an approximate equation of the form3

$$N_x = 7.6 + 14.4 x \tag{2}$$

where N_x is the average number of spheres with surface separations less than a distance x around a central or datum sphere. This approximation holds from x=0 to about x = 0.5, so that

$$\frac{\mathrm{d}N}{\mathrm{d}x} = 14.4\tag{3}$$

We make the assumptions that every gap smaller than x_r in a wet sphere packing is spanned by a bridge, and

Table 1. BRIDGE RUPTURE SEPARATIONS RELATED TO $F_{
m max}$

Neck radius R	$\frac{F_{\max}}{2\pi R\gamma}$	x r
0.168	0.931	0.046
0.213	0.912	0.074
0.261	0.890	0.103
0.310	0.868	0.134
0.360	0.845	0.165
0.410	0.821	0.197
0.460	0.797	0.228
0.508	0.772	0.259
0.555	0.749	0.288
0.599	0.725	0.316
From Fi	Extrapolated from	
		Fig. 1

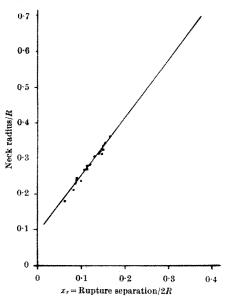


Fig. 1. Extrapolation of experimental rupture separations to larger liquid bridges.

that all bridges have equal volumes. x_r is dependent on bridge volume and hence on overall moisture content.

The total force of all the bridges acting on any sphere

total force =
$$7.6 F_{\text{max}} + \int_{0}^{x_r} \left(F - F_{\text{max}} \frac{x}{x_r} \right) 14.4 \, dx$$
 (4)

 $= F_{\max} \left(7.6 + 7.2 x_r\right)$

In tensile strength, only the force components in the direction of tension are involved. Thus the tensile strength o is equivalent to the average force exerted on a unit area of a sphere surface, therefore

$$\sigma = \frac{F_{\text{max}}}{4\pi R^2} (7.6 + 7.2 x_r) \tag{6}$$

where R is the sphere radius.

The attractive force F_{max} may be obtained from Fisher's normalized force which is equivalent to F_{max} $2\pi R\gamma$, and x_r may be obtained from Fig. 1 using Fisher's neck radius results. The moisture content of the granular material may be calculated knowing the number and volume of bridges in a packing. It is expressed as the percentage of liquid volume to solid volume in the packing. The results of these calculations are shown in Table 2. It is noticeable that σ remains roughly constant at about 3.65 γ/R dynes/cm² for a wide range of moisture content. This is about five times greater than experimental values and does not account for the apparent change in tensile strength with moisture content. This is because the decrease in the strength of the bridges with increasing moisture content is almost exactly countered by an increase in their number. Thus the original hypothesis

Table 2. Some tensile strengths of a bed of wet spheres (packing density, 0-625) calculated for different moisture contents using equation (6)

Fisher's olume of half each bridge R^3	$\frac{F_{\max}}{2\pi R \gamma}$	Extra- polated from Fig. 1	Number of bridges/ sphere = 7.5 + 14.4 xr	$\sigma \times \frac{R}{\gamma}$	Percentage of liquid by volume
0.000679	0.93	0.046	8-22	3.67	0.13
0.00178	0.91	0.076	8-67	3.70	0.367
0.00407	0.89	0.103	9.08	3.75	0.882
0.00826	0.87	0.134	9.53	3.73	1.88
0.0154	0.85	0.165	9-98	3.74	3.67
0.0265	0.82	0-197	10.44	3.68	6-60
0.0426	0.79	0.228	10.88	3.65	11.60
0.0647	0.77	0-259	11.33	3.63	17.40
0.0933	0.75	0.288	11.75	3.59	26.5

would seem to be inadequate for a quantitative explanation of experimental results. Our original assumptions might be a source of error. Presumably Carr's static method of forming wet beds of spheres would result in all the gaps below x_r being bridged, but the assumption of equal bridge volumes is more difficult to assess. If the bridges do reach a pressure equilibrium with each other. then the more highly curved bridges between contacting spheres would grow and decrease their curvature at the expense of bridges between non-contacting spheres. This should result in a decrease in tensile strength from that calculated here. Calculations using bridges of constant curvature rather than constant volume would yield quantitative information on this point.

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Recording the Initiation and Development of Failure in Timber

MICROSCOPIC examinations of fractures in materials have been restricted, in the past, to the detailed study of surfaces after failure has occurred; pre-failure development within the material has remained an elusive phenomenon. Thus the changes in structure which occur during stressing have usually been deduced from information obtained from a series of blocks each being subjected to different degrees of stress. While this approach can yield valuable information on the sequential changes in the internal structure of a material before failure, it lacks continuity, because it introduces both variations between samples and a degree of subjectivity in the interpretation of the sequence from the observed stages.

The need for continuous observation during stressing was evident from recent investigations into the changes in structure which occur in timber during compression stressing and fatigue, and a visual record of these has now been achieved by sandwiching thin radial longitudinal sections of wood between a 'Perspex' sheet which has a modulus of elasticity only one-half of that of wood, parallel to the grain. The sections, cut under carefully controlled conditions to eliminate the presence of artefacts induced during microtomy^{1,2}, measured 22 mm in length by 30µ in thickness. This thickness represented a compromise between the need for thin sections for photographic examination and thicker sections to provide enough support within the wood. A 30µ section contained fibres which possessed at least one radial wall in addition to the two tangential walls, while thinner sections would have included a number of cells without radial walls, thus permitting abnormal buckling of the cell walls during compression.

The 'Perspex' supports for the compression tests were cut from 0.8 mm sheet and were 6 mm wide, and waisted in the middle to 3.2 mm. The sections were mounted between supports using a chloroform-methacrylate cement. With two-thirds of the blocks, the centre portion of the section was embedded in immersion oil while the top and bottom parts were cemented, thus allowing the centre portion of the wood section to move independently of the Perspex' (Fig. 1). In the remaining blocks, the entire section was cemented between the 'Perspex' supports, but

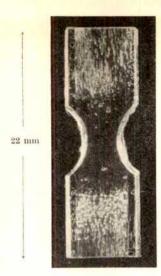


Fig. 1. Compression test piece containing a section of wood, the centre portion of which is immersed in oil while the ends are cemented.

tally between the jaws of the 'Instron', the microscope was placed at the side of the instrument and the load was transferred from the 'Instron' by fluid transfer to a compression plate attached to the mechanical stage of the microscope. This alteration provided greater flexibility in operation, particularly in microphotography, while still providing a stress-strain curve for the block.

The sequence of events occurring during compression was recorded on 16 mm ciné film and on 35 mm still film. A series of still photographs is shown in Fig. 2. Detailed results will be presented and discussed in a

later paper

This technique was designed primarily to obtain qualitative evidence to support results deduced from the examination of blocks subjected to different degrees of stress. Quantitative interpretation of the results in terms of stress is not possible, because neither the amount of interaction between the wood and the 'Perspex', nor the effective cross-section of the wood, is known. The approximate calculation of strain, however, is more feasible, because 40 per cent of the change in length of the block occurs in the waisted region. The first signs of dislocation within the fibre walls occurred at a strain of

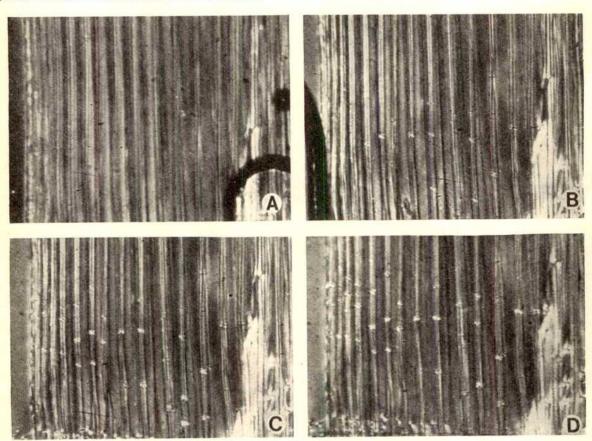


Fig. 2. Changes in the cell walls of Norway spruce timber subjected to increasing compression stress along the fibres. (A) Initial condition; (B) load 13 lb., slip planes just appearing; (C) load 21 lb., further development of these dislocations; (D) load 30 lb., preferred distribution in horizontal planes becoming apparent.

the evidence obtained from observation of these under compression indicated that at least part of the section was usually acting independently of the supports.

These blocks were compressed longitudinally in an 'Instron' testing machine, operating at a cross-head speed of 0.02 mm/min, and were viewed microscopically using a polarizing microscope and 150 W quartz iodine light source. Polarization microscopy is essential to observe the changes which occur in the orientation of the microfibrils in the cell wall. Because of difficulties in reducing the amount of vibration with the microscope slung horizon-

0.5 per cent and a stage corresponding to complete failure of the fibres occurred at a strain of 1.5 per cent.

This method is currently being used to study the changes in structure which occur during the fatigue of wood.

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BOOK REVIEWS

SCHOOL PHYSICS

Physical Science

A Study of Matter and Energy. By Verne H. Booth. Second edition. Pp. xvi+742. (New York: The Macmillan Company; London: Collier-Macmillan, Ltd., 1967.) 70s.

If the Schools Council has its way with the curriculum of the sixth form and reduces the number of subjects to be studied and externally examined to two, students entering the science and technology departments of colleges and universities might well have followed a course of the type which Dr Booth has devised for "non-science majors in colleges". Such a course is very different both in degree and in kind from those now followed in sixth forms and also from those now being tested by the "A" level teams of the Nuffield Science Teaching Project.

This American course exhibits a considerable and welcome breadth, even though it is limited to a study of matter and energy. Its design is well revealed by the sequence of section headings: the solar system; force and motion; molecules and energy; the electrical nature of matter; atomic structure and chemical combination; matter and energy in the study of the Earth; the energy within nuclei. Within these sections, the treatment is largely historical. For example, the first sections trace the growth of the theories of planetary astronomy from the early days to the time of Newton, interpolating a chapter on simple mathematical ideas and symbolism (ratio, proportion, graphical representation, vectors but not the calculus). Only after the simple kinetic model of an ideal gas has been considered is any aspect of chemistry treated. This is a familiar historical unfolding of the development of the atomic hypothesis from Boyle to Mendelyeev. Subsequent chemistry includes a lengthy treatment of the Bohr atom with little of later developments, and a descriptive and very brief chapter on carbon chemistry. Indeed, a somewhat critical chemist who examined the book was convinced that it must have been written by a physicist; on the other hand, a physicist was especially impressed by the section on the Earth sciences.

In a course of this nature, omissions are inevitable and must be welcomed. The debate must concern the criteria for inclusion and the author would seem to have had the question "What must I include?" in mind rather than the desperate "What dare I leave out?". Designers of new courses which the coming revolution in the sixth form will enforce would do well to follow his example in this respect. But it is to be hoped that no course developed on this side of the Atlantic would carry so little evidence that laboratory work by the student is an essential part of education in science.

MODERN IDEAS IN CALCULUS

Theoretical Analysis

By Lester J. Heider and James E. Simpson. (Saunders Mathematics Books.) Pp. xii+379. (Philadelphia and London: W. B. Saunders Company, 1967.) 59s. 6d.

THE Committee on the Undergraduate Program in Mathematics (USA) has recommended a course in calculus which shall emphasize the logical structure and the nature of mathematical proof. The authors of the present text, while attempting to meet this requirement, are careful to

explain that their book does not replace standard texts in advanced calculus, for skill in technique and applications must still be acquired; overemphasis of abstract concepts may reduce the average undergraduate to mathematical sterility. The reader is expected to have had at least a good first course in calculus.

The underlying notion is that of functional analysis, in metric or normed spaces. Thus the first six chapters lead to the Riemann and Riemann–Stieltjes integrals, as providing an ordered linear topological space with a norm (the integral) determining its topology. But this space is not complete, and the next step is to embed this space in a complete space by means of the Lebesgue integral. From the L^2 space, Hilbert space is easily reached in a chapter which goes as far as the famous functional extension theorem of Hahn and Banach. There is a short chapter on multiple integrals, and a final chapter tracing connexions with classical calculus: orthogonal polynomials, the Fredholm integral equation, the spectrum of a self-adjoint operator.

This is a very stimulating volume; but stimulants must be employed with care, and an external motivation might have to be strongly urged to save the weaker students from a hangover. The honours undergraduate interested in pure mathematics should be able to digest most of the book readily enough, especially as the discussions in the earlier chapters are taken quite slowly and in full detail. The authors also hope that teachers of mathematics in schools may find help here in bringing their knowledge up to date.

T. A. A. Broadbert

ELECTRON DIFFRACTION

Interpretation of Electron Diffraction Patterns

By K. W. Andrews, D. J. Dyson and S. R. Keown. Pp. xi+188+5 plates. (London: Hilger and Watts, Ltd., 1967.) 105s. net.

This book is intended for those who observe high energy electron diffraction patterns, particularly in a transmission electron microscope.

The first section contains sixty-three pages devoted to the theory and interpretation of electron diffraction patterns from crystalline materials. When discussing the determination of unknown crystal structures the authors only consider numerical methods and make no reference to Bunn charts. Moiré fringes are mentioned but are not explained. This subject is of sufficient impertance to merit detailed consideration. Alternatively a reference should have been made to a suitable book such as that by Hirsch et al.¹. The subject matter of this section is not always presented in a manner which would be readily intelligible to a beginner; for example, Laue zones and reciprocal lattice streaks are discussed before the intensity formula is given from which such effects may be deduced.

The second section contains seventy-seven pages of well presented information about plane spacings, interplanar and interzonal angles, angles required for the construction of stereographic projections and standard diffraction patterns with and without double diffraction. The information applies to tetragonal, hexagonal, rhombohedral and cubic crystals. Allowed diffraction maxima are listed for the various cubic lattices (primitive, b.c.c. and so on) and many stereographic projections are given. These illustrate, among other things, twinning in cubic crystals and the various inter-relations between lattices in martensitic transformations. A table of electron scattering factors is not given.

The third section contains information of the type given in the previous section, but it applies to specific materials such as metals, metallic carbides and intermetallic compounds. The relation between cementite and ferrite is considered. The book, which concludes with a description of a stereographic plotting table, contains fifty-seven references and an adequate index. It is a useful addition to the literature of high energy electron diffraction.

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¹ Hirsch, P. B., Howie, A., Nicholson, R. B., Pashley, D. W., and Whelan, M. J., Electron Microscopy of Thin Crystals (Butterworths, London, 1965).

THEORETICAL PLASMA PHYSICS

Theoretical Methods in Plasma Physics

By N. G. van Kampen and B. U. Felderhof. Pp. ix + 215. (Amsterdam: North-Holland Publishing Company, 1967.) 160s.

THE authors do themselves less than justice in their preface to this book. They imply that this is a book for the theoretical rather than the plasma physicist, in which plasma physics is seen not so much as a subject in its own right but rather as a vehicle on which to exercise a variety of theoretical methods. This generated, for me, a bias against the book which was only dispelled when going through the text.

It is true that such a formal approach leads to an emphasis on general consequences at the expense of those details which yield a physical insight into plasma behaviour, but this fault is partly overcome by the choice of explanatory problems (with hints on their solution) which occur appropriately throughout the text and not

at the end of the chapters.

The authors admit that they have chosen topics which strongly reflect their own research interests; they are known for their work on the Vlasov equation and discuss this extensively together with its application to plasma wave theory. On the other hand, such subjects as turbulence and radiation emission, where the insight and guidance of theoreticians are so valuable, are missing.

A plasma is treated initially as a continuum fluid; the equations of magnetohydrodynamics are developed and solutions, dependent on and independent of time, are discussed, as well as instabilities. The rest of the book considers the plasma in terms of individual particles. Three chapters on the statistical mechanics of an ionized gas, two component and multi component theory, are followed by five chapters based on the Vlasov equation.

A theoretician reading himself into plasma physics could well start with this book; it can also be recommended to any worker in this field as a theoretical treatment to be followed without too much difficulty.

JOHN PAIN

QUANTUM MECHANICS AND NMR

Structure of High-Resolution NMR Spectra

By P. L. Corio. Pp. xi+548. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 120s.

This well produced volume results from the expansion of a widely read review article which appeared in 1960. The first eight chapters deal with the theory, which is illustrated by some opportune examples, of single quantum transitions in the presence of a linearly polarized rf field. The final chapter provides an introduction to multiple quantum transitions, double resonance and a new section on spin-echo experiments. A collection of numerical data for some two-group systems forms an appendix. This is based on the original article, but it should be noted that the numbering of some of the transitions has changed and a new spin system, A₄B, has been added.

All of the mathematical apparatus required for the analysis of high resolution nuclear magnetic resonance (NMR) spectra is thoroughly developed. Few books adequately cover this aspect of the subject, and so this

volume should appeal to research workers who may wish to advance their knowledge of quantum mechanics and applications to high resolution NMR. It should also be stated that the mathematically inept will not find it easy reading on the first occasion.

It is unreasonable to expect the theoretical and practical aspects of the subject to be treated in depth in a single volume, and so it is appropriate for this book to concentrate on the former and leave the more detailed aspects of the latter to others. The book has adequate author and subject indices and each chapter has a bibliography, but there is no reference to spin systems containing more than three groups of nuclei.

On the understanding that the book could be used either in a course in high resolution NMR or in an elementary course in quantum mechanics it is to be recommended.

G. A. WEBB

FAILURE OF A MISSION

Crganic Chemistry of Macromolecules

An Introductory Textbook. By A. Ravve. Pp. xiii + 498. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1967.) 126s. net.

In his preface the author sets out his objectives in writing this book. It is intended to be an introductory textbook covering "the most important and interesting aspects of organic chemistry of macromolecules" which, it is anticipated, "will be useful in an advanced course in polymer chemistry". "Some brief excursions into physical chemistry (kinetics, molecular weight determinations, and so forth) are undertaken to facilitate the discussions." "No attempt has been made to present an exhaustive review of the literature. What is offered instead is a selection of references which will present each subject; this was done, of course [sic], in an arbitrary fashion." "Greater attention is given to synthetic macromolecules than to naturally occurring ones. This is done on the assumption that most organic chemists are more concerned with synthetic materials."

Many readers will be disappointed at the scant treatment allotted to natural macromolecules (fifteen pages on polysaccharides, nineteen on proteins, seven on polynucleotides) and all will be alarmed at the admittedly apparently arbitrary selection of references. Nevertheless, a really good guide to the organic chemistry of synthetic macromolecules, a subject so important to the chemical industry and so neglected in most university courses, is badly needed and in undertaking the writing of such a book the author gave himself an opportunity of making chemistry and chemists deeply indebted to him. Unfortunately, he has not succeeded in his task and the book he set out to write still needs to be written.

The general plan of the book (an introduction, mainly on physical properties; mechanisms of polymerization reactions; addition polymers; condensation polymers; naturally occurring polymers; reactions of polymers) is

good, but its execution is not.

The book contains, without doubt, a mine of information on its subject; unfortunately the mine has not been worked. The presentation is unclear, jumbled and confused; for example, the third chapter, on the molecular weights of polymers, leaves one little wiser after reading than one was before. The following verbatim quotations are a few examples of obscurity: page 3, "The opinion of the majority of that day was that Raoult's solution does not apply to materials in colloidal state"; page 33, "At that point the minimum takes an upturn"; page 40 "Thus arises the need to work with concentrations below 1 per cent and in many cases down to several tenths o 1 per cent"; page 111, "Ethylene was then bubbled into this suspension for perhaps 30 minutes, sometimes a room pressure or at a few atmospheres"; page 113

"Generally, most such heterogeneous systems are active at relatively high temperatures (room to 100° C)". There are also plenty of what I assume to be uncorrected misprints, for example on page 24, "polyacrylonite"; page 39, "a polymer of glucose ($C_6H_{12}O_{6n}$); and page 47, "where π is the constant 3·214". I am reminded of the first draft of a PhD thesis by a student whose native tongue is not English. Such a student is made to rewrite and rewrite; it is a pity the publishers did not demand as much of the author of this book.

Far from being stimulated by this book, those who read it are more likely to be repelled, and what might have been a real service to organic chemistry has a good chance of turning out to be a real disservice. The best one can hope is that this book will quickly be forgotten and that someone else will do what this author has so singularly failed to do.

H. N. RYDON

MORE SNELL AND SNELL

Colorimetric Methods of Analysis

Including Photometric Methods. Vol. 4A. By Foster Dee Snell and Cornelia T. Snell. Pp. ix+645. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1967.) 140s.

Any analyst who has to deal with a wide diversity of samples and finds colorimetric or spectrophotometric methods sufficiently accurate for his purpose is bound to have Snell and Snell within arm's reach of his bench. Others who have occasional need to deal with samples outside the narrow range of daily routine will have Snell and Snell on their library shelves. And it is to Snell and Snell that the analyst will first refer colleagues seeking advice on particular determinations. This work is, in short, the established source for analytical procedures based on colour reactions. Such are the convenience and popularity of spectrophotometric methods that the flow of papers describing new methods or reagents or determinations of new products or of old products in new contexts continues to increase. To keep abreast of the literature is a formidable task indeed, while to make any sort of critical evaluation is wellnigh impossible. Developments in the subject have, of course, meant that some of the material in the four original volumes of this work has been superseded by improved methods or new methods, and advances in science and technology have posed new problems, solutions to which are not to be found in the early volumes of this edition. The heroic feat of updating the second and third volumes has been completed, and is here in part accomplished for the fourth volume. But so extensive is the literature to be covered that it has been found necessary to split the supplementation of this fourth volume into two parts, and volume IVA covers the advances of the past twelve years relevant to the first seven chapters of volume IV, dealing with organic compounds of non-cyclic nitrogen. The six chapters deal with nitrates, nitrites and nitro compounds; aliphatic amines and amides; amino-acids; proteins; aromatic primary, secondary and tertiary amines and amides; and azo compounds, nitrogen containing cycles and other systems. A selection has been made of some 1,383 references for detailed treatment, but many more citations are made in

The treatment is terse and severely practical. General discussion is sharply pointed and principles are stated with the utmost economy. Sample treatment and determination procedure for each compound are given tersely but in full working detail such that a technician, in consultation with an analyst during the initial stages, can work directly from the text. The wealth of information compressed into this volume makes it very good value, and it will be particularly welcomed by clinical, pharmaceutical, agricultural, public health and biochemical

workers as well as by organic chemists in general. Neither praise nor criticism of so compendious a work is viable, one can only admire and return thanks for the industry of the authors and the high standard they have maintained.

E. BISHOP

AUTOIMMUNITY

Autoimmunity: Clinical and Experimental

By J. R. Anderson, W. W. Buchanan and R. B. Goudie. (A Monograph in the Bannerstone Division of American Lectures in Living Chemistry.) Pp. xv+485. (Springfield, Illinois; Charles C. Thomas, 1967.) \$19.50.

The great expansion of interest in all aspects of immunology which has occurred during the last decade has been particularly dramatic in the case of autoimmunity which appeared so paradoxical to the classical immunologist. It would be premature to suggest that this paradox is completely resolved, yet it is obvious that the study of autoimmunity promises to be very fruitful to the immunologist and is also becoming increasingly relevant to clinical medicine. This new book by three of the Glasgow group who have contributed so much to the development of the subject reflects the diversity of the problems as well as the variety of disciplines which autoimmunity encompasses.

Although the subject matter is necessarily similar to that of other recent books on autoimmunity, the organization of the material is somewhat novel. The book is in three sections, the first of which is mainly concerned with the fundamentals of immunology and autoimmunity. The basic concepts and nomenclature of immunology are introduced in a clear and simple way before the experimental and theoretical aspects of autoimmunity and its relation to disease are considered in more detail. Possible mechanisms of autoimmunity are then discussed with particular regard to the role of the thymus.

The correlation of autoimmunity with disease occupies the rest of the book and is divided between the organspecific diseases and the connective tissue diseases. Work on all the immunological aspects of these diseases is thoroughly surveyed and where possible significant correlations are made. The total amount of information is very great, but there is still insufficient evidence to allow firm conclusions to be drawn about the pathogenesis The authors, however, have of autoimmune disease. critically assessed the relevance of the existing evidence and have been able to draw limited conclusions. Further progress has been achieved since this book was prepared, but this does not substantially detract from an excellent survey of the subject presented in a clear and stimulating style. The printing and photographic reproductions are excellent and the twelve hundred references should provide J. E. FOTHERGILL adequate further reading.

INSIDE HYDRA

The Cell Biology of Hydra

By Th. L. Lentz. Pp. xi+199. (Amsterdam: North-Holland Publishing Company, 1966.) 40 guilders; 80s. This is primarily a research monograph recounting the author's own observations. While these are mainly concerned with the fine structural morphology of hydra, there is material on enzyme histochemistry, nematocyst discharge and the role of the nervous system in regeneration. The author thus does not attempt to provide a balanced review of the cell biology of hydra and he is very uncritical not only of his own work but also of that of others. For example, when dealing with regeneration and growth of hydra, he presents, in the main, only his observations which he suggests point to the presence of a growth stim-

ulating or form regulating substance in neurosecretory granules, which is intimately involved in growth and regeneration. This view is based in part on the effect of neuropharmacological agents on regeneration, which I would regard as remarkably unimpressive and inconclusive. The most useful material in the book is the electron microscope observations, which provide a convenient and detailed structural description; the summarizing diagrams are particularly clear. There are also some interesting suggestions and observations on the control of nematocyst discharge.

For the research worker dealing with hydra this is quite a useful but uninspiring book. It cannot be recommended for other biologists. The many cell biology problems posed by hydra remain largely unexplored and even the problems of form, growth, regeneration, behaviour, communication and osmoregulation are undefined. If one is to write a book on a topic of considerable interest based mainly on one's own research work, then this work ought to be seminal, or at least well above average. The work described here is not.

L. WOLPERT

TRACKING BRITISH MAMMALS

Mammals of Britain

Their Tracks, Trails and Signs. By M. J. Lawrence and R. W. Brown. Pp. 223+32 plates. (London: Blandford Press, 1967.) 30s. net.

APPEARING so soon after the Handbook of British Mammals, a new book on the subject calls for some justification by way of fresh information and novel treatment. useful book passes the test by reason of its concentration on drawings of the footprints and droppings of mammals and inclusion of many more drawings of skulls and teeth. The basic information about each species being less than is given in the Handbook, one still inevitably turns to the latter as a reference book, but Lawrence and Brown have produced a handy companion to the larger work. Another different feature is the distribution maps given for some (but not all) of the wild species (domestic animals are also included). These, I feel, do not always come off; some of them, such as otter, pine marten, Leisler's bat and mole. merely illustrate how very difficult it is to put imperfect information into map form. The authors are not, of course, to blame for the imperfection of the information, which is the fault of all British mammalogists, but they have tended to give an impression of precision where vagueness should have been the order of the day. And an attempt to show vagueness on a map usually ends in

I am also struck by how hard it is nowadays to produce a really up to date book on British wildlife. New facts are constantly emerging or being discovered, and no book can be completely up to date, even given perfect distribution of new knowledge. In this book, for instance, one naturally looks first to the mink, the grey long-eared bat and the coypu, and in each case events have moved on since the book was written, doubtless 18 months or so before it was published. But by the same token the authors are that much more up to date than the *Handbook*, published in 1964.

R. S. R. Fitter

OBITUARIES

Sir Cyril Hinshelwood

Sir Cyril Hinshelwood died at his London home during the evening of October 9. A few hours earlier he had talked with one research student and had written to another to arrange a discussion about a joint publication His death came as a shock to his many friends who have seen him so relaxed and happy during recent years when free from administrative duties, he was able to enjoy his laboratory work and pursue his many other interests.

Hinshelwood was recognized internationally as one o the finest intellects of our time, a polymath, a descendanof those men described in John Evelyn's diary as "My lords virtuosi"—the early Fellows of the newly born Royal Society, over which he was to preside with distinction. Born in 1897, he was educated at Westminster City School, went to Balliol College, Oxford, in 1919 with a Brackenbury scholarship, and gained distinction in the shortened post-war course in 1920. After a year as a fellow of Balliol, he became a fellow and tutor of Trinity and in 1937 succeeded Professor F. Soddy as Dr Lee's professor of physical and inorganic chemistry. On retirement in 1964, he returned to his native London as a senior research fellow of Imperial College. During his professorship, physical chemistry at Oxford reached full bloom. The move was made from the cellars and outhouses of Trinity and Balliol to new laboratories, and among his staff were nine Fellows of the Royal Society leading research groups in diverse fields.

From 1916-19 he worked at the Queensferry Royal Ordnance factory on explosives, where his remarkable ability was quickly noticed. This work stimulated his interest in the mechanisms of chemical change, which was to be the main theme of nearly all his scientific research. Throughout, he showed a mastery in planning simple measurements to answer questions of great complexity. His earliest papers dealt with the velocity of decomposition of solid substances and of reactions of gases on metallic catalysts. He soon turned to homogeneous gas reactions with special reference to theories of molecular In 1926 he interpreted some first order activation. decompositions of organic molecules as quasi-unimolecular processes involving activation by collisional mechanisms. Soon afterwards, from kinetic measurements he established the main characteristics of thermal chain reactions. The discovery of critical explosion limits, similar to those found by Semenov in other systems at about the same time, led to new ideas about straight and branching chains. The effects of catalysts such as iodine on some of these gas reactions, of inhibitors such as nitric oxide or propylene, and of deuterium were used to clarify other details. In those early years, the origin and nature of the chain carriers were obscure, and Hinshelwood sought, with meticulous care, to establish their identity. final account of the hydrogen-oxygen reaction, given in the Bakerian lecture (1946), was the culmination of a long exemplary application of the scientific method to disentangle the intricacies of this process. Later, he studied the mechanism of thermal decomposition of hydrocarbons, and the polymerization of some unsaturated compounds. Over many years, too, he examined the kinetics of a variety of reactions in non-aqueous solvents and in the liquid phase. Here again, the main aim was to establish the significance of the constants in the Arrhenius equation and the functional relationships between them. He analysed the different approach in his own work and that of others using the so-called transition state theory, but emphasized clearly the fundamental relationships between the different methods. His book on The Kinetics of Chemical Change appeared, in a kind of evolutionary way, in four editions between 1926 and 1940.

In 1938 he used the kinetic approach to study physicochemical aspects of bacterial growth, and this subject perhaps remained his major love to the end. The influence of added substances on the different phases of the growth curve, the adaptation of bacteria and their resistance to drug action caused by changes of media, the take-up of alkali metals or of phosphorus and the effect of different carbon sources, were examined. The living cell was con-

ceived as a complex assemblage of chemical reactions, each subject to the same laws. "One of the sources of the each subject to the same laws. "One of the sources of the richness and subtlety shown by the behaviour of living matter," he wrote, "is the way in which, in the individual, physico-chemical laws can play variations on the fundamental theme expressed by the genetic code, while populations can also respond in another way to rarer and more catastrophic changes in the code itself." He discussed these laws in many papers and in two books, The Chemical Kinetics of the Bacterial Cell (1946) and (with A. C. R. Dean) Growth, Function and Regulation in Bacterial Cells (1966). The question of cellular regulation was a recurring theme which found expression in what he called "the principle of total integration" and the "network theorem". These expressed in mathematical terms the mutual interdependence of all the components in the intact functioning cell. He had been asked for a non-mathematical presentation. Two days before his death, he wrote of his intention to do this, "pointing out where, which is in most places, the model is not in conflict with other descriptions, but adds something of its own". He was not unaware that his incursions into biology had encountered some scepticism among a few of the more traditional professional biologists. In his elegant book The Structure of Physical Chemistry (1951) he presented a unified, coherent story in his own critically philosophic way. His first book, Thermodynamics for Students of Chemistry (1926), is another, less known, classic.

Hinshelwood was in turn president of the Chemical Society, Faraday Society, Royal Society, the British Association, and of the Classical Association; chairman of the Research Committee of the Gas Council, a delegate of the Clarendon Press, chairman of the Council of Queen Elizabeth College, London, a trustee of the British Museum, and on the court of the Goldsmiths Company. He served on many governmental advisory committees. His honours included the Davy, Royal, Copley and Leverhulme medals of the Royal Society, the Longstaff and Faraday medals of the Chemical Society, the Nobel Prize for Chemistry (shared with Semenov) and many medals given by foreign organizations. He received honorary doctorates from about a dozen universities, was a member of many foreign academies, and an honorary fellow of four Oxford colleges. In 1960 he received the Order of Merit.

To his fluency in numerous European languages there was added a competence in Russian and Chinese, with a little Arabic. He had a wide knowledge of English and foreign literature, of operatic music, of Chinese porcelain and Persian carpets, and was an accomplished painter in oils. He often contributed papers to the Oxford Dante Society. He was regarded with affection and respect by his colleagues, and by undergraduates in both the sciences and the humanities. In all his lectures and writings there was an unmistakable mark of the natural philosopher. None was more fitted than "Hinsh" to drink the traditional toast of the Royal Society's dining club: "Arts and Sciences". H. W. T.

Dr Pierre Jacquet

PIERRE JACQUET and his wife died tragically at sea near Puerto de la Selva, Spain, during a trip in their cabin cruiser on September 6, 1967. Jacquet was born in 1906 at Saint-Mande (Seine), graduated as a chemical engineer from the Institut de Chimie, Paris, in 1926, and obtained his doctorate in 1938. He was well known throughout the world as the father of electropolishing, a phenomenon which he discovered in association with H. Figour in 1929.

He first described this process in the Comptes Rendus in 1935 (201, 1473), and spent the rest of his scientific life investigating fundamental aspects of the phenomenon, applying it to a wide range of metals and alloys, and encouraging its industrial application. This work is

recorded in more than 200 papers published in many journals in France and elsewhere. It is no exaggeration to say that electrolytic polishing has revolutionized not only the practice of metallography by providing the most scientific way of preparing polished surfaces free from strain and blemishes, but also methods of surface preparation in industry where it is now extensively used for polishing and machining. In addition, the present techniques of electron microscopy of metals and alloys are almost entirely dependent on methods pioneered by Jacquet. In recent years Jacquet developed the technique further so that it could be used in situ on larger structures or objects, thus initiating a new approach in non-destructive metallography. A detailed list of the fields in which Jacquet has made effective contributions would include most of the important aspects of physical metallurgy. Much of his early work was done in the Research Laboratory of the Société, le Materiel Téléphonique, but since 1945 he worked in the Research Laboratory of the French Navy, from which he had only recently retired.

His scientific work has been recognized by many awards including the Berthelot Medal (1956), the Jaffe Prize of the French Academy, and the Le Chatelier Medal of the Société Française de Métallurgie. He was a Chevalier de la Légion d'Honneur and an Honorary Foreign Member of the American Academy of Arts and Sciences (1956).

Jacquet will be long remembered as a modest, retiring man who by firm conviction and a long scientific career at the laboratory bench made outstanding contributions to metallurgical science and technology.

R. W. K. HONEYCOMBE

Dr Francis Silsbee

Francis Briggs Silsbee, who died on August 21, aged 78, retired as Chief of the Electricity Division of the US National Bureau of Standards in 1959. He had made many contributions to electrical engineering.

Silsbee graduated in electrical engineering from the Massachusetts Institute of Technology in 1910 and obtained a master's degree the following year. In 1915 he obtained his doctorate from Harvard University. He joined the National Bureau of Standards in 1911, and continued to act as a consultant after his retirement, when he was interested in the history of the scientific work of the bureau. He was chairman of a committee whose work led to the establishment of the NBS Museum. which exhibits instruments made during the 66 years of the bureau's existence.

Silsbee's contributions to electrical engineering included involvement in the design of alternating current resistors of exceptionally low reactance. He also invented the test set which is used by industry to compare working current transformers with standard transformers of known ratio and phase angle. During the Second World War, he investigated lighting hazards to aircraft for the National Advisory Committee of Aeronautics. As chief of the Electricity Division at the National Bureau of Standards he was concerned with establishing electrical units and standards, and took part in committee work with the International Electrotechnical Commission and other standardizing organizations. In 1956 Silsbee was sent to Ethiopia by the International Co-operation Commission to advise the government on the establishment of a national system of measurement standards.

Silsbee was a fellow of the Institute of Electrical and Electronics Engineers, a fellow of the American Physical Society, and a former president of the Washington Philosophical Society and the Washington Academy of Sciences. In 1963 he received the Morris E. Leeds Award for his contribution to the methods of improving the accuracy of commercial electrical measurements. He also received Exceptional Service Awards from the Department of Commerce and the Naval Bureau of Ordinance.

University News:

Bristol

DR J. B. CHAPPELL, reader in biochemistry in the university, has been appointed to the second chair of biochemistry, and Professor D. L. Dinely, professor and chairman of the Department of Geology in the University of Ottawa, has been appointed to the Chaning Wills chair of geology.

Liverpool

DR A. D. BRADSHAW, reader in agricultural botany in the University of North Wales, Bangor, has been appointed to the Holbrook Gaskell chair of botany, and Dr H. D. Parbrook, at present reader in charge of the Department of Building Science at Liverpool, has been appointed to the chair of building science.

Sussex

MR J. F. Scott, at present reader in mathematics and statistics at the university, has been appointed to a personal chair of applied statistics.

Weizmann Institute of Science

PROFESSOR M. FELDMAN, dean of the institute's Feinberg Graduate School, has been appointed the first holder of the Klutznick chair in developmental biology. This chair was established in honour of Mr P. M. Klutznick on his sixtieth birthday.

Appointments

DR S. C. DAUBIN, head of the Marine Sciences Section of General Motors Corporation at Santa Barbara, California, has been appointed senior scientist and chairman of the Department of Ocean Engineering in Woods Hole Oceanographic Institution.

Announcements



PROFESSOR P. M. S. BLACKETT has been appointed a member of the Order of Merit, the tenth scientist of a possible membership of twenty-four.

SIR ERIC ASHBY, vice-chancellor of the University of Cambridge and Master of Clare College, has accepted an invitation to become president of the Society for Research into Higher Education, in succession to Lord Fulton. Sir Eric will take the chair at the society's third annual conference to be held in Senate House, University of London, on December 14.

THE inaugural meeting of the Fisheries Society of the British Isles was held on October 21 at the Zoological Society of London and the following officers were elected: President, Dr J. W. Jones; Vice-president, Mr P. H. Tumbleson; Secretary, Dr L. E. Mawdesley-Thomas; Treasurer, Mr A. Wheeler. The society's first meeting will be on April 26, 1968, at the Zoological Society and further details can be obtained from the Hon. Secretary, Department of Pathology, Huntingdon Research Centre, Huntingdon.

THE Royal Society has awarded the three Royal Medals for the current year to the following: Professor C. E. Tilley, emeritus professor of mineralogy and petrology in the University of Cambridge, for his contributions in all branches of petrology; Professor J. Z. Young, professor of anatomy in University College, London, for his researches correlating neural structure with function; Sir Joseph Hutchiason, Drapers professor of agriculture in the University of Cambridge, for his work on the genetics and evolution of crop plants with particular reference to cotton.

THE president and the council of the Royal Society have awarded the following medals: the Copley Medal to Professor B. Katz, professor of biophysics at University College, London, for his contributions to knowledge of the fundamental processes involved in transmission across the neuromuscular junction; the Davy Medal to Professor V. Prelog, head of the Laboratory of Organic Chemistry, Eidgenössische Technische Hochschule, Zurich, for his work in the development of stereochemical concepts and on the structure of alkaloids and antibiotics; the Buchanan Medal to Sir Graham Wilson, director of the Public Health Laboratory Service of England and Wales from 1941-63, for his work on the medical aspects of bacteriology and immunity; the Sylvester Medal to Professor H. Davenport, Rouse Ball professor of mathematics in the University of Cambridge, for his contributions to the theory of numbers; the Hughes Medal to Dr K. A. G. Mendelssohn, reader in physics in the University of Oxford, for his contributions to cryophysics, especially his discoveries in superconductivity and superfluidity.

THE Ramsay Memorial Fellowships Trustees will consider in March 1968 applications for one Ramsay travel grant not exceeding £350 for the year 1968–69. The grant will be limited to junior academic chemistry staff of universities and colleges of technology or advanced technology in Scotland. Further information can be obtained from the Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, London WC1.

CORRIGENDUM. In the communication "Tetragonal and Hexagonal Iron–Manganese Carbides" by M. J. Duggin (Nature, 216, 362; 1967), the heading of the eighth column of Table 4 should be a, of the fourth and ninth columns should be c and of the tenth column should be a/c. The penultimate sentence of the last paragraph should refer to an "increase of approximately 15·3 per cent in the cell volume of the hexagonal carbide".

ERRATUM. In the article "Amide Cotton Effects of Heparin" by Audrey L. Stone (Nature, 216, 551; 1967), refs. 1–5, not 2–5, should be mentioned at the end of the third paragraph. In the third line of the legend to Fig. 1, 30 sec/period should read 30 sec pen-period. In the fifth line of p. 553 absorption should read rotational, and the sentence beginning on the twenty-third line of the next paragraph should end ". . . however, at low ratios of histamine to heparin the ORD changes in the π - π region are similar to those with decreasing pH".

EBRATUM. In the article "More About Chain Initiation". by our Cell Biology Correspondent (Nature, 216, 638; 1967), the seventh sentence of the second paragraph should have read "Once the initiation complex has formed, a 50S sub-unit couples with it to produce a 70S ribosome attached to mRNA and with the chain initiator in place."

ERRATUM. In the issue of November 4 (Nature, 216, 425; 1967) Professor R. J. Smeed was misquoted as saying that road accidents cause 8 per cent of all male deaths. Although the proportion varies widely—and is as high as 50 per cent in the 15 to 19 age group—only 2 per cent of all male deaths are caused by road accidents.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 27

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2) -Colloquium on "Energy from Natural Gas".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 1.15 p.m.—Professor L. Pearce Williams: "Physics and Philosophy in the Early Nineteenth Century".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr C. S. E. Phillips: "Computers and Adaptive Radar".

INSTITUTION OF ELECTRICAL ENGINEERS (Joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Systematic Approach to Design" opened by Mr R. L. Latham and Mr G. J. Terry.

Tuesday, November 28

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 1 p.m. Dr W. H. Brock: "William Bollaert, Faraday and the Royal Institution".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, SW7), at 1.30 p.m.—The Reverend Professor C. F. Evans: "A Scientific View of the Gospels" (further lectures on December 5 and 12).*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Coding in the Visual Nervous System" opened by Mr A. Robertson.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5.30 p.m.— Sir Lawrence Bragg, FRS: "How we Hear, Speak and See". (Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on November 29, and December 5 and 6.)

UNIVERSITY OF LONDON (at the Institute of Child Health, Gullford Street, London, WG1), at 5.30 p.m.—Dr V. Eisen: "Kinin Formation in Human Disease". (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Watford College of Technology, Watford), at 6.30 p.m.—Mr F. J. M. Laver: "Computers".

SOCIETY FOR ANALYTICAL CHEMISTRY, PARTICLE SIZE ANALYSIS GROUP (in the Physics Lecture Theatre, Imperial College, London, SW7), at 6.30 p.m.—Second Annual General Meeting, followed by an Ordinary Meeting on "Light Scattering Methods".

Tuesday, November 28-Wednesday, November 29

INSTITUTE OF WATER POLLUTION CONTROL (at Church House, Westminster, London, SW1)—Symposium on "Water Pollution Control".

Wednesday, November 29

SOCIETY FOR ANALYTICAL CHEMISTRY, THIN LAYER CHROMATOGRAPHY GROUP (at the Borough Polytechnic, Borough Read, London, SE1), at 2.15 p.m.—Third Annual General Meeting, followed by Scientific Papers.

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, WC2), at 2.30 p.m.—Mr G. Ordish: "Scientific Pest Control and the Influence of John Curtis" (John Curtis "Woodstock" Lecture).

INSTITUTION OF MEGHANICAL ENGINEERS, LUBRICATION AND WEAR GROUP (joint meeting with the Institute of Physics and the Physical Society; the Institution of Metallurgists; and the Royal Institute of Chemistry, at 1 Birdcage Walk, Westminster, London, SWI), at 4 p.m.—Discussion Meeting on "Tribology—Its Inter-Disciplinary Challenge".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr D. J. Williams, Mr L. J. Allen and Dr T. A. Davies: "Contacts in Telephony—a Search for the Ideal".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr W. J. Nicholls and Mr F. G. McDonald: "Aluminium Conductors for Overhead Lines".

UNIVERSITY OF LONDON (In the Anatomy Theatre, University College. Gower Street, London, WC1), at 5.30 p.m.—Dr G. A. Horridge: "The Compound Bye: Being an Analysis of what Arthropods See, as based upon the Electrophysiological Analysis of the Retina of Apposition Eyes and of the Optokinetic Response" (further lectures on November 30 and December 1).*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 8-9 Bedford Square, London, WC1), at 6 p.m.—Professor K. W. H. Stevens: "Generation, Propagation and Applications of Microwave Acoustics".

UNIVERSITY OF LONDON (at the Institute of Neurology, National Hospital, Queen Square, London, WC1), at 6 p.m.—Mr V. Logue and Dr H. Davson: "Cerebrospinal Fluid: Hydrocephalus and Mechanics—Clinical and Biochemical Aspects".

Thursday, November 30

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m. Professor R. J. Harrison: "The Living Cell" (Civil Service Lecture).

ROYAL SOCIETY (at 6 Carlton House Terrace, London, SW1), at 2.30 p.m.-Anniversary Meeting.

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr D. A. Price Evans: "Genetic Factors in Drug Therapy". (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Planning and Progressing in Site Construction".

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP (at 14 Belgrave Square, London, SW1), at 6.15 p.m.—Dr A. Spicer: "Fats in the Baking Industry".

UNIVERSITY OF LONDON (at Wye College, Wye, near Ashford, Kent), at 8.15 p.m.—Miss Sylvia Crowe: "Design for a Changing Landscape".*

Thursday, November 30-Friday, December 1

ROYAL PHOTOGRAPHIC SOCIETY OF GREAT BRITAIN (at the Institution of Electrical Engineers, Savoy Place, London, WC2)—Symposium on "Photographic Granularity".

Friday, December I

INSTITUTE OF BIOLOGY (in the Wellcome Lecture Hall, Royal Society. 6 Carlton House Terrace, London, SW1), at 10.30 a.m.—Conference on "Improving the Contacts Between Industry and University".

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p.m.—Dr B. Broklehurst: "The Naphthalene Dimer Cation $(C_{10}H_9)^+_3$ ".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2). at 5.30 p.m.—Dr A. Fettweis: "Resonant Transfer".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at Savoy Place, London, WC2), at 5.30 p.m.—Mr D. D. Lipman: "Experiments in the Use of Digital Systems for Air Traffic Control at the Eurocontrol Experimental Centro".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 9 $\rm p.m.$ -Professor Hermann Bondi, FRS: "Gravitation".

Saturday, December 2

BRITISH MYCOLOGICAL SOCIETY (at the Linnean Society of London, Burlington House, Piccadilly, London, W1), at 2 p.m.—Annual General Meeting. followed by Professor J. L. Harley, FRS: "Fungal Symbiosis" (Presidential Address)

Iollowed by Professor J. L. Harley, Eds. Funga: Symbols (Address).

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3.30 p.m.—Miss P. M. Sears: "George Baxter of Sydenham—the Victorian Colour Printer".*

Monday, December 4

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Institution of Heating and Ventilating Engineers, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "The Commissioning of Engineering Services in Large Buildings" opened by Mr P. A. Flanigan and Mr W. C. Jeffreys.

SOCIETY OF ENGINEERS (at Burlington House, Piccadilly, London, W1), at 6 p.m.—Mr Brian J. Bell: "Reinforced Plastics".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, SW1), at 6.30 p.m.—Professor C. F. Cullis: "Recent Developments in the Controlled Oxidation of Hydrocarbons".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:
GRADUATE IN BIOCHEMISTRY (or other biological subjects with a wide general interest in biology) in the DEPARTMENT OF MEDICINE, for research work on the synthesis or secretion of hormones—The Secretary, King's College Hospital Medical School, Denmark Hill, London, SE5 (November 30).

HRAD TECRNICIAN (preferably with experience in both human and animal physiology) in the DEPARTMENT OF PHYSIOLOGY, University of Nottingham Medical School—The Registrar, The University, Nottingham (November 30).

ASSISTANT LECTURER (with special qualifications or interests in pharmaceutical chemistry) in PHARMACY—The Registrar, University of Manchester. Manchester, 13, quoting Ref. 169/67Na (December 2).

BRITISH EMPIRE CANCER CAMPAIGN RESEARCH WORKER (science graduate with experience in cytogenetics) to be a senior member of a small team working on problems related to leukaemia—The Secretary, King's College Hospital Medical School (University of London), Denmark Hill, London, SE5 (December 2).

LECTURER (preferably graduate in veterinary medicine, pharmacology or physiology) in Veterinary Pharmacology—The Secretary of the University Court, The University, Glasgow (December 4).

LABORATORY MANAGER (with previous laboratory and administrative experience) to be responsible for the supervision of all laboratory services. Including stores, assist in recruitment and training, and the general supervision of junior laboratory staff and to maintain liaison with the Institute service departments—The Secretary, Animal Virus Research Institute. Pitright, Surrey (December 5).

LECTURER IN THE DEPARTMENT OF PHYSICS, University of the Witwaters and, Johannesburg, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (South Africa and London, December 8).

LECTURER (With an honours degree in civil engineering and at least five years' professional experience, and preferably experience in the use of

The Secretary of the University Court, The University, Glasgow (December 15).

Lecturer of Assistant Lecturer in the Department of Mathematics—The Secretary, University of Edinburgh, Old College, South Bridge Edinburgh (December 15).

Lecturers/Assistant Lecturers (with at least a good honours degree or equivalent, plus suitable teaching and research experience) in Chemistry at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Kuala Lumpur and London, December 17).

Research Assistants (2) in the Department of Ceramics with Refractories Technology, for a programme of research into the factors controlling the constitution and microstructure of polyphase ceramics and the influence of these variables on their temperature properties, including hot strength and creep resistance—The Registrar, University of Sheffield, Sheffield (December 22).

CHAIR OF BIOCHEMISTRY—President of the Board of the Swiss Federal Institute of Technology, CH-8006 Zurich, Switzerland (December 31).

SCIENTIFIC OFFICIER (with at least a good honours degree in an appropriate agricultural science) for research on methods of growing sugar-beet root and seed crops and assist in managing the farm; and a SCIENTIFIC OFFICIER (with at least a good honours degree in botany or agricultural botany) to study the factors affecting the quality of sugar-beet seed, at Brooms Barn Experimental Station, Higham, Bury St. Edmunds—The Secretary, Rothamsted Experimental Station, Harpenden, Hertfordshire (December 31).

at the London School of Economics and Political Science—The Academic Registrar (N), University of London, Senate House, London, WC1 (March 1).

HEADSHIP OF THE DEPARTMENT AND CHAIR OF PHYSICS—The Academic Registrar, The City University, St. John Street, London, EC1, quoting Ref. P/N.

PINCIPAL LECTURER (preferably with a special interest in bacteriology or virology or some other aspect of microbiology) in Microbiology—The Secretary, Wolverhampton College of Technology, Wolverhampton, quoting Post No. 01305.

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PRINCIPAL SCIENTIFIO OFFICER and a SENIOR SCIENTIFIO OFFICER (citizen of the United Kingdom or the Republic of Ireland, preferably with an honours degree in zoology or a medical degree, and postgraduate research experience in tropical parasitology) at the East African Institute for Medical Research, Mwanza, Tanzania, to plan and carry out research on schistosomiasis and methods of control and also to train newly appointed science graduates in research methods—The Ministry of Overseas Development, Room 403 (Y), Eland House, Stag Place, London, SW1.

PROFESSOR OF THEORETICAL CHEMISTRY—Professor R. McIntosh, Head of the Department of Chemistry, Queen's University, Kingston, Ontario, Canada.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Great Britain and Ireland
British Airports Authority. Annual Report and Accounts for the period
2nd August 1965 to 31st March 1967. Pp. 1x + 99. (London: British Airports
Authority, 1967.) [89
British Medical Bulletin, Vol. 23, No. 3 (September 1967): Symposium
on "Intestinal Absorption". Pp. 205-296+5 plates. (London: The British
Council, 1967.) 30s. [89
Comparison, Reform, and the Family. By Professor L. Neville Brown.
(An Inaugural Lecture delivered in the University of Birmingham on 27th
April 1967.) Pp. 19. (Birmingham: The University, 1967.) 2s. 6d. [89
London and Home Counties Regional Advisory Council for Technological
Education. Science Education in the Region. Pp. 33. (London: London
and Home Counties Regional Advisory Council for Technological Education,
1967.) 5s. [89
The Zoological Record, Vol. 102, Section 1, 1965: Comprehensive Zoology.
Compiled by Dr S. Markowski. Pp. 31. 12s. 6d. Vol. 102, Section 12, 1965:
Arachnida, together with Merostomata, Pantopoda, Pentastomida, Tardigrada, Myrlapoda and Onychophora. Compiled by Ernest Browning. Pp. 99.
30s. (London: The Zoological Society of London, 1967.) [89

Other Countries

Commonwealth of Australia. Department of National Development: Bureau of Mineral Resources, Geology and Geophysics. Bulletin No. 73: Cretaceous Stretigraphy and Palaeontology of the Northern Territory. By S. K. Skwarko. Pp. 135+15 plates. Bulletin No. 76: Volcanic Cauldrons, Ring Complexes, and Associated Granites of the Georgetown Inlier, Queensland. By C. D. Branch. Pp. xii+158+42 plates. Bulletin No. 86: Strati-

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The Regional Research Centre of the British Carlbbean at the University of the West Indies, Imperial College of Tropical Agriculture. Soil and Land-Use Surveys, No. 22: Montserrat. By D. M. Lang. Pp. 32. (Trinidad, W.L.: The University of the West Indies, 1967.) 10s. [19] India: Council of Scientific and Industrial Research. Research Survey and Planning Organization. Occasional Paper Series, No. 3: Scientific Research in India—an Analysis of Publications. By Dr. B. V. Rangarao. Pp. 11. (New Delhi: Research Survey and Planning Organization. Occasional Paper Series, No. 3: Scientific Research in India—an Analysis of Publications. By Dr. B. V. Rangarao. Pp. 11. (New Delhi: Research Survey and Planning Organization. Occasional Paper Series, No. 3: Scientific Research in India—an Analysis of Publications. By Dr. B. V. Rangarao. Pp. 11. (New Delhi: Research Survey and Planning Organization. C.S.I.R., 1967.)

United States Naval Observatory, Circular No. 116: Total Solar Eclipse of 22 September, 1968. By Julena S. Duncombe. Pp. 15. (Washington, D.C.: United States Naval Observatory, 1967.)

Metropolitan Life Insurance Company, Statistical Bulletin, Vol. 48. June 1967: Rapid Metropolitan Population Growth Con

Scientific and Technical Information, N.B.S., US Department of Commerce 1967.) \$3. [49]
Skrifter fra Danmarks Fiskerl- og Havundersøgelser, Nr. 27: Fisheri undersøgelser I 1966 ved Danmark, Faerøerne og Grønland. Ved E. Bertelsen og Paul M. Hansen. Pp. 86. (København: I Kommission Hos Andr. Fr. Høst and Son, 1967.) 9.75 D.kr. [54]
Norsk Polarinstitutt. Skrifter Nr. 141: Storbreen Glacler in Jotunhelmen, Norway. By Olav Liestøl. Pp. 63. (Oslo: Norsk Polarinstituth 1967.) [55]

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Is There a Technology Gap?

THE statement by President De Gaulle on November 27 about the prospects for British entry into the European Economic Community is bound to set in train a new flurry of interest in the phenomenon known as the technology gap. Indeed, even without the intervention of the President of France, this well established subject for debate seemed destined for another round of popularity. In Turin two weeks ago, the Twentieth Century Fund and the Agnelli Foundation organized a conference on the subject, and this served as a reminder that it was Signor Fanfani who did much to draw public attention to the technology gap more than a year ago. The council of the EEC has also been taking leisured steps to decide what should be done about technology in Europe, and has produced a declaration of good intentions towards European collaboration in policy making. That development, which followed the meeting of the council in Luxembourg a few weeks ago, seems oddly to have inspired the declaration of Mr Harold Wilson, the British Prime Minister, in his speech at the Guildhall, that the need to close the technology gap, whatever it may be, is now so urgent that he would not wait for British membership of the EEC before taking steps to stimulate more effective collaboration between Britain and the mainland. Nothing much has been heard about the proposal since those brave words were spoken, although the devaluation of sterling is by itself enough to account for that.

A more serious problem, however, is that nobody is quite sure what the technology gap consists of. It is much easier to decide what it is not. For example, it is quite certain that Europe as a whole and European nations individually are not at a disadvantage compared with the United States in their capacity for innovation. That is simply another way of putting the familiar dirge that Europe invents and the United States exploits. It is also plain that the technology gap cannot be measured simply by the numbers of scientists and engineers at work on both sides of the Atlantic. By this simple test, indeed, Britain compares quite well with the United States, and a good many other European countries are not far behind. So why is it that Europe is so much at a disadvantage in the exploitation of new technology? And what can be done to change that state of affairs?

The first thing to acknowledge is that the essential difficulty is somehow to decide which projects to pursue and which to leave alone. This, for example, has been the outstanding problem in Britain since the

Second World War. Should there be a massive investment in aircraft, nuclear energy and computers all at the same time? And what should be done about transport and the development of the chemical indus-Successive governments have been driven by the conviction, now commonly shared in Europe, that it is too dangerous for a forward-looking nation to stand aloof from forward-looking technology of any The result has been that resources have been too thinly spread for new developments to be pushed ahead with the excellence and speed which competitiveness demands. But in the understandable belief that it is necessary at all cost to foster new technologies, by breaking into whatever markets are naturally available to them, successive governments in other European countries as well as Britain have found themselves trying to sell new products in national markets which are too small to be viable on their own. Thus, for example, what really cripples the British aircraft industry is that its domestic market is too small to be profitable. The kinds of nuclear power stations now being built in Britain would be enormously more attractive as commercial propositions if there were a real chance of selling as many again elsewhere. It is sensible also to acknowledge that one awkward accompaniment of this aspect of the problem is that by trying to spread resources over the whole field, European companies have often been less able to manufacture goods of high quality than their American competitors. It is fashionable to blame the supposed spin-off from the programme of space research in the United States for this disadvantage, but that is not an explanation, only an excuse.

It follows from this, of course, that the most certain way-the only long-term way-of making sure that European industries compete with their rivals in the United States is somehow to ensure that European nations can safely be more selective in the jobs they tackle. It must, for example, come to seem quite proper that even the largest of the nations in Europe should be able to refrain from manufacturing aircraft, nuclear power stations, computers or even motor cars. No amount of negotiation between countries on the sharing of technical expertise will help if the partners to these agreements then insist on making everything for themselves. It is true that agreements like these may increase the intellectual productivity of the scientists and technologists concerned, but they will not by themselves allow manufacturing organizations to reap the benefits of concentrated resources and large scale production. This is an awkward truth which everybody, but particularly Mr Harold Wilson, should squarely face. The technology gap is not technological at all but a consequence of the scale and structure of European industry.

Two important principles follow from this, and both of them will be crucial in the years immediately ahead. In the first place, it is doubtful whether there can be much sense in an attempt to anticipate British membership of the EEC by negotiating agreements with European countries on technical collaboration. Indeed, one of the principal objectives of those who formed the EEC in the early 50s was precisely that of finding some device for making the structure of European industry more effective. As the community has emerged, it is precisely the kind of framework within which nations could concentrate their resources on new developments without damaging their pride. Thus the rules of the EEC allow for the free movement of capital and of work-people as well as the more obvious benefits of a market which is as extensive as the whole of Europe.

Yet it remains a sad truth that the EEC has so far been comparatively slow at creating the kinds of institutions needed to exploit these potential advantages of scale. The scale of European communities does not yet match the scale of their opportunities. Regulations of patents and the like are still much in need of co-ordination. Governments still favour companies in their own territories. In other words, it seems as if the EEC itself has still a long way to go along the road to full exploitation of its own resources. In the circumstances, it is hard to see what scope there is for separate action by the United Kingdom.

This raises the issue of what Britain should do if, as seems likely, entry into the EEC is substantially delayed. The first thing to be said is that there is some scope for the fostering of specialization within the framework of the European free trade area. The opportunities for industrial symbiosis with this group may not be as considerable as on the mainland of Europe, but they are not altogether unimportant. But it will also be necessary at some stage for the British interest in advanced technology to become more specialized and concentrated. If this means getting out of aircraft and leaving computers, there will be a good many tears to be shed, yet choices of that kind will be inevitable. It is sensible that the British Government should be looking for bilateral agreements that will help somehow to postpone these nasty decisions but there are obvious limits to what can be done.

Whose Finger on the Drawing-board?

THE first report of the Select Committee of the House of Commons on Science and Technology is not so much a bombshell as a squib. It will startle a good many people, which is by itself not necessarily a disaster. Indeed, by bringing into the open some of the courses of action towards the development of nuclear power in Britain, and towards the Atomic Energy Authority in particular, the select committee has done a public service. Ordinary mortals, like the rest of us, too easily slip into the habit of believing that institutions are assured of a permanent existence once they have provided themselves with the appearances of permanence-headed notepaper, some publicity films and a few laboratories in the country. All this is good, and it is also a great benefit that the Minister of Technology will no longer be able to postpone a decision on the future of the Atomic Energy Authority. Evidently it would have been a great discourtesy if he had rushed ahead with a radical reorganization, although there is a great deal that he could have done in the fields with which the select committee was not con-

If, however, the appearance of the committee's report will pave the way for lasting decisions, there can be little confidence that its own recommendations will quickly be enacted. The trouble is that even though the report itself is packed with the kind of information that will keep specialists poring over it in the months ahead, the argument which sustains the principal conclusions is so lacking in conviction that nearly half of the members of the committee were unconvinced.

In the circumstances, it is only fair to recognize that the committee did successfully cover a good deal of contentious ground. The committee does, for example, take the view that the strategy for atomic energy in the years ahead, in countries such as Britain, should spring from a determination to win the greatest economic benefit. To its credit, the committee is a cheap energy committee, and clearly not overawed by Lord Robens, the chairman of the National Coal Board. There is also a refreshing flavour in its argument that atomic energy should be regarded with some favour because it does less than fuels based on carbon to pollute the atmosphere. These, as the committee quite rightly says, are increasingly important considerations. If this is what foreign travel does for tired Members of Parliament, then the Foreign Office has more than the obvious reason to be sure that it offers help and not obstruction in the committee's future investigations.

But what about the organization of the nuclear energy industry in Britain? This is the nub of what the committee has to say. This is where it is divided among itself. This is where the report is least convincing. Briefly (see page 854) the committee wishes to create a single "commercial organization" to undertake the design and construction of the "nuclear boilers" in future nuclear power stations. By this proposal, the committee echoes the suggestion of the Atomic Energy Authority, which put forward in evidence a scheme in which the authority itself would join with interested companies in a commercial venture to

manufacture the essential parts of nuclear power stations. The committee and the authority agree that it would be necessary for the design and development facilities of the authority to be transferred to the new company. The committee goes further than the authority by taking the line that the authority should be dismembered into parts with separate functions. Although Lord Penney himself acknowledged that a "radical reconstruction" of the authority is necessary, he does not seem explicitly to have advocated such a drastic step.

Because the committee has been bold and because its principal conclusion smacks a little of a tidy paper scheme intended to get rid of "duplication" or some other illusory economic defect, it will be tempting to assume that it has been entirely naive. It is therefore only proper to acknowledge that some of the most obvious pitfalls have been spotted and that suggestions have been put forward for not falling into them. In its defence against the charge that a single design and manufacturing organization would necessarily acquire monopoly powers—one of the points made by the Central Electricity Generating Board—the committee replies that one essential feature of the scheme should be that the electricity boards are empowered to consider tenders from outside the United Kingdom. By the same test, the argument goes, the new organization would have to be prepared to lose—or at least to risk-quite large amounts of money in its own commercial dealings in the export market—a radical notion for the use of public funds. The difficulty is, of course, that these theoretical safeguards may not in practice be safeguards in any real sense. It is hard, for example, to see a government which is consistently reluctant to let the nationalized airlines buy aircraft overseas acquiescing permanently in a scheme that would allow other nationalized industries to spend very much larger sums of money on capital equipment from abroad. At the same time it is hard to see the British Government being prepared to relax its interest in the uses to which substantial sums of public money have been put. In other words, the scheme which the select committee and the Atomic Energy Authority have between them hatched is somewhat idealistic, to say the best of it.

But does it follow that every idealistic proposal is nonsense? That would surely be an unreasonably cynical view. But the difficulty which the committee should have faced much more squarely is that, in the advocacy of idealistic notions, it is essential to make a closely argued case. In particular, it is necessary to answer detailed questions such as the precise meaning of the word "commercial". Would the proposed nuclear boiler company be able to move into the manufacture of computers if it considered there were better profits to be made in that direction, for example? Would it be compelled by its constitution to accept all intending participants as shareholders, and, if not, how could the granting of this franchise be squared with the Monopolies Act? How would the supposed boiler company respond to technical change? Would it, for example agree to disband itself if, at some time in the future, the electricity boards could easily order the components of "nuclear boilers" separately? To ask all these questions does not imply that none of them can be answered. The committee itself, or perhaps Lord Penney or his successor, may have perfeetly acceptable answers. But the proposal in its present form is altogether too imprecise. Among those who think of it as a bogy, for example, will be some who regard it as a cloak for nationalization and others-Lord Penney, perhaps?-who see it as a means by which the essence of the Atomic Energy Authority might continue to exist a little more remotely from the pressure of detailed scrutiny by the Government. And, above all, there is bound to be a nasty suspicion that the scheme might not work for some unsuspected reason. What this implies is that the select committee should either have done a more detailed study of these points or should, alternatively, have put forward the proposals much more tentatively.

There is, too, always a doubt whether any reorganization scheme which does not command a fair degree of political agreement has any chance of success. This is not to argue that select committees and ministers should never set in train radical or provocative schemes. but merely to point out that in matters like these. political truths are just as significant as commercial ones. From the attitude of Conservative members of the committee, it is fair to assume that any Conservative government in Britain would oppose the establishment of a monopoly supplier. But it would be unlikely to unscramble an organization which was already working efficiently, particularly if it had achieved some measure of export success. Any move towards the central design authority, if it is to be permanent. should therefore be made with all speed, for Mr Benn cannot be unaware that there will be an election in 1971. If the industry is still in the process of reorganization then, a Conservative promise to return it to private hands is likely to prove an irresistible tempta-Quite apart from the merits of the case, this would not be a way of ensuring success for the industry -rather the contrary. And because of the outstanding contracts, the last of which is to be finished in 1973, rapid change may be the last thing the industry is willing to contemplate.

These are the points on which the committee is vulnerable to criticism. A close reading of the report will show that the committee has worked hard, and covered as much ground as could reasonably be expected of it. If in the future it is given technical assistance on the right scale, it could become a power in the land. But as things are, it has skimped on thinking, with the result that the document is much less persuasive than it could have been. From this it does not follow that the select committees are ineffectual, but rather that they need even more help and encouragement than they have had so far. This particular committee will need all the help it can get when it comes to enquire into defence research establishments in the weeks ahead

There remains the question of what the Minister of Technology should do about the Atomic Energy Authority. Simple men will hope that he will now be emboldened to do at least those simple things which stare him in the face. For one thing, he should relinquish responsibility for thermonuclear fusion, which should now be with the Science Research Council on an elastic budget and not short commons by ministerial decree. The weapons laboratory at Aldermaston should be transferred from the AEA to one of the ministries, possibly the Ministry of Defence. problem of what to do with Harwell is daunting by comparison, but there is a case for considering novel -solutions such as the creation of several autonomous laboratories, with separate sources of finance, in one physical environment. The trading functions of the AEA, including the sale of fuel elements and of isotopes for research, could be separated into one commercially conducted nationalized industry, but this is hardly an urgent matter. The central need is to decide just what part will be played in the future development of nuclear energy by the men now engaged on design and development for the AEA. The solution which the select committee has proposed is only one of many. Another, for example, is that the AEA might be divided between the electricity boards and the industrial companies. For even though the select committee is probably right in saying that there is no pros--pect of anything approaching competition in the present -circumstances in which three commercial enterprises compete for what little business there is to be had, and although everybody will agree that the companies should be more closely concerned with design and development, it is by no means obvious how this should best be done. Plainly the Minister of Technology will have to start where the select committee left off. He had better make haste.

Japanese Science Policy

Science in Japan comes under scrutiny in a recent study by the Organization for Economic Cooperation and Development (OECD, £1 10s.). This is the seventh review of national science policy which the OECD has published; the others cover Sweden, Greece, Belgium, France, Germany and the United Kingdom. The review concludes that Japan suffers from an unwieldy advisory structure and a multiplication of laboratories too small to justify themselves. Advice is supplied by two principal organizations—the Council for Science and Technology, and the Science Council of Japan. The first of these, the report says, is too concerned with government affairs, while the second, whose relations with the government have for several years been in a permanent state of crisis, is too involved with academics. There are in addition four specialist advisory agencies, covering atomic energy, radiation, marine science and space.

Much of the direct administration of science in Japan is carried out by the Science and Technology Agency. The position of the agency is complicated, the report says, because it also acts as secretariat for the Council for Science and Technology and the four specialist

agencies. Sometimes it is in the position of being both judge and advocate. Research conducted by other government ministries "presents a picture of extraordinary dispersal of effort". Often the effort is too small to meet the threshold requirements for concerted programmes of research. There is further danger, the report suggests, in the sharp division between funds for university research and for other research. This division represents a major obstacle in the development of coherent science policy.

Despite the success of Japanese industry since the War, the report suggests that there is still an overheavy reliance on imported technology. "There is still some way to go before Japanese technological innovation commands respect from those nations whose technology has been the basis for Japanese development." If Japan really does want more technological independence, it will have to pay more for it, by stimulating domestic innovation. The recent establishment of a Research Development Corporation to underwrite high-risk and development projects could help, the report concludes.

Japanese witnesses to the review body gave interesting details of a project intended to collect together a large number of the government's research institutes in a research park at Mount Tsukuba. The plan sounds ambitious: the park will have several universities and some forty government institutions, as well as some private ones. The park will be some 50 kilometres from Tokyo, and an express highway will be built between the two. At the moment land is being obtained, and construction will begin next year—within ten years the park should be complete. The park will include a great variety of institutions, including pure science, architecture, mechanical engineering, disaster prevention, medicine and agriculture.

Fewer Claims on Oxford

by our Oxford Correspondent

APPLICATIONS for admission to Oxford University fell by 8.5 per cent this year. It is, of course, too early to say whether this was caused by a loss of interest in the older universities among sixth formers, or indeed whether the decrease is significant at all. There may not have been any change in the quality of candidates—examination scripts are only being marked now—so that the fall in numbers may merely result from stiffer pre-selection of candidates by the schools.

But, in any case, long before the fall in the number of applicants became known, the university decided to increase its contacts with the schools so as to encourage sixth formers to apply to Oxford. At large and well equipped schools, such as Manchester Grammar School, which sends 70 to 80 boys on to university each year, Oxford, with its college system and academic and administrative idiosyncrasies, is very much a known quantity. Such schools need no proselytes; candidates from them are many and well informed. But at smaller schools, where a tradition of entrance to universities has not been established, Oxford may be regarded with a certain amount of awe, a distant and perhaps unsympathetic institution, little interested in applicants from unknown schools. One head master, for example, has commented on the fact that some Oxford women's colleges used not to make past examination papers

All Change on Aldabra

The Under-Secretary of State for Defence for the Royal Air Force (Mr Merlyn Rees): "... I want to make it abundantly clear at the outset—and this to a very large degree limits my remarks this evening—that no decision has yet been made whether or not to use the island of Aldabra for defence purposes. As my right hon, friend the Secretary of State for Defence assured the House on 5th July, the scientific issues at stake will be taken carefully into account in reaching any decision." Hansard, Col 1845, October 25, 1967.

The Prime Minister (Mr Harold Wilson): "... In overseas terms, we have decided not to proceed with the Aldabra project—(Interruption)—the establishment of a staging post in the British Indian Ocean territory. As far as we can at present estimate, it should mean that expenditure next year will cut defence spending to the level which last July, with great difficulty, we had scheduled for 1970–71. It is an advance from 1970–71 to 1968–69—I think a remarkable achievement." Hansard, Col 1341, November 22, 1967.

available to applicants, so that only schools which regularly sent in entrants could collect papers for the aid of future candidates. (This anomaly has now been cleared away. From this year onwards the Colleges Admissions Office will deal with women's applications as well as men's.) Relations between schools such as these and the newer universities have been much stronger: Leeds University has been particularly active in sending members of its staff to talk to sixth formers. At the University of East Anglia, a report on a student's work in his first year is sent back to his school, and Sussex University gives an account to the school of how its candidates fare at the entrance interview.

This is not to say that Oxford has been entirely idle, for more and more candidates have been coming from state schools in recent years. But recruitment has largely been left to individual colleges. Last summer, the Colleges Admissions Office began arranging with a number of schools that Oxford dons should come and talk to sixth formers or staff about any problems there might be in applying to Oxford. It is hoped that the first visits may be made next term so that it might be possible to gauge the success of the scheme by the entrance figures for next year.

More Big Spending

Britain should go ahead and support the plan to build the CERN 300 GeV machine, according to Professor P. M. S. Blackett, President of the Royal Society. In his presidential address at the anniversary meeting of the society on November 30, Professor Blackett said that the big machine would provide a natural follow-up to the "brilliantly successful" 28 GeV machine at Geneva. There was wide agreement on scientific grounds that the machine should be built, he said, but doubt had arisen because of its great cost. "Some scientists fear that the fifteen years or so forward commitment which such a big project requires could put at risk the provision of adequate finance for the great bulk of little science, which cannot be planned far ahead. After careful thought on all these matters, I am convinced that Britain should decide to join in this great and exciting European venture—and the sooner the better."

Professor Blackett went on to suggest that in reality there was no real conflict between big and little science. Of course, it was possible that the demands of big science would starve little science of funds, but in Britain this had not happened. The big spenders—nuclear physicists, high energy physicists, space scientists and radio astronomers—had beaten a path to the Treasury door and made it easy for little science to follow. "In short, the big sciences have raised the scale of expenditure on research to a new level, and this has carried little science in its wake."

Professor Blackett spoke with great enthusiasm about the increased level of exchanges within Europe, and the new arrangements for which the Royal Society is acting as a clearing house. And, in spite of the other demands on national resources, Professor Blackett said he was convinced that the rate of growth of the science budget should remain high for a long time yet. This called for very careful selection of projects for support, and some scientists were bound to feel frustrated—the biggest protests, he said, were likely to come from those who had been best fed in the past. A close comparison with manufacturing industry could be made-many firms work very hard to make their tenders for a given contract. But only one firm wins it. So it must be with British scientists, Professor Blackett concluded.

Royal Society Blooming

The move to new premises seems to have left the Royal Society in a cheerful frame of mind even if the appeal for funds to pay for the capital cost of conversion is still some £200,000 short of the target of £850,000. In practice the programme of exchanges with European countries seems to have captured a good deal of the society's enthusiasm. The society describes its operations in the year past as "a very successful start to a new programme". There seems every prospect that the work of the past year will be increased in the months ahead and that European countries will come forward with proposals for spending money which will allow the Royal Society to draw more heavily on the British Government in support of the programme of exchanges. In the year ahead, £50,000 has been made available by the Government for this work.

Government spending through the society is increasing steadily. In the year to last April, a total of £564,000 was spent in this way, roughly a quarter of it on the support of scientific research. The society spends roughly the same amount of government money—£146,300—on participation in international ventures, mostly through the International Council of Scientific Unions.

The government contribution in the current financial year will amount to £657,000 and will include for the first time a contribution towards the Naples Zoological Station. It had been agreed before devaluation that the Royal Society will be the vehicle for the British Government contribution of £28,000 a year (50 million lire) towards the station so as to qualify for a seat on the administrative council of the station. Presumably the cost will now be higher.

Among the new ventures undertaken by the fellows of the Royal Society in the year past is the setting up of a study group on modern population, chiefly as a result of a discussion held at the society on May 17 this year. The chairman of the study group is Dr H. A. Cole.

Big Biology

Consideration of the philosophical bases of the International Biological Programme (IBP) has so preoccupied scientific leadership in the United States that observers elsewhere have wondered when American biologists might actually get down to specific projects, and what these might be. A stream of stately essays has been the main output so far; yet Phase 2, or the operational part of the IBP, was supposed to start last July. At that time, however, the National Committee was in the midst of Congressional hearings on IBP and its funding.

It now seems that, having scrutinized the underlying concepts of IBP more thoroughly than any other community, the United States may now undertake work that is proportionately more significant. This is clear from the most recent report of the National Committee, Report No. 3, Part 1, now published. Of the greatest interest are the major integrated research projects specially developed within IBP guidelines by the National Committee and directly sponsored by it. The radical sweep of some of these programmes makes most of the efforts of other countries look very small beer. None is expected to cost less than \$2 million and several will cost a great deal more.

Six such integrated programmes have already been adopted by the US National Committee and were discussed in detail in the committee's report to the House of Representatives Subcommittee on Science, Research and Development. These are: (1) an aerobiology programme to study on a world-wide scale the dispersal of airborne bio-material such as pollen, spores, algae, pathogens, and insects, to make the prediction of crop diseases and other pests more reliable and control more effective (cost \$16 m); (2) large ecosystems analysis of which six contrasted regions have been chosen including a polar environment and a South American tropical forest (cost \$45 m); (3) a joint Canadian study of Eskimo populations based on at least three widely separated population centres in Alaska, Arctic Canada and Greenland whose communities all stem from the same origin but which have become differentially adapted (cost \$2 m); (4) intensive work on the Hawaiian "evolutionary explosion" while it remains identifiable—this rich flora and fauna has all evolved from 700 immigrant ancestors in the few million years that the islands have been isolated. but the area has already lost more land species than the whole North American continent put together through the accelerated impact of man (cost \$2 m); (5) phenology within the United States, or the impact of climate and seasons on animals and plants, is expected to lead to a series of maps of the country in terms of nest-building, fish-spawning, bud development, seed production, useful for biological prediction (cost \$2 m); (6) ecology of migrant populations, primarily concerned with the effects of urbanization on rural peoples and particularly those migrating from rural southern states to large city receiving areas such as Chicago (cost \$10 m). All these programmes now have a research director and some sort of headquarters, although none have yet secured funds. The committee shows confidence that they will be obtained, and hopes for House sympathy over requests.

In addition, nine other major research operations are under review and a whole series of high-level "workshop" meetings are taking place this month and next to bring these to a head. They cover the adaptation of peoples to high altitudes; experimental biogeography of the sea; control of insects by plants and plants by other plants; biological control of pests, insects and other organisms; (in conjunction with Japan) adaptive processes in hybrid human populations; adaptability of primitive peoples; convergent and divergent evolution, and the physiology of colonizing species; plant gene pools; nutrition and new foods.

Apart from these committee-sponsored moves in a grand strategy, the recent report lists over 100 individually proposed schemes relevant to the IBP, many of which are going on anyway and are already financially secure. The number has now risen to a total of about 170. This section of the report is quite a rag-bag, and the items very uneven in value and interest. This section much more resembles the national programmes of other countries such as Britain where the impression is left that the IBP has provided a new system for indexing research projects already in progress.

Some of the written answers to the Congressional Sub-committee's enquiries are revealing. consider what effect a lack of funds would have on the international programme as a whole and on American scientific prestige and US standing generally, the witnesses answered: "The IBP National Committee feels that this would not greatly affect the prestige of American science. At the same time, it points out that experience in the last 15 years has demonstrated that, if any international scientific programme is to be successful, the US must take a strong and vigorous role. It applies the analogy of 'critical mass'; with added fuel the programme will 'go'; with decreased fuel the programme will falter . . ." Summing up the committee evidence: "Inadequate US support would adversely affect this international research programme. Inadequate US support and participation would delay urgently needed worldwide ecosystem research.'

Anniversary in Chicago

The twenty-fifth anniversary of the first controlled release of nuclear energy rolls around this week. The University of Chicago is holding two days of ceremonies to celebrate the birth of the nuclear age in a squash court under its football stadium on December 2, 1942. The stands at Stagg Field have now been demolished, and five of the fifty who witnessed the scene are now

dead, including the team's leader Enrico Fermi, Arthur H. Compton and Leo Szilard. Among those to be honoured by the university this week will be Robert Duffield, director of the Argonne National Laboratory.

The enthusiasts for commercial exploitation of the nuclear reaction have, in the United States at least, further cause for celebration. This year marks the tenth anniversary of the first sale of electricity generated by a nuclear power station. (The Schippingport reactor in Pennsylvania, with a new core, is still serving Pittsburgh today.) The boom in orders for new power plants is continuing; twenty-six were made known in the first three-quarters of this year compared with twenty-three in the corresponding period last year. And the contract has just been signed for southern California's combined nuclear power and desalination plant, expected to be not only the world's biggest, but one of the cheapest in terms of the delivered cost of its desalted water.

Moreover, next week should see the first nuclear explosion sponsored jointly by the Atomic Energy Commission and private industry to test the peaceful (that is, commercial) possibilities of such explosives. Project Gasbuggy, as someone has thought fit to label it, will consist of a 26 kiloton explosion 4,000 ft. down in the sandstone formations near Farmington, New The AEC and the El Paso Natural Gas Company want to see whether the blast can release the gas locked up in an otherwise unprofitable gas field. The AEC has at least six other commercial experiments scheduled—one to get at oil, another at copper. Conceivably some day the commission could be in such heavy demand as a blaster that it might have to contract out the business of setting off nuclear explosions to a private company.

Discovery of X-rays

On November 8, 1895, W. C. Röntgen, professor of physics at Wurtzburg University, observed a phenomenon which led to his discovery of X-rays. Almost immediately after the discovery, applications blossomed at a remarkable rate, and some fell even within the ambit of the original discovery itself. Even today, when the importance of the rapid application of new ideas is widely appreciated, this rate of application is rarely if ever equalled. Dr D. Chilton of the Science Museum discussed the reasons for the rapid application of Röntgen's discovery in a paper to the history of science discussion group at the Royal Institution on November 15.

One reason for Röntgen's success seems to have been the remarkable speed at which he worked. Dr Chilton quoted from a speech by Professor Ewald—"Röntgen was a character who hated to part with an unfinished experiment by publishing it. He made the chance observation on November 8 and, working feverishly in the next six weeks, found nearly all the properties of X-rays which were to be known within the next ten years . . . Physicists and medicals alike tried to find out more about the properties of these rays than Röntgen had indicated in his ten-page pamphlet which he sent out to his friends as a New Year's gift." The reaction was certainly remarkable; Dr Chilton said that by the end of April 1896, there had been sixty-one references to X-rays in the pages of Nature, an average

of three to four each week. By February 1, the Lancet was reporting that the invention was so far advanced that in Belgium it was being brought into practical use in the hospitals. This was just one month and three days after the first announcement of the dis-

Other applications were also quickly realized, Dr Chilton said. Röntgen himself noted the way in which X-rays showed up lack of homogeneity in a metal structure, now the basis for industrial testing of metals. S. P. Thompson showed that gem stones and their glass imitations differ in their absorption of X-rays. All this, Dr Chilton said, was derived almost directly

from Röntgen's first paper.

Röntgen's "chance observation" came when he was working on electric discharges within an evacuated Working in a darkened room with the glass tube. tube enclosed in black paper, he noticed that a film of barium platinocyanide, a well-known fluorescent material, showed a bright fluorescence whenever the tube was operating nearby. But if the discovery was a piece of luck, from then on Röntgen left nothing to chance.

New Building at Teddington

MR ROBERT MELLISH, Minister of Public Building and Works, cut the traditional sod on November 28 to mark the site of a new laboratory building at the Ministry of Technology's National Physical Laboratory at Teddington.

The laboratory, which will be known as Petayel Building after Sir Joseph Petavel, a former director of the NPL, should cost about £650,000. The NPL is already the third largest research establishment in Britain and the new three storey building will provide an extra 34,000 sq. ft. of laboratory space and 21,000 sq. ft. for offices. The main building will consist of a central reinforced concrete core containing the main staircase, lift and common services. Surrounding this will be the laboratories which, on each floor, will be divided into seven zones, each sub-divided into areas by low partitions. Although the laboratories will be without windows, thereby permitting an economical design for the engineering services, the artificial lighting and air conditioning will, however, provide a stable environment for research. The offices will be located on the perimeter of the building and will have natural light and ventilation.

Petavel Building, which should be completed by September 1969, is a departure from the normal type of research building. It has been essentially designed to meet the changing needs in research; as Mr Mellish put it, "Petavel Building is, we believe, a good answer to the request for a general purpose laboratory which would provide the conditions required for a wide range of the National Physical Laboratory's work and which would also be capable of modification to provide other conditions with a minimum of expense". The contractors began work on November 29, and it looks as if the ministry is determined to have the building finished on time.

Protein from Petroleum

Interesting results have been obtained from experiments in which yeast cells have been used to produce protein concentrate from petroleum hydrocarbons. The pioneer work in this field was done at Lavera, France, and for the last five years the Shell BP group has been engaged in a research and industrial programme aimed at protein production on an industrial scale, chiefly at Grangemouth, Scotland.

Protein concentrates can be made by two processes: the product is an off-white, free flowing, tasteless powder which has a relatively high lysine content but which is low in methionine. One process starts with refined petroleum hydrocarbons and the other with gas or diesel oil. These unusual substrates are fermented by yeast cells grown in conditions of favourable pH and temperature and provided with adequate substrate and minerals. The fermented product is centrifuged and treated to produce a cream suitable for the drying From each ton of hydrocarbon, a ton of protein concentrate is produced. This is suitable for use in animal foodstuffs and will possibly compete in food value and cost with more familiar protein concentrates such as fish meal and soya. Laboratory and early field-scale testing of the nutritional value of the product is being supplemented by toxicological studies using albino rats. Stringent safeguards have to be satisfied before the protein can be used for human consumption, but it has been suggested that it could be included in bread and biscuits. Furthermore, the protein can be extracted and coagulated to form a material with a structure resembling meat such as veal or chicken.

These results are shortly to be published in detail in the *Journal of General Petroleum*.

A Ship from the Bottom

The raising and restoration of the seventeenth century Swedish warship the Wasa is a strikingly brilliant piece of marine archaeology, and the small but informative Wasa Exhibition at the Science Museum in London does it justice. The exhibition, organized by the Wasa Museum and the Swedish National Maritime Museum under the auspices of the Swedish Institute, traces the history of the ship from its construction and sinking on its maiden voyage in 1628 to the location of the hull in 1956 and its recovery in 1961.

The Wasa, for some unknown reason, capsized and sank before it had left Stockholm harbour. Most of the heavy cannons were recovered in the seventeenth century by divers using the most primitive of diving bells and the ship was then forgotten. In 1956, however, it was relocated at a depth of 100 feet, completely silted up with mud, and the Swedish Government decided to raise it. This posed enormous technical difficulties which have been brilliantly overcome. The exhibition shows with models and photographs how the hull was lifted in seventeen stages until it was at a depth of 60 feet and finally raised to the surface in April 1961.

Once on the surface, 1,000 cubic metres of mud was removed and sifted, yielding about 24,000 objects including clothing (see illustration), food, coins, sails and utensils. From these an authentic picture of life aboard a seventeenth century warship can be reconstructed. The woodwork of the hull was remarkably well preserved as several photographs and replicas show. Fortunately, the salinity in the Baltic is too low for wood boring animals such as Teredo and this

more than anything else accounts for the good state of the woodwork.

Preservation of the hull is an enormous task which is still going on. It is the largest water-logged object ever to be preserved and the Swedish authorities are sparing neither time nor expense to make sure it is done properly. To prevent the wood from shrinking as it dries out it is being impregnated with polyethylene glycol. All the movable parts are being stewed in a 60 per cent solution at 60° C and then carefully dried. The main bulk of the hull—190 feet long—is being continuously sprayed with polyethylene glycol solution and this operation will not be completed until 1971. It will then be dried out under carefully controlled humidity and eventually all the pieces reassembled.



The seaman's clothing on this model was recovered from the hull of the Wasa. (Science Museum photograph.)

The exhibition, which whets the appetite for the real thing, will stay in England until 1969 during which time it will be shown at most provincial museums. Similar exhibitions are touring North America, Australasia and the continent of Europe.

Irradiating Food

Some estimates indicate that as much as 20 per cent of the world food supply is wasted by spoilage—in some areas this figure may reach 50 per cent. Clearly any technique for reducing this waste would be welcome, which explains the considerable interest in food preservation by irradiation. One investigation of this sort is being carried out under an agreement between the Austrian Atomic Energy Agency, the European Nuclear Energy Agency and the International Atomic Energy Agency. The work is done at the Reactor Centre at Seibersdorf in Austria, and the project has just published details of progress up to the end of 1966.

The report shows that the project has been slow to get off the ground. In 1960, the ENEA set up a committee to examine the feasibility of the process; in 1961 Austria indicated willingness to do the experimental work, on fruit and fruit juices. In 1962 the draft programme was drawn up; in 1963 a draft agreement was submitted, and it was finally signed in September 1964. Work began in January 1965, but the irradiation facility was not finished until September this year. Initially work will be concentrated on fruit juices, both because of their convenience and because the yeasts which are mainly responsible for the spoilage of fruit juice are a well studied group of micro-organisms. The experimental work is divided into three main fields: radiosensitization studies on yeasts and moulds, technological studies including radiation chemistry and wholesomeness tests.

Like other methods of food preservation—smoking, drying, canning or freezing-irradiation affects the taste of the food. Results at Seibersdorf with grape juice have not yet established which compound is responsible for irradiation taste, but the indications are that the undesirable taste is not caused by protein degradation. Some evidence indicates that flavour changes can be minimized by irradiation at very high dose rates, and preliminary results from work in Switzerland with an accelerator indicate that apple juice can tolerate a high dose rate without significant flavour change. Of rather more importance is the work necessary to establish that the new process is safe for human consumption over long periods. At Seibersdorf animal feeding trials are being made with three types of animals—rats, mice and miniature pigs.

Work with apple juice has established that only one compound, furan or a furan derivative, was specifically produced by the irradiation process. But compounds already present in the unirradiated juice, like acetaldehyde, may be increased. The concentration of acetaldehyde continues to increase during storage, while cupronaldehyde, present after irradiation, appears to decline in concentration during storage. The irradiated apple juice, the report says, could be stored successfully at room temperature for 200 days.

Architects and Engineers

THE building industry has for a long time been troubled by differences that exist between the members of the various professions that work together in a building team. Architects and engineers have tended to become isolated from one another because of differences in their professional training. An attempt is now being made to find ways of breaking down the barriers between them, so that the skills and total knowledge of individuals can be used more effectively than at present. A joint education group was set up in 1966 by the Council of Engineering Institutions (CEI) and the Royal Institute of British Architects (RIBA) to find ways of improving the understanding during training between the disciplines of architecture and engineering, and to consider the possibility of training people to be both engineers and architects. The nine-strong group of two architects, four engineers and three RIBA staff has just produced its interim report, which covers the first of these problems and gives a preliminary statement of the second.

Much of the present lack of communication between

architects and engineers is caused by differences in the educational systems. Both professions require at least six years' training, but the academic content varies. Engineers take a three-year academic course followed by three years' practical training, while architects have a year of practical training after their third year, in the middle of their academic course, so that theory and practice are interwoven. But the most fundamental difference is in the approaches of the professions. Engineers are given highly technical training with little concern for the human side of the problems. Architects, on the other hand, consider the human factor to be of prime importance, but are sometimes short on technical expertise. Some subjects that are common to both disciplines have up to now been treated differently. In structures, for example, architects are concerned with strategy, and engineers with tactics. This means that architects study the causes and effects of certain choices, while engineers are there to see that the choice which is finally made stays up. Despite his greater technical knowledge, the engineer must leave design to the architect.

The problems of communication are not new, but The CEI, which there are now signs of progress. represents the majority of the engineering institutions, is aiming to provide a common theoretical basis for all engineers which can then be applied to any specialist field. This approach does not encourage integration with architecture, but the fact that the CEI is involved on the education project indicates its awareness of the situation. The Council for National Academic Awards, which awards degrees to students at polytechnics, has the power to accept or reject courses into the degree category. It therefore has considerable influence on the content of non-university courses. The group has several suggestions to make for increasing understanding and co-operation, based on the idea of students working together. Project work could be carried out by architecture and engineering students together, and some parts of the syllabus could be shared. The group believes that "orientation" courses for engineers entering the building field are worth investigating. Teachers from one discipline who appreciate the problems of the other could also help. The ultimate solution as described by the group rests on the establishment of educational institutions where architects and building engineers can work together. This would require engineers to commit themselves to the building field at an early stage—to good purpose, the group thinks.

Free Enterprise under the Sea

AMIDST all the rumours of new Government support for oceanography in Britain, it is refreshing to find some young men who are prepared to back their hunches and build for the future under the sea without waiting for the cat to jump.

At Lintott Engineering Ltd's Horsham works last week, Britain's first mobile submersible was given its first demonstration. Called SURV, for Standard Underwater Research Vessel, it was initially the concept and design of M. J. Borrow, R. E. Lloyd and J. M. Metcalf of Underwater and Marine Operations Ltd (Woking), who have seen it through to the fully engineered prototype constructed by Lintott and demonstrated last week after two months of sea trials.

It is intended as a broad purpose underwater tool for the engineer and research worker at continental shelf depths, and carries two men. The makers claim that its performance "covers all aspects of undersea work including bottom surveys of sea-bed conditions before, during, and after laying of oil and gas pipelines and submarine cables". They also see applications for geology, marine biology, fish farming, surveillance, and as a search and recovery vehicle. There are provisions for outside manipulations and coring. (It might well have come in handy as an aid in the hazardous operation to recover the Royal Navy Buccaneer that plunged into 300 ft. of water off the Lizard a couple of years ago.)

To do all this, a cylindrical pressure vessel of 51 ft. diameter with 11 in. thick mild steel walls has been chosen. This has a theoretical collapse depth of 3,500 ft. The pressure hull is enclosed in a glass fibre shell to make SURV as rugged to surface treatment as a glass fibre boat. The prototype has been rated for 260 p.s.i., equivalent to 600 ft. depth, and is expected to be progressively reclassified down to 1,000 ft. which lies within its design safety factor. The crew environment is "near normal", that is, they breathe air at atmospheric pressure, and submerged endurance is 36 h which in an emergency could be extended to 48 h if the crew sit still. Arrangements for neutral buoyancy and two independently rotatable motors mounted on each side give the craft exceptional manoeuvrability. It can move forward and backward, turn and keep station at a given depth—in addition of course to vertical manoeuvres-all of which was convincingly demonstrated in the Lintott tank last week. It is not exactly nippy, however. Maximum speed is 2½ knots submerged or at the surface. For surveying this is still a considerable gain on the free diver's performance. In air, SURV weighs 6.1 tons excluding crew, not likely to impose much strain on harbour facilities or support ships and so qualifying it for operations in almost any dart of the world, the sponsors hope.

The cost of preparing the first SURV for operations is understood to be about £60,000—roughly 50 per cent of the cost of similar American submersibles, of which General Dynamics' vehicle is the nearest in design. At present SURV's sponsors look to hire rather than outright purchase to recoup their outlay. Hire price, which includes their own "driver", is negotiable but likely to fall within the range £300–£400 per day. So far, they have enquiries but no firm offers. The presence of two members of the National Institute for Oceanography at the demonstration was considered encouraging.

World Health

Within the past few years there has been unhappy evidence that some diseases, far from declining as public health standards improve, are actually increasing. Cholera El Tor, plague, yellow fever, trypanosomiasis, ancylosomiasis, viral hepatitis and venereal disease have all increased, according to the third report on world health, compiled by the World Health Organization (WHO, Geneva, £1 15s.). Against this must be set some real achievements—tuberculosis, for example, which killed one person in nine in the United Kingdom at the turn of the century, caused only one death in 180 between 1960 and 1964. But there is a great

discrepancy between the achievements of the developed countries and those of the underdeveloped world. Europe has been free of cholera since 1923, but during the past ten years it has flared up again in India and Africa. Until 1960 there was a decline, from 212,000 cases in 1950 to 33,000 in 1960, but since then the disease has come back strongly, with a total of 94,000 cases in 1964; the worst hit countries are India and the Philippines.

There is clearly no room for complacency when diseases can re-establish themselves as strongly as But the underlying trend is more hopeful; infant mortality rates, always a good indicator of public health standards, show improvements almost everywhere. In some cases the improvement is dramatic, with mortality rates down by 50 per cent or more. Other countries show smaller improvements, in some cases because mortality rates for babies were already low, but many developed countries which have achieved low rates have reduced them even further. Zealand shows a reduction in infant mortality of 18.7 per cent since 1954, and Canada a reduction of 17.6 per cent. Crude death rates also show a reduction in recent years, with some interesting exceptions. The United States, Argentina and Cuba all show increases of death rate since 1954, and in Europe death rates have tended to remain much the same over the past decade. Elsewhere, decreases have been common.

In developed countries, cardiovascular diseases account for about 40 per cent of all deaths. High blood pressure, the report reveals, is almost universal; only a few very primitive populations and populations living at high altitude are free from it. Cancers of the respiratory system have also become a serious health hazard; in the UK deaths from cancers of this type have increased by 69 per cent since 1954. But there are some striking variations between countries which might be expected to be similar; in Finland the death rate per 100,000 for respiratory cancers was 58·1 in 1963, while in Norway the figure was only 18·7.

Accidents, while not strictly a health problem, are becoming more and more important as a cause of death. In the age group from 1 to 35, they now rank as the leading cause of death. In developing countries, accidents are less significant, but they are beginning to increase in importance, and sometimes rank as the sixth or seventh commonest cause of death.

Response to Aldabra

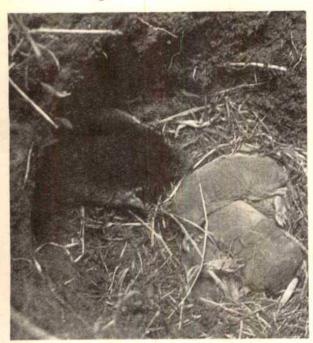
Those who have been campaigning, in the weeks past, to dissuade the British Government from using the island of Aldabra as an air staging post seem now to be entirely willing to press for the facilities that will be needed if the island ecosystem is to be properly studied. It seems, however, to be agreed that the first step should be a clarification of the political status of the island. Administratively, the island comes under the umbrella of the British Indian Ocean Territory, which was originally created for defence purposes. Obviously the British Government could do much to reassure the ecologists by formally detaching Aldabra from that organization.

As yet there is no plan for the long-term conservation of Aldabra, but the Royal Society is eager to help in preparing one. The Nature Conservancy is also a

natural participant in this work, but there would have to be an extension of its terms of reference to allow the conservancy to operate outside the United Kingdom. An official of the Royal Society offers the Charles Darwin Foundation Research Station on the Galapagos Islands as a model for what might be done on Aldabra. It seems to be understood that the Smithsonian Institution, urged on by the National Academy of Sciences in the United States, will be a willing partner in this part of the operation. Two of the nine members of the Royal Society's expedition to Aldabra this summer were from the Smithsonian Institution.

The international research station is a long-term objective, however. The expedition now returned from Aldabra was also impressed with the need for more urgent steps to be taken. One problem is to prevent the fishing of the waters around the island. At present the arrangement is that a Seychellian has been licensed to fish off Aldabra, with the result that there is a danger of the green turtles becoming extinct. Dr D. R. Stoddart of the University of Cambridge, who has just returned from the island, says that the way in which the fishermen live on the island temporarily may be almost as great a danger. In the year ahead, the Royal Society plans to send another expedition to Aldabra in January, and one member of the party of twenty—Mr M. J. Penny, an ornithologist—will probably stay there until March 1969. Everybody hopes that plans for the permanent conservation of the island will by then be complete.

Moles Underground



This picture, the first ever taken of moles underground, won the Wildlife Cameraman of 1967 competition organized by the Council for Nature and Wildlife and the Countryside magazine. The picture, taken by Mr Peter Stafford, shows a parent mole entering its nesting chamber three feet beneath a field in Sussex. The young are lying in a nest of dead grass, with at least two tunnels leaving the chamber. The feeding habits and activity of moles were described by Kenneth Mellanby in Nature (215, 1128; 1967).

Parliament in Britain

Foot and Mouth Disease

MR P. GORDON WALKER stated that research on foot and mouth disease was concentrated at the Animal Virus Research Institute, Pirbright, where it made up about three-quarters of the work of the institute. Grants from the Agricultural Research Council to the institute during the last five financial years had been as follows: 1962–63, £536,587; 1963–64, £248,863; 1964–65, £390,462; 1965–66, £499,379; 1966–67, £522,521. Net expenditure, and hence the size of grant needed, varied with the revenue from the sale of vaccines overseas, which in turn depended on outbreaks of the disease overseas. (Written answer, November 20.)

Dounreav

Two employees have left the Dounreay Experimental Reactor Establishment to go to the United States during the last six months. Mr A. Wedgwood Benn, giving the figures, stated that one was a senior machine operator accompanying her American husband on his return home. The other was a scientific officer who mentioned better pay as his main reason for leaving. (Written answer, November 21.)

Seals

THE Minister of Agriculture, Fisheries and Food, Mr F. Peart, announced that as the National Trust was unable, on the basis of its existing information, to agree to continue culling seals on the Farne Islands, the Natural Environment Research Council suggested that it would be desirable to have a review of the scientific facts and their interpretation by a wide range of scientific opinion. The council was arranging a meeting of scientists as soon as possible. (Oral answer, November 21.)

Defence

MR DENIS HEALEY came in for some vigorous criticism when he announced full details of the defence cuts which the British Government has made as part of the devaluation package. The word betrayal was mentioned by at least one member, but others thought Mr Healey had not done enough. What he has done is bring forward the date for the withdrawal of the aircraft carrier Victorious, saving £4 million, cancelled 8 Buccaneer aircraft, saving £6 million, deferred some Army equipment and cancelled an order for helicopters from the United States, saving another £11 million. There will also be a further reduction of £8 million in research and development expenditure, on top of the £30 million cut Mr Healey made in the last financial crisis. Nuclear weapon development will be cut by £2 million, and other savings will be made by not replacing wastage of civilians in defence establish-The Government had also decided not to build the staging post on Aldabra, although Mr Healey admitted that a decision had been made to go ahead, subject to approval from the American government. Aldabra would have been a valuable addition to British strategic flexibility, he said, but the Services must now forgo it. "I dare say that other sections of the community may see some benefits in this decision." The cancellation would save only £4 million this year, he said; the bulk of the reductions comes in the form of minor cuts and deferments. More than £60 million, he said, would be saved in this way. (Debate, November 27.)

Orbital Weapons

Orbital weapons may have the advantage of surprise, but would they be accurate?

A MONTH ago, on November 3, Mr Robert McNamara, Secretary of Defense in the United States, announced that he has evidence that the Soviet Union has developed a weapons system which consists of a rocket system for launching warheads into orbits about the Earth and then returning them on to predetermined targets. Studies now carried out in Britain suggest that the obvious advantages of such a system, chiefly surprise, will be to some extent at least outweighed by the disadvantages of increased inaccuracy.

According to reports from Washington, the evidence for believing in what is called the fractional orbital bombardment system consists chiefly of nine rocket launchings from the Soviet Union in the past few months. These have all consisted of brief flights in an orbit inclined at 50 degrees to the Equator and with a perigee and apogee of 145 and 210 kilometres respectively. The satellites which have caused particular concern are those known, from Russian announcements, to be Cosmos 139, 160, 169, 170, 171, 178, 179, 183 and 187.

It is also relevant, of course, that the then prime minister of the Soviet Union, Mr N. Kruschev, said in 1962 that the Soviet Union was to develop a rocket system which would enable warheads to be delivered on to their targets from any direction.

To function efficiently, a system of this kind must obviously involve an orbit which is low but yet sufficiently high to avoid serious consequences due to air drag. The element of surprise is the immediate attraction of such a system. Such a missile would be above the horizon from its target for about three or four minutes, but a ballistic missile on a course which spans a distance of 5,000 nautical miles would be visible to direct radars for the whole of 12 minutes—at least four times as long. But it is also plain that attacks with orbital weapons could be launched from any direction, at least if sufficiently powerful launch vehicles are available. With this system, it is also difficult for a defence to predict the point of impact even after an attacking missile has been identifiedeverything depends on when the retro-rocket is fired roughly 10 minutes before impact.

The snags, however, are considerable. For one thing, an orbital system would need a greater launching velocity than a ballistic missile, and the need to carry a retro-rocket implies either a larger launching vehicle or a smaller payload. Detailed calculations of the seriousness of these effects necessarily depend on the detailed characteristics of the rockets concerned, but a rough estimate would suggest that the payload of a rocket vehicle would be halved if it were converted to use in the orbital mode. In this rough estimate, the need for extra launching velocity seems to be decisive and responsible for three-quarters of the reduction of payload.

To make up in part for these disadvantages, it is of some importance that the orbital system could benefit from eastward launching directions. The benefit to be won in this way from the rotation of the Earth seems to be equivalent to roughly an extra 10 per cent of payload. In practice, of course, it would only be possible to take the fullest advantage of this small benefit

The chief disadvantage, however, of the fractional orbital ballistic system seems to lie in the increased inaccuracy likely to be involved. There are two sources of inaccuracy—the inaccuracy due to the inertial guidance system during the launching phase and the period of travel in a low orbit about the

by sacrificing a good deal of the element of surprise.

and the period of travel in a low orbit about the Earth, and the inaccuracy of the retro-rocket. The errors attributable to guidance seem to be greatest 180 degrees from the launching point and least in the

last quarter of the first orbit.

Naturally, the aiming errors depend very much on the types of errors involved in launching, but the sensitivity of the satellite orbits to errors at launching seems to be much greater than the sensitivity of ballistic rocket trajectories. Satellites intended for circular or nearly circular orbits are understandably affected to a quite remarkable extent by errors of vertical speed—a few feet per second can mean as many nautical miles of error at the end of the journey.

The errors due to the retro-rocket itself are likely to be greater. For the simplest retro-rockets based on solid propellants, miss distances of six nautical miles are possible. It is, of course, possible that the designers of an orbital weapons system would seek to diminish the importance of this source of error by carrying better retro-rockets into orbit, in which case the payload would be still further reduced. Errors of yaw (but not pitch) of the rocket at the point at which the retro-rocket fires might be responsible for a further miss distance of more than one nautical mile.

It is also relevant that the errors associated with the drag of the atmosphere in low orbits about the Earth may be quite considerable.

In these circumstances, it would seem that the errors realistically to be expected of guidance systems would be such as to make the orbital system a blunt instrument for use against targets where the errors are greatest. Lumping all the sources of error together suggests that the greatest miss distances might be more than seven nautical miles for the orbital system compared with 1.2 nautical miles for a ballistic missile. Even if the inaccuracy of the retro-rocket were eliminated by improving the system, the errors due to other causes would still be close on four nautical miles. Although it is theoretically possible to improve on this performance by the use of guidance stations near to the point of impact—ships or earth satellites—the chances are that such devices will be for some time impracticable.

Ît is also possible that the accuracy of the orbital bomb could be improved by providing for the weapon to complete one orbit of the Earth before homing on to its target. Accurate tracking during the preliminary orbit would enable the final accuracy of the weapon to be markedly improved, but there would be a loss of the element of surprise which seems to be the principal advantage of the orbital bomb.

Finally, it is worth remembering that this whole discussion may in the end turn out to have been academic. Russia has denied any intention of building a weapon of the sort described here, and the denial should not go unrecorded.

NEWS AND VIEWS

Nation of Astronomers?

At various times in the past decade, the Isaac Newton telescope has seemed something of a joke. The fact that the mirror blank was cast at the same time as the blank for Mount Palomar has occasionally made it seem a little like an afterthought. And the telescope has taken a great time to assemble, even by the standards usually applied to telescopes. Yet, now that it is complete, it is the visionaries and not the scoffers who are justified by events. The past few years have seen a considerable resurgence of interest in astronomy among British academics. It would be wrong, of course, to suggest that the solid line of achievement which goes back at least as far as Newton has at any point been broken, yet it has been something of a surprise to see how buoyant astronomy has become in Britain.

The Isaac Newton telescope is only the most tangible evidence of this development. Just a few weeks ago, there was the opening of Professor Hoyle's Institute for Theoretical Astronomy at Cambridge. Plans are also being hatched for a new steerable radio-telescope, at once bigger and better than any now in service. With luck, a decision to go ahead with the construction of this machine should have been taken by the early summer. By then, there should also be some signs of progress with the Southern Hemisphere telescope in Australia, which will eventually serve not merely as an instrument which British astronomers can use from time to time but also as a strengthening of the link in astronomy between Britain and Australia. The path between Mount Stromlo and Greenwich is well trodden, radio-astronomers in the two countries have learned a great deal from each other (which is not in any way to diminish the importance of the special relationship between Sydney and Cornell) and even Woomera seems to have stimulated scientists, not just engineers. But hardware is always less than half the story, which is why it is every bit as important that astronomy is coming to play a respectable part in academic studies of all kinds. In a sense, there is a kind of boom.

Why should this be? It is too much to hope for a simple explanation, for several disparate influences have played a part in what has been happening. The early start in radio-astronomy, at Manchester and Cambridge, has turned out to be immensely stimulating. Not merely do the radio-astronomers need an optical complement to their instruments, but the kind of work which they have done is itself suggestive of problems which other astronomers can tackle with optical telescopes or just with pencils and paper. But British astronomy has also somewhat paradoxically benefited from the lack of a gigantic space programme.

What has been happening elsewhere has sometimes stimulated interest and curiosity in extra-terrestrial phenomena, but harsh reality has suggested that traditional astronomy might be the most practical means of coming to grips with them. And sometimes, of course sheer perversity has made people strive, with splendid results, to do from the ground what other people say can only be attempted from an orbit about the Earth. At the same time, the influence of individuals has been important. The astronomers tend to be an energetic band of people, and they have in any case a natural cause on behalf of which to make propaganda. The theoreticians have been particularly well served by their advocates.

The administrative arrangements for the support of astronomy deserve particular attention, if only as models of how the development of other branches of science may be stimulated. The astronomers have been lucky. The old Department of Scientific and Industrial Research learned quite early on, from the radio-astronomers, that branches of science other than nuclear physics could be expensive. The department and the Science Research Council, which succeeded to its responsibility for astronomy, have been consistently far-sighted in their policies towards this branch of science. The fact that the scale of spending on astronomy has increased by what may be an order of magnitude in a decade is perhaps less important than the way in which the administrators have been willing to countenance sensible procedures—the arrangements by means of which British astronomers now have access to optical telescopes in South Africa and Italy are splendid examples of this flexibility. Although it would be wrong to suggest that all deserving causes are now adequately supported, the Science Research Council deserves a good deal of credit for what it and its predecessors have accomplished.

Certainly the agreement by which the SRC supports the Radcliffe Observatory in South Africa will be useful to British astronomy, and it is perhaps now unfair to complain about the long delay which preceded agreement on the Southern Hemisphere observatory.

Unfortunately it does not follow from this that the successes of the past few years will automatically be repeated in the future. One cause of difficulty is, of course, that like many other intellectual activities, astronomy is about to change quite radically. So much is clear from the article by the Astronomer Royal on page 855. Electronics has at last caught up with observational astronomy, with the result that the managers of telescopes can look for a substantial

improvement of the productivity of their instruments. The immediate goal seems to be to make the fullest use The instruments at present of image intensifiers. available—or promised—offer an advantage of a factor of twenty or thereabouts in sensitivity. In other words, half an hour of observation with a conventional photographic plate may be the equivalent of just over a minute with an image intensifier in the circuit. This is an enormous saving, and is recognizable as such even in places like California which are not so often plagued by clouds as the United Kingdom. The fact that image intensifiers are most advantageous in the red, where photographic plates are least efficient, may turn out to be a particular advantage. But the overriding gain is one that brings pleasure to the cost-benefit analysts. There can be few applications of electronics where so little equipment can bring such great benefits.

Yet it is too soon to throw hats a long way in the air. For one thing, the image intensifiers have to be fitted into observatory routine. Reliability is a problem that will be comparatively easy to deal with. There are greater problems in turning telescope domes into physics laboratories. And the image intensifiers are only a beginning. Soon people will be wanting to count photons—that could bring a further improvement of an order of magnitude. Then the time will no doubt come when people will ask whether it would not be better to make some telescopes largely automatic in their functioning. People, especially research students, may be cheap computers, but there is likely to be a strong flow of innovation from the techniques now being developed for earth satellites into the design of ground based telescopes. In other words, the time will soon come when the mirror blank is almost a trivial component of a telescope. Astronomers then will be cheerful people, but they will also be expensive. There are some signs that the Science Research Council is fully aware of the opportunities. The question it will have to answer soon is whether it will be able to back its judgement with the necessary funds.

Russian Machines

Some hints about the way Russian engineers are developing machine tools were given in a lecture to the Institution of Mechanical Engineers on November 22 by Professor I. I. Artobolevskii. Professor Artobolevskii, who is head of the Department of Applied Mechanics in the Institute of Machine Research of the USSR Academy of Sciences, had just been presented with the James Watt Medal, the institution's principal award. Because his predecessors include such eminent capitalists as Henry Ford, Academician Artobolevskii was understandably delighted to be the first Soviet scientist to get the award.

The chief problem facing industry in the next ten years, he said, was the transition to the complete automation of industrial processes. The whole concept of the machine itself had undergone significant change, now that it is possible to incorporate logical or control functions and to design cybernetic machines capable of replacing human organs. Professor Artobolevskii explained an important dialectical contradiction. "On

the one hand, automatic systems become more complex and specialized, the economy of operation of this expensive machinery being connected with the constancy of the design of articles and their mass production. On the other hand, technical progress involves constant innovation." This contradiction, he said, can be resolved by creating adaptable automatic systems capable of a rapid switch over from one product to another.

In years to come, he said, methods of chemical machining would come into operation which would eliminate many intermediate processes—die making and mechanical machining-which are characteristic of present day engineering. The problem of building automatic machines was closely connected with the problems of the theory of fluidics, and Soviet workers were developing a variety of pneumatic systems. He suggested that in the Soviet Union work was directed at the development of universal pneumatic systems on a modular basis for use in control systems. More recently, work on volumetric hydraulic drive had been considerably expanded; most of the calculations had been solved on electronic computers and simulators, he said, but on a much smaller scale than was desirable.

Trace Element Analysis

THE Society for Analytical Chemistry held a meeting on "Some Aspects of Inorganic Trace Elements Analysis" at the Atomic Energy Research Establishment, Harwell, on November 15. The ninety participants were welcomed by the head of the Analytical Sciences Division, Dr A. A. Smales, who pointed out that recent advances in materials science have emphasized the need for methods of measuring the distribution and chemical form of impurities in a wide range of materials, as well as for even greater sensitivity of measurement than is at present possible. Techniques particularly featured in the accompanying exhibitions included activation analysis, mass spectrometry, X-ray fluorescence analysis and emission spectroscopy.

During the afternoon, Dr R. K. Webster pointed out that although the isotope dilution technique based on thermal emission mass spectrometry is sensitive and accurate, only one or two elements are usually determined at one time. On the other hand, spark source mass spectrometry provides a wide coverage of elements in a single analysis, often with high sensitivity, but accuracy is limited unless suitable standards are available. He outlined a scheme for extending the isotope dilution method to the analysis of up to ten elements, including some of the alkali metals, and alkaline and rare earths, and showed that by applying the isotope dilution principle in spark source mass spectrometry the accuracy of the latter could be considerably improved.

Mr M. S. W. Webb described the use of a Q-switched ruby laser focused through a microscope to vaporize about a microgram of a sample from an area 50μ in diameter. By arranging for the vapour to short circuit two electrodes with an applied voltage of 2.5 kV, the spark spectra of the elements in the vapour can be recorded by conventional spectroscopy. ductors can be examined and the area to be analysed can be preselected. Examples were given to show the usefulness of the technique in assessing the homogeneity of materials and in examining inclusions.

There were also three papers on activation analysis. Activation by thermal neutrons is now well established. Because several active nuclides are usually present after irradiation it is necessary either to perform radiochemical separations or—in favourable cases—to use multichannel gamma spectrometry to measure active species. Mr D. Mapper described improvements in separation methods and the advance made possible by solid state detectors and the use of data processing and computer techniques.

There are some notable omissions from the list of elements which can be determined by thermal neutron activation. Dr C. A. Baker illustrated this with reference to three important impurities in metallurgy—carbon, nitrogen and oxygen. Irradiation with 30 MeV gamma rays produces radioactive neutron deficient isotopes—carbon-11, for example—which can be used to determine these elements at the p.p.m. level in samples weighing a few hundred milligrams.

Large and expensive equipment is required for activation by thermal neutrons and high energy gamma rays. Dr T. B. Pierce described the recent development of sealed-tube valve type neutron generators using the deuterium-tritium reaction. This makes possible a relatively inexpensive, compact system for the production of fast neutrons and is particularly suitable for the analysis of oxygen, aluminium, silicon, vanadium, chromium and titanium in intact metallurgical samples.

Inhibitory Synapses

from our Neurophysiology Correspondent

THE structure of the neuromuscular junction and of synapses in the central nervous system have been intensively studied by electron microscopists; it is fair to say that the physical basis for the storage and release of transmitter substances is well understood. Indeed, within the great diversity of synapses studied there is a "remarkable uniformity in the structures which are believed to be essentially concerned in their functional operation" (Eccles). Usually a presynaptic fibre expands to form a synaptic knob; within this, close to the presynaptic membrane, are large numbers of round vesicles between 300 and 600 Å in diameter. It is universally accepted (although most of the evidence is circumstantial) that the synaptic vesicles contain "packets" of transmitter molecules.

Recently Uchizono has suggested that there might be an anatomical basis for the recognition of excitatory and inhibitory synapses (Nature, 207, 642; 1965). Several authors have described synapses containing flattened, rather small, vesicles; Uchizono made the hypothesis that these were characteristic of inhibitory synapses. In the current issue of Experimental Brain Research (4, 97; 1967) he describes an attempt to correlate vesicular shape with synaptic function in the cerebellar cortex of cats. Eccles and his colleagues have used the cerebellar cortex as a model system because its structure is well known and conceptually simple. As they have been able to identify many of the gross anatomical features with specific physiological properties and in particular have shown which elements exert excitatory and which inhibitory effects, the cerebellum is ideal for Uchizono's purpose. The cerebellar cortex receives excitation through two sets of inputs—the mossy fibres and the climbing fibres. Mossy fibres synapse with granule cells in the "molecular" layer of the cortex: these give rise to axons which run vertically to the granular layer and there bifurcate, forming the parallel fibres which run along the long axes of the folds in the cerebellar cortex. As the course of the parallel fibres is so characteristic, their synapses are easily identifiable: They are known to be excitatory, and Uchizono shows that they contain spheroidal vesicles. The climbing fibres, which synapse with the dendrites of cerebellar Purkinje cells, are also known to be excitatory; their synapses contain spheroidal vesicles. On the other hand, the synapses of the basket cells, also easily identifiable, are known to be inhibitory: these contain ellipsoidal vesicles. Other synapses containing flattened or ellipsoidal vesicles are tentatively identified as belonging either to stellate or basket cells, both inhibitory.

So far, then, the evidence from the cerebellar cortex supports Uchizono's hypothesis. Several objections, however, have been made to it: Andersen and others have suggested that identification of the origin of a terminal axon can only be certain when supported by degeneration studies. However, it seems reasonable to accept Uchizono's claim of unequivocal identification on the grounds of our knowledge of cerebellar structure. It has also been suggested that ellipsoidal vesicles are fixation artefacts. While it is known that this can be so, several of Uchizono's electron micrographs show neighbouring structures containing round and ellipsoidal vesicles. A more serious possibility is that the same transmitter might be shown to have opposite effects in different synapses—as acetylcholine has on heart muscle and skeletal muscle—for presumably one type of transmitter inhabits only one sort of vesicle. Unequivocal support for Uchizono's hypothesis can only come from identification of the transmitter molecules in the two types of vesicle. Even so, it is at the moment more successful than the previous attempts to link synaptic structure with function.

Algal Toxins

from our Microbiology Correspondent

In comparison with bacterial and fungal toxins, algal toxins have had little attention. This is somewhat surprising because algae cause the well known fresh water and saline toxic blooms and "poisonous red tides", which present hazards to the health of humans, domestic animals and marine life. Indeed, some algal toxins are among the most potent known; that of Gonyaulax catenella, for example, has a lethality approaching that of botulin. Plankton-feeding shellfish have been implicated as vectors of algal toxins, but for the intoxication of other aquatic animals large scale toxin release induced by autolytic, bacteriolytic or algal virus action appears to be a pre-requisite. So far very few algal toxins have been characterized chemically: that of G. catenella is a substituted purine while Microcystis aeruginosa produces a cyclic polypeptide toxin containing D-serine. The mode of action of these substances involves the blocking of axonal and muscle conduction by disturbing sodium or calcium conductance. Apart from studying the production of algal toxins from the viewpoint of animal intoxication, it would also seem profitable to use these substances as tools in the investigation of excitable membranes and also to evaluate their possible antibiotic activities.

Studies in this sphere have been in progress for a number of years at the Hebrew University in Jerusalem, and Dr Moshe Shilo has reviewed recently the current state of knowledge (Bacteriol. Proc., 31, 180; 1967). The Jerusalem school selected the halophilic chrysomonad Prymnesium parvum as a model system, because it is an alga which produces an exotoxin complex responsible for high fish mortality in Europe. Toxic effects include haemolysis, disturbance of gill permeability and cytopathogenicity; other less well defined toxic components induce numerous pharmacological activities in nerve and muscle preparations. The toxins are formed during late exponential and stationary phases of growth, and light is essential for their production (heterotrophically grown cultures in the dark are non-toxic). One of the most interesting and intensively investigated Prymnesium toxins is ichthyotoxin. This toxin requires a divalent cation co-factor and its activity is stimulated by polyamines such as spermine. These co-factors do not function additively and ichthyotoxin activity is the resultant of their complex interaction. Ichthyotoxin induces marked and rapid changes in gill permeability and sensitizes fish to many other non-specific poisons. This toxin has many of the properties of surface active agents and anionic detergents clearly simulate its action. The activated toxin is considered to form a polymolecular micelle and thereby resembles plant

Prymnesium is killed by very low levels of ammonia and other weak electrolytes including acetic acid, and this sensitivity has provided the basis of control measures under field conditions. The electrolyte activity is pH-dependent, which suggests that undissociated molecules and not ions are involved in the killing of the alga. Shilo proposes that osmotic imbalance is established in the alga by the retention of undissociated electrolyte molecules and that this leads to lysis and death. It is significant that other halophilic micro-organisms such as luminous marine bacteria also are sensitive to weak electrolytes and this sensitivity may be a general feature of cells which develop under conditions of extreme osmotic stress.

Ultraviolet Light and Cell Growth

from our Cytogenetics Correspondent

IT is well known that ultraviolet light can cause damage tonucleic acids and that in some cases this damage can be repaired in visible light. Ultraviolet light also prevents cell division and growth, but the physiological basis of this effect and whether it too can be repaired is less well understood. Employing a microbeam of ultraviolet light, Brown and Zirkle (Photochem. Photobiol., 6, 817; 1967) attempted to characterize precisely what is damaged when cell division is halted. A beam 8µ in diameter was focused onto the cytoplasm of cultured axolotl cells approaching metaphase. By varying the wavelength of the light, an action spectrum for the arrest of chromosome movement at anaphase and mitotic spindle destruction was drawn up. The most effective ultraviolet wavelength in arresting both these processes was found to be 277.5 mµ, and the action spectrum follows fairly closely the ultraviolet absorption spectrum of proteins containing tyrosine and tryptophan. So it is possible that some protein in the cytoplasm is damaged by the microbeam and this results in an impairment of function of the nearby mitotic spindle. Could the protein be an enzyme essential for spindle integrity? The action spectrum fits quite well the spectrum for inactivation for at least one enzyme, aldolase, so enzyme damage is a clear possibility.

The mitotic spindle has much in common with the sub-units of flagella. Earlier this year Hipkiss reported in Radiation Botany (7, 347; 1967) that when the unicellular alga Chlamydomonas was irradiated with sub-lethal doses of ultraviolet the flagella immediately become detached from the cell. Probably structural proteins at the base of the flagella are damaged causing the latter to break off from the cell.

The effect of near ultraviolet (365 m μ) on growth of Ginkgo pollen culture and human HeLa cells has been investigated by Klein and Edsall (Photochem. Photobiol., 6, 841; 1967). They find this wavelength most effective in repressing growth of both cell types. Repression of the HeLa cells can be reversed by a period of darkness, but only if it is longer than 15 minutes within an hour-long dark-ultraviolet light cycle. Green light also repressed growth of the two cell types; this cannot be reversed by dark but can be reversed by red light. Other differences between near ultraviolet and green light inhibition are that in both cell cultures ultraviolet light inhibits the early phases of growth while green light inhibits the later phases; and in onion roots Wolff, Fives and Klein (Bull. Torrey Bot. Club, 94, 411; 1967) report that near ultraviolet light slows down the prophase stage of mitosis but green light slows the anaphase stage.

To complicate matters, however, Klein, in earlier publications this year, showed that near ultraviolet light can reverse the effect of ultraviolet light at 254 mµ on leaf fall in Coleus plants (Ann. N.Y. Acad. Sci., 144, 146; 1967). Also, in a number of other plant systems, including the Ginkgo pollen cultures, the repression of growth by ultraviolet light at 254 m μ can be reversed by blue light (Amer. J. Bot., 54, 904; 1967). So far there are no adequate explanations for any of these findings. In the plant systems investigated it is likely that ultraviolet light accomplishes some of its effects through damaging internal auxin (growth hormone) functions. Only a fuller investigation of the effect of light of different wavelengths on proteins, enzyme function and nucleic acid metabolism will reveal more positive answers.

Polymerase Activity and Dissociation

from our Molecular Biology Correspondent

An interesting example of the control of enzymic activity by an association-dissociation equilibrium is provided by the important enzyme, DNA-dependent RNA polymerase. This is a large protein with a sedimentation coefficient, under the usual conditions of preparation, of about 24S. It has been shown that the enzyme will dissociate in response to changes in ionic strength or pH. Smith et al. (Biochemistry, 6, 3057; 1967) have now examined the effect of the template on the association equilibrium; for this purpose it is necessary to use a small template and not a high molecular weight DNA, which would be large compared with the enzyme itself and would contain many initiation sites. Using oligomers of deoxycytidylic and adenylic acids, the authors have made the interesting discovery

that addition of such templates causes a displacement in the association equilibrium in favour of a dissociated form. Thus with a ratio of nucleotide to enzyme of 0·4, a 19S component appears, and this is followed at higher ratios by one of 13S. At limiting nucleotide concentrations, only the last is present. Removal of the oligonucleotide by digestion with a nuclease reverses the reaction. These new components of the enzyme have been recognized before, and it has been supposed that the 19S and 24S states represent the dimer and trimer respectively of the 13S unit. The 13S form is evidently highly active, for it is observed that at elevated salt concentrations, at which the enzyme dissociates, its activity relative to a native DNA template is increased.

This latter observation is confirmed by So et al. (Proc. US Nat. Acad. Sci., 58, 1739; 1967), who have demonstrated enhanced activity in 0.2 M KCl solutions, using several DNA species as templates. Moreover, when the enzyme concentration is very high indeed, no such stimulation is elicited. So et al. suggest that under these circumstances all the initiation sites on the template DNA may already carry a polymerase molecule, so that an increase in the number of independent enzyme molecules through dissociation no longer has any effect. Alternatively, of course, the dissociation of the enzyme may be substantially less at such high concentrations; dissociation will in any case not be favoured by the low template to enzyme ratio.

It is also noted that the reaction at high salt is no longer subject to product inhibition, and so does not cease after a relatively short time, as it does at low salt concentrations. A further effect of the potassium chloride appears to be the suppression of non-specific RNA synthesis (polymerization without correct copying).

with denatured DNA it was observed that when the enzyme in 0.2 M KCl was added to the reaction mixture, addition of KCl now failed to produce the expected result. This is attributed to the instantaneous binding of the dissociated enzyme to the single strands; once bound, it is unable to aggregate again, so that no further disaggregating effect ensues on introduction of potassium chloride. If, on the other hand, the enzyme is added from a solution of low ionic strength, so that it enters the reaction mixture as 24S particles, the enhancement of activity by added potassium chloride is again observed. These results are not incompatible with those of Smith et al.: both high template to enzyme ratios and the added salt are factors which promote dissociation and activity.

So et al. note finally that different univalent cations vary in the degree in which they stimulate the polymerase activity. Whether this arises from differences in their efficacy in promoting dissociation or some other kind of action is not known.

Dynamics of RNA Synthesis

from our Cell Biology Correspondent

Schlessinger's group has shown that the ribosomal population $E.\ coli$ consists of free 30S and 50S sub-units and polysomes without a pool of free 70S ribosomes, and as I mentioned two weeks ago (see Nature, 216, 638; 1967), these observations agree with the latest ideas about chain initiation. In the current J. Molecular Biology (29, 395; 1967) Mangiarotti and Schlessinger

report further kinetic studies of the formation and lifetime of $m{\rm RNA}$, ribosomal sub-units and polysomes which provide more evidence for many of the now almost conventional ideas about transcription and translation.

Mangiarotti and Schlessinger uniformly labelled all the stable RNA in a fragile strain of *E. coli*, which is easily lysed, with C¹⁴ uracil and then switched the cells to a medium containing H³ uracil. The ratio of H³ labelled RNA to C¹⁴ labelled RNA is then a measure of the ratio of new to old RNA molecules. Under their growth conditions complete chains of ribosomal RNA are synthesized in 1 to 2 minutes and it takes at least another 5 minutes to convert the ribosomal RNA into 30S and 50S sub-units by the addition of protein. They claim, unlike earlier workers, that it takes the same time to make either type of sub-unit. Not surprisingly, synthesis of ribosomes in *E. coli* is much quicker than in mammalian cells (see *Nature*, 214, 963; 1967).

The specific activity of the label in free 30S and 50S sub-units and that in sub-units of the 70S monomers of polysomes increases at an identical rate. This means that there is rapid exchange between a pool of free sub-units and the sub-units of 70S ribosomes in polysomes. Furthermore, ribosomal RNA never appears in polysomes in an incomplete form. This is more evidence that ribosomal RNA does not act as mRNA (see Nature, 214, 963; 1967). It also means that the attachment of ribosomes is not necessary for the release of ribosomal RNA from its DNA template.

About 30 per cent of the total H3 uracil incorporated within 6 minutes of giving the label, in other words before new ribosomal sub-units have been completed, is in mRNA. Virtually all the mRNA-about 3 per cent of the total cellular RNA-detectable at any time is in polysomes. These results confirm other evidence that translation begins before transcription has been completed and moreover it shows the mRNA molecules must be functional for almost all their lifetime, which on average is 11 to 12 minutes. Under different conditions Forchhammer and Kjeldgaard (J. Mol. Biol., 24, 459; 1967) found that about half the mRNA in a mutant strain of E. coli has a half-life of about 5 to 7 minutes whereas the other half was relatively stable with a half-life of between 42 and 70 minutes. Clearly the stability of mRNA in E. coli is very dependent on the strain and growth conditions.

In eucells mRNA is generally more stable than in bacteria, but even so in several situations its stability depends on whether or not the cells are dividing; stability increases as the cells lose their mitotic activity. Stewart and Papaconstantinou have just reported an interesting example of this (J. Mol. Biol., 29, 357; 1967). Bovine lens epithelial cells synthesize three closely related proteins, α , β and γ crystallin. Actinomycin D completely inhibits crystallin synthesis in embryonic cells but not in adult cells, although in both cases all RNA synthesis is inhibited. The embryonic cells actively divide whereas the mitotic index of the adult cells is low. Stewart and Papaconstantinou infer from this that as epithelial cells age the mRNA molecules which specify crystallin become stabilized and this stabilization may well be related to the decrease in mitotic activity of the ageing cells, but how it is effected is a mystery.

Recipe for Nuclear Power

NIGEL HAWKES

The Select Committee argues in favour of a single design authority —but not everyone agrees.

THE Select Committee on Science and Technology makes one bold recommendation in its report on the British nuclear power programme, published last week (HMSO, £2 19s.). It says that future nuclear power stations should by designed and built by one central organization, which would incorporate parts of the Atomic Energy Authority and the existing nuclear consortia. Whether or not the committee would back such a central organization was the main doubt behind its deliberations, because the idea divides the nuclear power industry in Britain down the middle. The select committee itself was in two minds and finally decided on the central authority by a majority vote, 7 to 5, with one Labour member abstaining. For the rest, the recommendations are uncontroversial, even mundane.

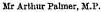
At times, the report gives the impression of a committee so anxious to pick a winner that it was prepared to back every horse in the race—and some not even running. High temperature reactor development should be intensified, with particular emphasis on the Dragon system. Water reactors, and the steam generating heavy water reactor at Winfrith, should also be speeded up. Yet another survey, this time by the Ministry of Technology, should be made of fusion research, although if the committee wants more support for fusion, it should have asked another body to do the job—the ministry has already made its attitude perfectly plain. And the committee also finds the courage to say a few kind words about marine nuclear propulsion; here it recommends a departmental committee to look into the possibilities.

These detailed proposals can, of course, be quietly pigeon-holed if they do not suit the ministry's ideas. It is far less easy to dodge the central recommendations which the committee makes. For one thing, it is probable that the move away from turnkey contracts already set gently in motion by the Central Electricity Generating Board will be accelerated by the committee's approval. And the British Nuclear Export Executive, in existence for only 18 months, always seemed likely to become the whipping boy for the industry's failure to export, although, until the Dungeness B assessment in 1965, the product was wellnigh unexportable except as a curiosity. BNX should be wound up, the report concludes, to be replaced by "an intensive survey" of overseas markets by the Board of Trade, the Atomic Energy Authority and the engineering companies.

It is in the debate about the future of the Atomic Energy Authority that the committee is likely to have its greatest impact. The Minister of Technology, Mr Wedgwood Benn, has looked in the last few months like a man waiting for his mind to be made up, and the report could just do the trick. This is not to say that he will necessarily agree with it in every detail, but certainly the central design authority now looks a likely bet. Under the com-

mittee's scheme, the AEA would be divided into four groups. Those parts of the authority which are concerned with the design and construction of nuclear boilers would go into the central authority or company. The pure research work of the authority would remain as a rump organization, with a stern admonition not to let its attention wander into fields outside nuclear energy. There would also be the fuel processing and fuel element construction side of the authority, which some companies in the private sector should be allowed to join, and a defence research organization continuing the authority's work in the nuclear armament field.







Dr J. M. Hill, Chairman of the AEA.

This bears some resemblance to the arrangements which the authority, in the person of Lord Penney, itself put forward. This may explain why the authority has given the report a cautious welcome. In the official wording—"The authority are gratified that a decision much like their own has been reached". But the authority also makes warning noises about the dangers of fragmentation, particularly in fuel element manufacture. And the detailed operation of splitting the authority into four might turn out to be even less good for morale than the long period of uncertainty which the authority has survived. There should be no doubt about what the proposals actually mean: the report says "The AEA as we know it today may well disappear". Mr Arthur Palmer, chairman of the committee, puts it this way—"What we're suggesting is the breaking up of the AEA".

If the proposal is adopted, much will hang on the exact proportion of the equity of the new organization which the AEA will hold. Mr Palmer said that the AEA would be in a "dominant" position, but later admitted

that this might be putting it too strongly. Mr Eric Lubbock, another member of the committee, believes strongly that the AEA should not dominate the organization. The sort of figures which are being talked about—and which alarm Conservatives—are that the authority should hold about 35–40 per cent of the equity of the nuclear boiler organization. But there is also some evidence that the Government is thinking again, and may modify this figure. Mr S. A. Ghalib, managing director of the Nuclear Power Group, thinks that the exact figure may not be important—"provided that it's less than 50 per cent".

50 per cent". The Central Electricity Generating Board is happy, naturally enough, that the report endorses its own views on the competitiveness of nuclear stations. The report also backs up the board in suggesting that the second nuclear power programme should be slightly larger, something which Mr Richard Marsh, Minister of Power, may be considering. The board accepts, too, that turnkey contracting is on the way out. But behind the assurances it is clear that the CEGB will not be very enthusiastic about dealing with a monopoly supplier of nuclear knowhow—and it is also likely now to claim that its expenditure on laboratories, which will give it technical independence. was doubly justified. Just how unhappy the board will be to deal with the single boiler company is difficult to assess -an official last week suggested that the board would not be particularly hostile. "But we would have preferred two competing groups," he admitted.

The report does not close the door entirely on commercial competition. It says that the CEGB must be willing to invite tenders from abroad for nuclear boilers—in effect, this will mean from the two largest groups in the United States, Westinghouse and General Electric. This is, in fact, a generous recommendation, because it is extremely unlikely to be reciprocated. The very restrictive United States trade policy, enshrined in various "Buy American" Acts, makes certain of this. For political reasons, therefore, a tender from the United States would be most unlikely to be accepted unless it was made through a British licensee—a loophole which may allow some independent British firms to stay in business. The committee also indicates that links between British and European firms might allow the market to be widened. Mr

Ghalib points out that TNPG already has arrangements with Belgonucléaire in Belgium and Snam Progetti in Italy, and is clearly hoping that this sort of agreement may be a way of maintaining competitiveness. But it must be admitted that the removal of commercial competition at home, combined with an acceptance of foreign tenders, is a rather contradictory position for the committee to have adopted. If competition is good internationally, it ought to have at least some merits nationally. Most of these points are made by Conservative members of the committee, in an amendment proposed by Mr David Price which sought to overturn the recommendation for a single authority.

With the publication of the report, the nuclear power industry moves from one period of uncertainty into another. However quick Mr Wedgwood Benn may be in coming to a decision, reorganization cannot take place overnight. The existing consortia will presumably have to work out their present contracts, because a changeover to a new organization halfway through a contract would really be rather wasteful of time and effort. It is sobering to realize that there are still two Magnox stations, Oldbury and Wylfa, to be completed. After that will come Dungeness B in 1970-71, Hinkley Point B in 1972, and Hunterston B in 1973.

One of the interesting aspects of the report is the light it sheds on relations between the select committee and various government departments. The Ministry of Technology seems to have been very correct, except in its refusal to publish the report of the committee which recommended the reductions at Culham Laboratory. The Ministry of Power, on the other hand, is sharply criticized for failing to produce quickly enough a document promised to the committee by Mr Richard Marsh. And the Foreign Office seems to have been deliberately obstructive when the committee sent two sub-committees abroad, although the embassies abroad—and particularly in Bonn were very helpful indeed. The report of the subcommittee which went to Europe has a terse reference to the scale of expenses allowed-which, although it is not quoted, is believed to be £1 a day—forcing the members to entertain out of their own pockets. "The members to entertain out of their own pockets. financial provisions must be improved in future," the report says.

The Isaac Newton Telescope

by
Sir RICHARD WOOLLEY
Astronomer Royal

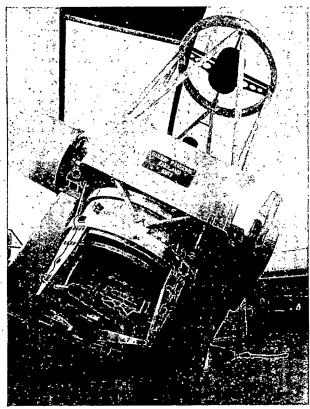
The largest telescope in Britain has been inaugurated in the past week.

The Isaac Newton telescope, which was inaugurated by the Queen on December I, 1967, is a parabolic reflector of 98 in aperture and a primary focal ratio of 3.03. It is equipped with a Cassegrain focus at f/15 and a coudé focus at f/32. At these foci the scales are 27.5"/mm, 5.5"/mm and 2.7"/mm respectively.

That the telescope has been built is a tribute to the advocacy of Professor H. H. Plaskett, then President of the Royal Astronomical Society, as long ago as 1946. Although agreement in principle by the Treasury and the Admiralty to provide funds for the instrument was reached in the following year, the project was delayed for a number of years and the contract for its construction, by the firm of

Sir Howard Grubb, Parsons and Co., was not placed until 1959, since when the manufacture and erection have proceeded at the rate which has been accepted as normal in all large telescope projects. The Admiralty having transferred the administration of the Royal Greenwich Observatory to the newly formed Science Research Council, the council has become the authority responsible for the Isaac Newton telescope. The council has vested the responsibility for the maintenance of the telescope in the Royal Greenwich Observatory, in whose grounds at Herstmonceux the telescope stands; but the observatory has no special claim on the time available for observing with it. In fact the Science Research Council has set up a Large

Telescope Users' Panel, which will consider applications for the use of the telescope from all British astronomers. It is expected that the panel will be in a position to allocate observing time in the last quarter of 1968, for which it will be prepared to consider applications at Easter, 1968.



The 98 in. Isaac Newton telescope.

The telescope has a fork mounted in a novel way, for it is based on a large disk (22 ft. in diameter) mounted in the equatorial plane rather than on a polar axis. The use of the disk has enabled the designers to use very massive tines for the fork, and it is anticipated that these will suffer very little flexure. A second novel feature is the use of an air bag for the support of the mirror. The mirror must, of course, be located at three points, but if these three points sustained the entire weight of the mirror there would be intolerable flexure, resulting in deformation of the optical surface. This difficulty, which has, of course, been recognized for a century, has been overcome in the past by the provision of a number of floating supports—that is to say, mechanical levers. The ideal support is flotation, which is, of course, ruled out, since the telescope must point anywhere from the zenith to the horizon. Support with an air bag achieves the same uniformity as liquid flotation but suffers the difficulty that the air pressure must be made to vary from zenith (bearing the full weight of the mirror) to the horizon, where the weight to be borne is zero. The Isaac Newton telescope mirror support incorporates a system which is designed to adjust the pressure in an air bag under the mirror to the correct value. The mirror must clearly have a side support as well as a back support, and in order to avoid deforming the mirror surface it is necessary to arrange that the two supports do not compete; the permitted coefficient of friction is less than 1 per cent.

The primary mirror weighs four tons. The blank is of 'Pyrex' glass and was cast at the same time as the blank for the 200 in. telescope at Mount Palomar. It was presented to the Board of Management originally respon-

sible for the telescope by the trustees of the McGregor Fund through the offices of the late Astronomer Royal, Sir Harold Spencer Jones. The mirror can be lowered from the observing floor to the ground floor—where there is an aluminizing tank permanently located—by means of a crane which is carried by the dome of the telescope. The secondary mirrors are all made of quartz.

Despite the comparatively modest aperture, the telescope carries a prime focus cage in which an observer can sit and carry out direct photography. At the Cassegrain focus there is an observing chair attached to the telescope, so that the observer can follow the telescope wherever it goes in a long exposure. No problem of locating the observer arises, of course, at the coudé focus.

The Royal Greenwich Observatory has completed the manufacture of a special Cassegrain spectrograph for use in conjunction with the Isaac Newton telescope. This will shortly be adjusted and mounted on the telescope. There is a large space provided for a coudé spectrograph. This has been designed and some part of the manufacture has been commenced, but it is not expected that the coudé spectrograph will be in operation before the end of 1968.

It is, of course, expected that image intensifiers will play a dominant part in the use of the Isaac Newton telescope in years to come, and active plans to bring these into practical operation are currently being made by the Royal Greenwich Observatory in close co-operation with Professor McGee of Imperial College, and with Dr Wynne of the same college. Efficient use of the new sensors demands the design and construction of special cameras to combine low focal ratio with ease of access to the focus, and Dr Wynne will be designing these.

It is not possible to say how the telescope will be used, as it is open to any astronomer to make any proposal to the Large Telescope Users' Panel. The facilities already mentioned (direct photography, Cassegrain spectrograph, coudé spectrograph, possibly image intensifier spectrographs) may be generally available to investigators in 1969 or 1970. It appears very likely that direct photography of interest to radio astronomers will be undertaken at an early date.

The climate of Herstmonceux is, of course, inferior for astronomical purposes to the climates of desert and semi-desert areas in lower latitudes, but it is likely that some work can be undertaken on at least 40 per cent of the nights of the year. It is not advisable to set out on a long exposure unless the night sky is clearer than usual and the weather forecast is good—conditions which obtain on perhaps 20 per cent of all nights. Observers who have had time allotted to them will be expected to maintain a constant watch for breaks in the weather, if they are not actually working, and the SRC will maintain at all times at least one of its own personnel in the dome with instructions to see that the telescope is properly used and not put at risk. It is intended to set up arrangements to accommodate outside observers at Herstmonceux Castle for their observing periods.

The telescope may be expected to give a powerful stimulus to British optical astronomers, and to produce results of first-class scientific value, if due attention is paid to sophisticated instrumentation.

As has already been announced, the Science Research Council is co-operating with Australian authorities in setting up an Anglo-Australian telescope of 150 in. aperture. This telescope will be located on Siding Spring mountain, near Coonabarabran in New South Wales. Some of the time on this telescope may be reserved for the use of a permanent staff, and the remainder will be divided between United Kingdom and Australian observers. It is intended that the UK share in the time of this telescope will be distributed by the Large Telescope Users' Panel. It is unlikely that the Anglo-Australian telescope will be commissioned until about 1975. Its existence will, of course, enlarge still further the opportunities available to British astronomers of the future.

Actions of Thrombin and Other Coagulant and Proteolytic Enzymes on Blood Platelets

by M. G. DAVEY* E. F. LÜSCHER

Theodor Kocher Institute, University of Berne, Switzerland The action of thrombin on fibrinogen is not responsible for its effect on platelets. Thrombin does, however, have an enzyme effect on a platelet constituent: this requires an unsubstituted serine hydroxyl group and a group susceptible to acetylation.

THROMBIN is a proteolytic enzyme resembling trypsin in specificity1. It can be postulated that both its coagulative action and its effect on platelets follow proteolytic reactions. Fibrinogen, the principal natural substrate of thrombin, is present in platelets² and it has been suggested that the platelet "release reaction" follows the hydrolysis of susceptible arginine-glycine peptide bonds in fibringen on the platelet surface3. To establish this mechanism it is desirable to show that other enzymes capable of clotting fibrinogen will cause a release reaction when applied to platelets. Such enzymes are trypsin4, papain⁵, and a number of coagulative substances in snake venoms, particularly of members of the family Crotalidae^{8,7}. We have reported studies of the actions of a number of these venoms8,9: two which were potent coagulants of fibrinogen caused no changes in platelets and five with similar coagulative actions also caused a release reaction. At the time we proposed that both effects could be caused by one thrombin-like constituent. We now report further studies which have proved that this proposal was wrong, and we also report some experiments with other proteolytic enzymes and with a "thrombin" formed by the action of a bacterial coagulase.

Suspensions of washed human platelets were prepared in isotonic media buffered with *tris*¹⁰. The morphological changes and the release of nucleotides after various agents were added were studied as before. The total amino-acid content of perchloric acid extracts of platelets and supernatants was determined by a ninhydrin reaction¹¹, using glycine as a standard. We studied venoms of the yafara, Bothrops jararaca, and the himehabu, Trimeresurus okinavensis (both from Koch-Light Laboratories, Colnbrook, Buckinghamshire), the shore pit viper, T. purpureo-maculata, and the tropical rattlesnake, Crotalus t. terrificus (gifts from Professor R. G. Macfarlane), the Malayan pit viper, Ancistrodon rhodostoma (given by Dr M. P. Esnouf), and the eastern diamond-back rattlesnake, C. adamanteus (Ross Allen's Reptile Institute, Silver Springs, Florida). All contain coagulants of fibrinogen and all but those of A. rhodostoma and C. adamanteus provoke a release reaction⁹. Venom solutions which were kept for any period were stored at -20° C. Bovine thrombin (63 N.I.H. units/mg) was given by Hoffmann-La Roche, Basle.

The enzyme preparations used were crystalline trypsin (C. F. Böhringer, Mannheim), papain and α-chymotrypsin (Worthington Biochemical Corp., Freehold, New Jersey) and "pronase" (Streptomyces griseus protease, grade B, Calbiochem, Los Angeles, California). Fresh enzyme solutions were prepared for each experiment. Human "coagulase thrombin" was prepared at the Centre National de Transfusion Sanguine, Paris, by the activation of partly purified human prothrombin with staphylocoagulase¹²—kindly given by Professor J. P. Soulier. The preparation contained the equivalent of 8 N.I.H.

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clotting units of thrombin/mg of protein. Hirudin (VEB Arzneimittelwerke, Dresden) was a gift from Professor F. Markwardt. Diisopropylfluorophosphate (DFP; Calbiochem) was dissolved in analytical grade isopropanol at a concentration of 0·2 moles/l. This was added to aliquots of venom or enzyme solutions (1–2 mg/ml.). The greatest final concentration used was 0·01 molar DFP (5 per cent isopropanol) and mixtures were kept for at least 2 h at room temperature (20°–22° C) before testing. Fibrinogen clotting times were determined as before. Esterase activities were measured by a formol titration method using toluene-p-sulphonyl-1-arginine methyl ester (TAMe) as substrate¹³.

Electrophoretic separations of venom components were performed in layers of 1 per cent agarose (Serva, Heidelberg) in 0.05 molar veronal-hydrochloric acid buffer, pH 8.2, on glass slides. Proteins were stained, after fixation, with amido black 10B. Esterase activity was detected by covering the unfixed layers with 0.2 molar TAMe containing 0.1 per cent phenol red; yellow zones indicated hydrolysis. Other unfixed layers were cut into 4 mm strips which were eluted with 0.3 molar sodium chloride, and the cluates were assayed for coagulant and esterase activities and actions on platelets.

esterase activities and actions on platelets.

Reaction of thrombin with DFP inhibited its effect on platelets to the same extent as its esterolytic and coagulative actions. The venom of B. jararaca behaved similarly but with the venoms of C. terrificus, T. okinavensis and T. purpureomaculata the esterolytic and coagulant activities could be abolished without reducing their release of platelet nucleotides (Table 1). On electrophoresis, each of the Trimeresurus venoms showed four or five protein bands, two of which had esterase activity. Coagulant activity was eluted with the faster moving esterase fraction in each case. Material which produced a release reaction was eluted independently of the coagulant in both. The thrombin preparation showed a number of protein bands, but esterase, coagulant and nucleotide-releasing activities were all found in the same fraction (a β -globulin).

With the help of Dr M. P. Esnouf (Radcliffe Infirmary, Oxford), the substance in T. okinavensis venom which

Table 1. INHIBITION OF THROMBIN AND VENOM ACTIVITIES BY DEP

Test	Fibrinogen clotting time (sec)		TAMe esterase (µmoles/mg/h)		Platelet nucleotide release (per cent)*	
(0.04 mg)	Intact `	DFP	Intact	\mathbf{DFP}	Intact	DFP
Thrombin B. Jararaca venom C. terrificus venom T. okinavensis venom T. purpureomaculala venom	5 37·2 65·6 26·3 71·0	> 2,400 > 2,400 2,000 > 2,400 1,200	65 280 101 827 210	0 5 6 8 4	61 46 62 55 56	24 33 62 53 56

^{*} Means of three experiments: Control, 20-25 per cent. Test substances were incubated with aliquots of platelets (14-19 mg total protein) for 15 min at 37° C. Concentrations of DFP were 5 mmolar in original solutions of thrombin and venoms, 0.1-0.2 mmolar in incubation mixtures.

releases nucleotide has been separated from the coagulant and other esterolytic and proteolytic components: these results are to be published. So far, the material seems to be of quite high molecular weight (about 500,000), contains some protein and a large proportion of carbohydrate and induces the release of ADP from platelets in plasma at concentrations as low as five to ten molecules for each platelet. No enzymatic activities have been identified. No other fraction of the venom has any effect on platelets. including those with coagulant, esterase, protease and phospholipase A activities.

Table 2. Effects of protrolytic enzymes and thrombin on platelet nucleotide and amino-acid content

	Nucleotide release Free a (per cent) Supernatants		mino-acids (µmoles) Platelets Total	
Control Thrombin 10 units Trypsin 0·1 mg	21 57	1·8 2·6 4·4	5·5 4·9 4·8	7·3 7·5 9·2
Papain 0·1 mg Pronase 0·1 mg Chymotrypsin 1·0 mg	69 56 62 28	4·2 5·4 3·2	5·1 5·3 4·9	9·3 10·7 8·5

Test materials were added to platelet suspensions containing 32 mg total protein and 5 $\mu\rm moles$ CaCl₂ in 1 ml., and incubated for 15 min at 37° C.

Of the purified proteolytic enzymes tested (Table 2), trypsin, papain and pronase, in low concentrations, caused release of platelet nucleotides with associated platelet aggregation and contraction of aggregates. When larger amounts were applied, stable platelet aggregates did not form. Chymotrypsin, up to 1 mg/ml., had no such action. All four enzymes produced large amounts of free aminoacids and peptides in the supernatants but the amounts within platelets did not alter. The effects of the three active enzymes were inhibited in part by removing calcium from the suspensions or by adding hirudin. Trypsin and pronase were also inhibited by DFP, and papain by preincubation with N-ethylmaleimide. agulase thrombin, compared with equivalent coagulant amounts of bovine thrombin, did not alter washed platelets (Table 3). Finally, washed platelets were incubated with coagulants shown to have no nucleotidereleasing effect such as A. rhodostoma and C. adamanteus venoms. After washing, aliquots were exposed to thrombin. Preincubation did not modify the effects of thrombin in any instance (Table 4).

Some conclusions from these experiments are set out in Table 5. The Bothrops venoms are put in a doubtful category, because it has not been possible until now to separate their two coagulant activities (factor X activation and direct clotting of fibrinogen?) from their action on platelets. The latter may be the result of a thrombin-like enzyme or, as was earlier suggested, the activation of factor X, and thus of prothrombin, on the surface of platelets. Other studies of the release of platelet serotonin and nucleotides by snake venoms have also shown that venoms of a number of Bothrops and Crotalus species were active¹⁴. For C. terrificus, this activity could be distinguished from coagulant, protease and phospho-

Table 3. ACTION OF BOVINE THROMBIN AND COAGULASE THROMBIN ON

	PLAT	ELETS			
	Fibrinogen clotting time Aggregation (sec)		Platelets Retraction	Nucleotide release (per cent)	
Control Thrombin 2 units Congulase thrombin 2 units	12·2 9·4	++++	++++	16·7 43·7 17·5	

Mixtures with platelets (37 mg total protein) and 5 $\mu \rm moles$ ('aCl, in 1 ml, were incubated for 15 min at 37° C.

Table 4. ACTION OF THROMBIN ON PLATELETS TREATED WITH COAGULANT

	VENOM	S		
Preincubation with:	Aggregation thrombin	Retraction thrombin	(per	ide release · cent) · Thrombin
Buffer A. rhodostoma 0·1 mg C. adamanteus 0·1 mg	+ + + + + + + + + + + +	+ + + + + + + + + + +	35 30 32	63 60 60

Platelets (114 mg total protein) were incubated for 15 min at 37° C with buffer or venoms, washed, and aliquots of each sample (38 mg protein) incubated for 15 min at 37° C with buffer (control) or 10 units of thrombin.

Table 5. SUMMARY: ACTIONS OF ENZYMES AND VENOMS ON PLATELETS

Release reaction	No action	Doubtful
Thrombin Trypsin Papain	Coagulase thrombin Chymotrypsin	
Pronase	Venom coagulants	
Venom factors* C. terrificus	A. rhodostoma C. adamanteus C. terrificus	Venom coagulants B. atrox
T. okinavensis T. purpureomaculata	T. okinavensis T. purpureomaculata Venom esterases	B. jararaca
* Not congulative.	T. okinavensis	

lipase A activities also present in the venom; it was not inhibited by DFP.

These results make it impossible to maintain that the enzyme action of thrombin on fibrinogen is also responsible for its effect on platelets. They do, however, indicate an enzyme action of thrombin on a platelet constituent, and this is supported by other kinetic studies of the reaction (our unpublished results). This reaction requires an unsubstituted serine hydroxyl group because it is inhibited by DFP and also a group susceptible to acetylation, because acetylated thrombin, which has esterolytic but not coagulant activity, is inactive towards platelets¹⁵. A third part of the thrombin molecule is implicated by the failure of coagulase thrombin to react with platelets. This substance retains the enzymatic properties of thrombin, while differing from normally activated thrombin in physical and immunological properties12. Modification by staphylocoagulase of a site required for binding the enzyme to platelets is suggested.

It is now known that thrombin can hydrolyse peptide bonds in proteins other than fibringen 18,17. In these substrates, the peptide bonds hydrolysed are probably arginine-isoleucine, lysine-alanine or lysine-isoleucine, and not arginine-glycine as in fibrinogen. Furthermore, there is evidence that thrombin alters the activities of a number of coagulation factors8, all of which are known to be associated with platelets18. There is therefore a plethora of possible alternative substrates for thrombin. We can assume that trypsin and the similar enzymes which reproduce the effects of thrombin on platelets act on the same substrate. That a certain specificity is involved is evident from the inactivity of venom coagulants and other venom esterases, all trypsin-like in preference for synthetic substrates. Further study of this aspect may help identify the peptide bond(s) split in the reaction.

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Confirmation of the Genome Donors of Aegilops cylindrica

by

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Department of Agronomy, University of California, Riverside, California A method for identifying the C and D genome donors of Ae. cylindrica (CCDD) from the electrophoretic pattern of protein mixtures of Ae. caudata (CC) and Ae. squarrosa (DD) types may be useful in confirming the B genome donor of wheat.

The origin of the B genome of the tetraploid wheats (AABB) continues to be controversial. A was evidently acquired from the wild diploid $Triticum\ bocoticum\ Boiss.$ or its cultivated derivative, $T.\ monococcum\ L.$, and B presumably from a similar source or from a diploid of the related genus $Aegilops^{2-4}$. Morphological attributes on which the genome donors might be precisely identified, however, are tenuous. Furthermore, observed chromosome homology between the diploids and the tetraploids is only partial for both A and B. This lack of homology has been ascribed to differentiation of the tetraploid genomes following the incidence of amphiploidy^{2,5,6}.

Alternatively, diploid types of maximum homology with the tetraploid genomes may have failed to be identified because of the difficulty of adequately sampling the wild A and B populations by the methods of cytogenetics. This study of the tetraploid Ae. cylindrica Host. (CCDD) (Fig. 1) shows that unique C and D genome donor types can be readily distinguished in the variable population of its diploid progenitors, Ae. caudata L. (OC) and Ae. squarrosa L. (DD) by the relatively rapid method of protein electrophoresis.

Thirty accessions of the Aegilops species listed in Table 1 were increased in the field in southern California and were

documented by voucher specimens filed in the herbarium of the University of California, Riverside. Protein extracts of ground seeds made from these accessions with 70 per cent ethanol⁷ were freeze dried for prolonged storage.

Table 1. Geographical distribution and awn development in subspecies and varieties of three species of Aeqilops

Species Subspecies Variety the species Lemma range awns	Glume
Ae. squarrosa (DD) Eusquarrosa Typica General -	
,, ,, ,, ., Anathera	
., ,, ,, ., Meyeri	
, Strangulata Westward	
Ae. caudata (CC) Polyathera Eastward	
Tunian Wastward	
Ae. cylindrica (CCDD) Typica General	
., ., ., Pauciaristatu ., -	

The crude extracts were fractionated by disc electrophoresis (unpublished work of L. Ornstein and B. J. Davis) using 15 per cent acrylamide gel columns prepared with 3 molar urea and a gel formulation⁸ giving a running pH of 4·3 with β-alanine-acetic acid buffer. The sample applied to each column consisted of 0·5 mg of the dry powder dissolved in 0·1 ml. of the stacking gel solution. Optimum separation of the protein bands was obtained by electrophoresis for 2 h 15 min at 4 m.amp of current per column.

The gels were fixed and stained with 0.5 per cent annido black in 7.5 per cent acetic acid saturated with mercuric chloride, then electrophoretically destained with 7.3 per cent acetic acid and photographed. Bands in the electrophoretic patterns from different species were tested for homology (that is, equivalent migration velocity) by reference to the pattern obtained from a mixture of their proteins. The mixture pattern permitted a band by band verification of homologies between species two at a time and provided the basis for making small adjustments in the length of individual species patterns (Fig. 3) during the photographic enlargement, to compensate for small discrepancies in the speed of migration among gels in different experiments. The patterns were enlarged to conform with a standard length of 10 cm for that of Triticum dicoccum Schübl accession 497 (ref. 9).

Homologies between species were inferred mostly from the bands which had migrated -4.0 cm or farther because occasional inconsistencies of pattern can occur among the slower bands⁹.

The present range of Ae. cylindrica (CCDD) extending from the Balkan Peninsula to Sinkiang (Fig. 2) overlaps that of Ae. caudata (CC) in central Anatolia and that of Ae. squarrosa (DD) from the Transcaucasus area eastward. This suggests the upper Euphrates Tigris valley.

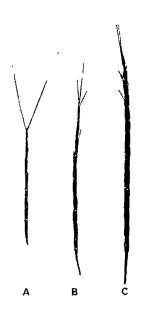


Fig. 1. Spikes of Aegilops caudata (A), Ae. cylindrica (B).
Ae. squarrosa (C).

where the diploids are not now in contact, as the probable site of origin of $Ae.\ cylindrica$. The cytological evidence of its parentage is conclusive^{10,11}, and its morphological intermediacy is apparent (Fig. 1). Early systematists¹² commented on its affinity with $Ae.\ caudata$ but not with $Ae.\ squarrosa$. A more precise identification of the C and D genome donors with recognized morphological types within the diploid species has not been attempted.

In Ae. squarrosa as in other species of the section Pachystachys Eig the lemmas are awned and the glumes unawned (Table 1). In Ae. caudata, as in other species of the section Macrathera Eig, the reverse is true. Systematically Ae. cylindrica, with less strongly developed awns on both glumes and lemmas, occupies a central position between the two. Eig¹² assigns it to the mono-

typic section Monoleptathera.

Of the three species, Ae. squarrosa is the most variable. Its subspecies eusquarrosa Eig (Table 1) comprises three varieties, typica, anathera and Meyeri. These are all generally distributed geographically but var. typica is the most common. The subspecies strangulata is a rare type occurring in the western part of the range. Lemma awn development varies from strong in var. typica to weak in var. anathera. In Ae. caudata the var. polyathera with glume awns on the lateral as well as the terminal spikelets is more common in the eastern half of the species range while var. typica with glume awns only on the terminal spikelets is more prevalent westward. In Ae. cylindrica lemma and glume awns occur throughout the spike in the common var. typica, but only at the apex of the spike in the less common var pauciaristata. Both varieties have a general distribution and transitional types occur.

All of the enumerated subspecies and varieties were represented in the electrophoretic studies except that Ae. caudata types, unequivocally referable to var. typica, were not available. Seventeen accessions of Ae. squarrosa were included from Afghanistan and Iran, six of Ae. caudata from Greece and Turkey, and seven of Ae. cylindrica from the upper Euphrates—Tigris valley and from the adjacent areas of Iran and Turkey where the tetraploid and its respective diploid progenitors intermingle. The selected gels shown in Fig. 3 illustrate the range in protein pattern observed in each species.

A characteristic electrophoretic pattern for each diploid is at once obvious. The *squarrosa* accessions (Fig. 3A-D) exhibit dense, massive bands in the faster as well as in the

slower half of the series, whereas the caudata accessions (Fig. 3E-H) show dense bands only in the slower half. All squarrosa accessions are alike with respect to a broad dense band centred at -9.7 cm which tends to fuse with a narrower one at -9.0. They all show less dense bands at -8.1, -6.7, -6.0, -5.0 cm and all have a dark leading band usually at -10.4 but exceptionally at -10.9 cm. The caudata accessions show a faint leading band at -10.4 or exceptionally at -10.6 cm. They all have discernible bands at -7.7, -6.3, -5.3, -4.2 cm, that at -5.3 being the most conspicuous.

In its general features, the *cylindrica* pattern can be visualized as a combination of the two diploid patterns. For example, it exhibits a *squarrosa* homologue at 9.7 and a *caudata* homologue at -5.3 cm. More detailed comparisons will be made after variability within the

diploid species has been considered.

The three varieties of subspecies eusquarrosa (Fig. 3A-C) can be distinguished one from another on the migration velocity of the fastest band and the degree of separation of the bands at -9.7 and -9.0 cm. Subspecies strangulata (Fig. 3D) differs from var. typica mostly with respect to the density of the bands between -3.5 and -6.0 cm. The gels illustrated in Fig. 3 were selected from ten accessions of var. typica, three of var. Meyeri, one of var. anathera and three of subspecies strangulata. Of the typica accessions six, represented by Fig. 3A, were identical except for differences in density among the slower bands, between 0 and -4.0 cm. The other four deviated towards var. Meyeri with respect to separation of the bands at -9.7 and -9.0 cm.

The six examined accessions of Ae. caudata were identified as var. polyathera. Two gave the protein pattern shown in Fig. 3E. A variation on this pattern consisted of an additional relatively dense band at -4.9 and a denser leading band at -10.4 cm (Fig. 3F). Another variation showed an extra, faint band at -5.8 cm (Fig. 3G) and a third variation showed two extra bands, at

-5.8 and -9.8 cm (Fig. 3H).

Of the three species Ae. cylindrica showed the least variability. It was represented by six accessions of var. typica (Fig. 3I-K) and one of var. pauciaristata (Fig. 3L). All of these had qualitatively identical electrophoretic patterns except possibly between 0 and -4.0 cm. The apparent discrepancy among the slower bands of var. pauciaristata is largely quantitative, because of differences in optical density. This uniformity suggests that Ae

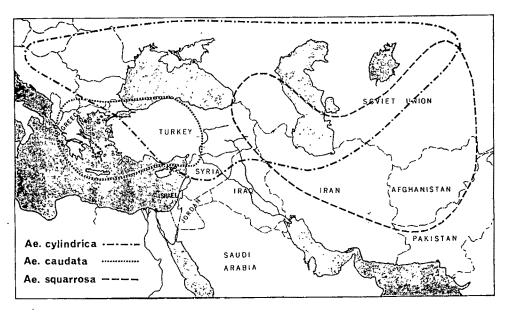


Fig. 2. Geographical distribution of Aegilops cylindrica and its putative parents, Ae. caudala and Ae. squarrosa.

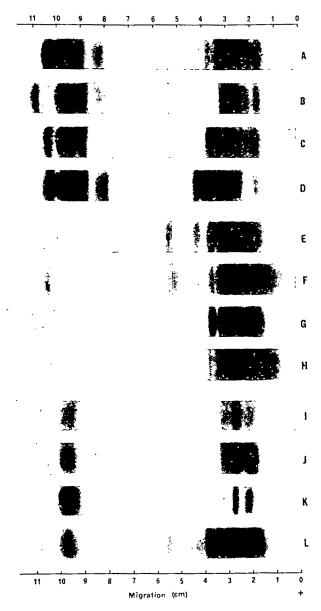


Fig. 3. Electrophoretic patterns on acrylamide gels. A, Ae. squarrosa var. typica (364). B, Ae. squarrosa var. anathera (754). C, Ae. squarrosa var. Meyeri (752). D, Ae. squarrosa subspecies strangulata (963). E-H, Ae. caudata var. polyathera (accessions 756, 592, 593, 591, respectively). I-K, Ae. cylindrica var. typica (accessions 403, 406, 401, respectively). L, Ae. cylindrica var. pauciaristata (605).

cylindrica originated with single amphiploidy event. It confirms cytogenetic evidence suggesting that neither the C nor D genome has been appreciably modified since the origin of the amphiploid. Consequently, the observed variation in the diploid patterns raises the question whether specific amphiploid progenitor types might be recovered in the populations of Ae. squarrosa and Ae. caudata. Protein mixtures from such types would be expected to duplicate the amphiploid pattern in detail.

The protein patterns of Ae. squarrosa var. typica accession 435 from Afghanistan, Ae. caudata var. polyathera accession 758 from Anatolia, and a 1:1 mixture by weight of their extracts are illustrated in Fig. 4A, B and C, respectively. These are compared with accessions 404 (Fig. 4D) and 403 (Fig. 4E) of Ae. cylindrica var. typica also from Anatolia. Except for a difference in optical density of the band at -4.3 cm and some uncertain band homologies between 0 and -4.0 cm, the mixture spectrum

is identical with that of Ae. cylindrica accession 404. Similarly, the electrophoretic pattern of accession 403 and of all the other cylindrica accessions was qualitatively essentially identical with the mixture pattern.

Similarly, any one of the six identical accessions 364, 434, 435, 436, 437, 962 of Ae. squarrosa var. typica in combination with either accession 758 or 947 of Ae. caudata var. polyathera effectively duplicated the cylindrica protein pattern. Other combinations of squarrosa and caudata accessions sufficiently promising to warrant their testing by the protein mixture method failed to do so. Variation in the cylindrica pattern was not enough to reflect any of the variation observed among other diploid accessions. Thus the enumerated accessions seem to represent unique D and C genome donor types, a conclusion which is consistent with their morphological attributes, geographical distribution and prevalence.

The ability of the protein electrophoretic method to pinpoint extant genome donor types of Ae. cylindrica in the variable populations of its diploid progenitors suggests that this technique may be useful in confirming the B genome source of the tetraploid wheats. The latter problem, obviously, is complicated by greater variability both at the tetraploid and diploid levels. At least 10 tetraploid species have been recognized. The Acgilops section Platystachys, where prevalent opinion places the B donor, comprises five species; and the A donor T. bocoticum includes at least two wild types plus cultivated

derivatives.

Diversity among the *Triticum* tetraploids may be a consequence of their postulated complex origin from primary amphiploids of different parentage¹³. Electrophoretic evidence so far^{9,14} suggests that the wild tetraploid *T. dicoccoides* Körn. may have had such a polyphyletic origin, in which case protein mixtures should be useful for identifying the various parental combinations which comprise the primary amphiploids. The primitive domesticated *T. dicoccum* Schübl with its cultivated derivatives apparently originated from one or a few dicoccoides types. The uniform protein pattern of these AABB tetraploids enhances the possibility of finding likely A and B genome donors.

The non-homology referred to between the tetraploid ${\cal B}$ genome and the genome of plausible diploid donors has

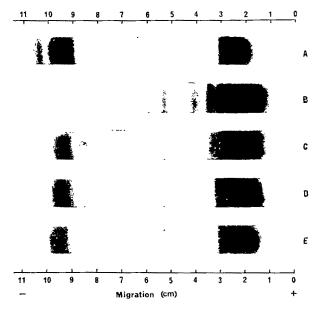


Fig. 4. Electrophoretic patterns on acrylamide gels. A. Ac. squarrosa var. typica (485). B. Ac. caudata var. polyathera (758). C. Protein mixture (1:1) from 435 and 758. D. E. Ac. cylindrica var. tupica (accessions 404 and 403, respectively).

been ascribed specifically to re-arrangements of chromosome structure and mutations at the tetraploid level² and to modification of the tetraploid B genome by introgressive hybridization among various B genome types. In the first case, chromosome re-arrangements involving only the two genomes of a given tetraploid would not be expected per se to obscure gene homologies between the tetraploid and its diploid progenitor as measured by the protein pattern. Gene mutations and, in the second case, non-homology among the tetraploids generated by introgression would be expected to show new gene combinations producing protein patterns unidentifiable with an existing individual donor. But, whether such types can be expected to have subsequently predominated to the exclusion of the primary amphiploids from the population is an open question.

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Proton Magnetic Resonance Studies of the Helix-Coil Transition in Polypeptides

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In proton magnetic resonance studies of polypeptides in solution, the spectral behaviour of the α -CH proton peak depends on the nature of the solvent system, the side chain and the molecular weight. It is considered a more straightforward indicator of conformation than the amide NH peak.

In a recent communication, Ferretti¹ published work on proton magnetic resonance spectra at 100 Mc/s of poly-βmethyl-L-aspartate and poly-L-leucine in trifluoroacetic acid/deuterochloroform solvent mixtures. These spectra showed that the amide NH and a-CH resonance peaks consisted of two components in solutions of partially helical polymer; one component was assigned to protons in the helical polymer and the other to protons in the random coil.

We have studied many homo- and co-polypeptides in the same solvent system and conclude that the spectral behaviour of the a-CH proton peak depends on the nature of the solvent system, the side chain and the molecular weight. Because the amide NH peak broadens more rapidly than the a-CH peak and is subject to additional rate processes, we consider the latter peak to be a more straightforward indicator of conformation. Several cases of behaviour may be defined.

(1) Helical and random coil sections interconvert rapidly; irrespective of molecular weight the α -CH peak will then be a single time-averaged resonance which remains sharp and shows an upfield shift as the helicity increases. This is seen in the spectra of poly-D-alanine shown in Fig. 1.4; the same behaviour has been noted elsewhere for this polymer²⁻⁴. The observation of single peaks for the amide NH and α -CH lines is not caused by the more limited resolution at 60 Mc/s (ref. 1) as can be seen from the 100 Mc/s spectra of several poly-D-alanine samples in 40 per cent trifluoroacetic acid/60 per cent deuterochloroform shown in Fig. 2. The poly-D-alanine samples are of different molecular weights (as judged by the reduced viscosity in dichloroacetic acid), and in 40 per cent trifluoroacetic acid/60 per cent deuterochloroform the b_0 value was about +300 for all samples. It can be seen, however, that as the molecular weight decreases some asymmetry appears in the line shapes, particularly that of the amide NH; the origin of these broader components in the poly-D-alanine spectrum is not clear.

If the helix content of poly-D-alanine is increased beyond 50 per cent by replacing trifluoroacetic acid with dichloroacetic acid, the resonance peaks broaden2. This is because of a slower interconversion rate of helical and random coil forms and the fact that, as helical segments increase in length, their correlation times become longer. The rapid interconversion rate of poly-D-alanine at helix contents as high as 50 per cent may result from the small alanine side chain and, if this is so, then the rate of interconversion for different polypeptides might be dependent on the size and nature of their side chains. For other polypeptides broadening and loss of area might therefore be expected at lower helix content. Evidence for this possibility comes from the behaviour of the a-CH resonance peaks of poly-L-leucine shown in Fig. 1B. This peak shows pronounced broadening at an appreciably lower helix content than is observed for poly-D-alanine. The single broadened peak for poly-L-leucine probably results from an intermediate interconversion rate of protons between the random coil sections and helical segments⁵.

The spectra given in Fig. 1B differ from those of Ferretti in that they do not show a double peak and this is possibly a result of differences in the molecular weight of the poly-L-leucine samples. This leads to a second type of behaviour of which Ferretti's spectra may be an example.

(2) Polypeptide chains which have a short overall correlation time, and in which the helical and random coil forms, do not interconvert rapidly. This is observed principally for low molecular weight samples and the slow interconversion rate may be a result not only of the nature of the side chain but also be a direct consequence of the short chain lengths. The short correlation time for the motion of the whole chain leads, for case (2), to two

components of comparable widths assignable to the helical and coiled forms. Examples we have studied which demonstrate this effect are given in Figs. 1C and 1D. These show the α -CH resonance of poly-D- α -amino-n-butyric acid and poly-D-norleucine in deuterochloroform/trifluoroacetic acid mixtures from 10 per cent to 40 per cent trifluoroacetic acid, a solvent range which covers a large change of b_0 values. The relative areas of these components alter in accordance with changes in helix content while the total line area remains constant. The form of these resonance peaks is very similar to those given by Ferretti¹, and, as he pointed out, the observation of the separate components puts an upper limit of $10^2~{\rm sec}^{-1}$ on the rate of interconversion between helical and coiled conformations.

The spectra given in Figs. 1C and 1D demonstrate that effects, similar to those observed by Ferretti at 100 Mc/s, can be observed at 60 Mc/s. Markley et al. have observed that the line width of the α -CH peak of poly- γ -benzyl-L-glutamate and poly-L-methionine goes through a maximum as the helix content increases. This effect was attributed to an increase in the rate of interconversion at high helix content, but it may be an example of case (2) because it can be seen from Figs. 1C and 1D that if the components were not resolved the overall line width would appear to go through a maximum when the components were of equal intensity.

(3) It is not possible in case (1) to relate the area of the backbone proton resonance peaks to the helicity because the areas remain unchanged over a large range of helix contents and in case (2) it is possible only if the two components are well resolved. There is a class of polypeptides, however, for which this correlation might be expected. These are polypeptides of high molecular weight in which the helical and random segments are either completely prevented from interconverting (for example, proteins, where the differing stabilities of helical segments could inhibit this process) or interconvert slowly either because of large side chains or because of side chains

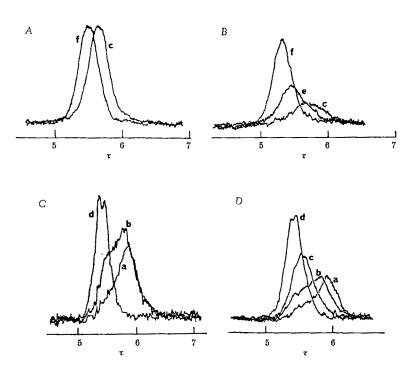


Fig. 1. The a-CH peak at 60 Mc/s of: A, poly-D-alanine; B, poly-L-leucine; C, poly-D-alanino-n-butyric acid; D, poly-D-norleucine, in the following trifuoroacetic acid/deutero-chloroform mixtures: (a) 10 per cent trifuoroacetic acid; (b) 20 per cent trifuoroacetic acid; (c) 30 per cent trifuoroacetic acid; (d) 40 per cent trifuoroacetic acid; (e) 60 per cent trifuoroacetic acid; (f) 100 per cent trifuoroacetic acid. b_0 values: Poly-D-alanine: +290 (c) +100(f); poly-L-leucine: -487 (c) -325 (e) -132 (f); poly-D-alanine: -hutyric acid: +300 (a) +100 (b) 0 (d); poly-D-norleucine: +400 (a) +314 (b) +146 (c) +72 (d).

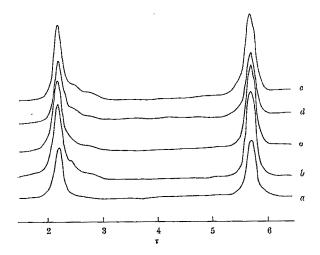


Fig. 2. The α -CH and amide NH peaks at 100 Mc/s of poly-D-alanine samples of different molecular weights. Reduced viscosities in dichloroacetic acid (l./residue mole): a,47; $b,24\cdot5$; $c,18\cdot46$; $d,8\cdot15$; e, poly-L-alanine initiated for 100-mer.

which interact strongly with the solvent. In these cases, the resonance lines from protons in helical segments will be broad and may be unobservable, while those from random coil regions will be sharp. The peak area will then be a measure of the random coil component. It is possible, however, that at low helix content the helical segments have sufficient mobility for the broad component to be observed; in these circumstances the width of the broad component would be greater than that of the narrow line. An example of case (3) for a homopoly-peptide may be the original observation of Goodman and Masudas that the ratio of the area of the amide NH peak to the terminal side chain methyl peak in poly-ethyl-L-glutamate followed the b_0 value. We have made similar

observations for poly-L-lysine-hydrobromide in aqueous solution, a system in which the side chain is not only large but also interacts with the solvent.

For proteins and polypeptides in case (3), nuclear magnetic resonance can give information on which residues are incorporated in helical segments from the behaviour of their side chain resonance peaks because the magnetic anisotropy of helical segments which have long correlation times will broaden the resonance peaks of groups close to the helical backbone. This effect has been observed for histones in aqueous solution at various salt molarities^{6,0} and for poly-γ-benzyl-L-glutamate^{4,10}.

It should be noted that cases (1) and (2) apply to polypeptides in trifluoroacetic acid/deuterochloroform: for this system the ra-CH peak shows a considerable chemical shift difference between helical and random coil forms which has been attributed to the magnetic anisotropy of the helical form as compared with the random coil form3,4. In aqueous systems, however, the shift is either much smaller as in the case of poly-Lglutamic acid4 or is absent, as we have observed for copoly (glutamic acid⁴², lysine²⁸, alanine³⁰) (ref. 7). Thus the magnetic anisotropy of the helical form is not the only factor involved in the large shift observed in trifluoroacetic acid/ deuterochloroform systems and we have suggested that an additional anisotropic effect may result from a specific interaction of trifluoroacetic acid with helical segments of the polypeptides². For aqueous systems a single α -CH peak is therefore observed and the fall-off in peak area as the helicity rises depends only on the rate of interconversion between the two conformations, unless the molecular weight is low enough for the overall correlation time to

be the line width determining factor.

It should be stressed that, because of the molecular weight dependence of some of the effects described, polypeptides used for nuclear magnetic resonance studies must be well characterized. Low molecular weight samples may explain reports that fully helical polymer gives rise to observable peaks for backbone protons, while it is our experience that fully helical polypeptides, as indicated by optical rotatory dispersion measurements, do not show these resonance peaks.

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Internal Organization of the Ribosome

bу

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The conformations of RNA in yeast ribosomes and in the free state are similar. The ribosomal proteins are believed to be in the interior and associated with non-helical parts of the RNA and probably with each other; the surface is largely RNA.

Although the conformation of ribosomal RNA in solution has been studied in some detail1-5, little has been established about the organization of RNA and protein in the native ribosome. We have been concerned primarily with establishing the nature and conformation of the macromolecular components of the yeast ribosome in situ and, as far as possible, their distribution between the surface and the interior of the ribosome. We have attempted, on the basis of our results, to construct a simple model to embody the gross features of morphology which can be deduced.

Ribosomes from the yeast Saccharomyces fragilis in the exponential growth phase were prepared as previously described. They were purified by chromatography on DEAE-cellulose, and the ribosomes thus produced were stable for some hours at room temperature and for long periods in the cold. Ribosomal RNA was extracted with phenol, and ribosomal proteins were extracted with acetic acid, also described earliers. Acridine orange was purified by the procedure of Lamm and Neville⁹. Thermal difference spectra were measured with a Beckman DK-2A recording spectrophotometer and melting curves were taken from these or were measured separately in a Beckman DUinstrument. For the standardization of concentrations, $\epsilon(P)$ for the RNA was taken as 7,400 (ref. 8), and $E_{1 \text{ cm}}^{1 \%}$ for yeast ribosomes as 113 (ref. 10). Optical rotatory dispersion was measured with a Bellingham and Stanley 'Polarmatic 62' spectropolarimeter, with cells of 1 cm path in a cell housing controlled by a thermostat. Precautions were taken to avoid stray light and band width errors by ensuring that measured rotations were independent of concentration or path length and slit width.

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Sedimentation coefficients were measured with a Spinco model E ultracentrifuge, using ultraviolet absorption optics. Viscosity determinations were performed with an Ubbelohde suspended-level capillary viscometer with a water flow-rate of 5 min.

Ribosomes sedimented at 42,000 r.p.m. in the standard tris-magnesium buffer gave a single peak with sedimentation coefficient, $s_{20,w}^0 = 80.4S$. The intrinsic viscosity, obtained from extrapolation according to the Huggins equation, was 0.046 dl./g, with a Huggins constant, k'=0.65. The partial specific volume of the ribosome was taken as 0.67 (ref. 10), and the molecular weight was calculated from the Scheraga–Mandelkern equation 11, taking $\beta=2\cdot12\times10^{-6}$ for a spherical particle. We obtained a molecular weight of 3.95×10^6 .

Early attempts to measure the temperature-absorbance profiles of intact ribosomes^{12,13} produced data only up to temperatures of 40°-60° C, because of heavy precipitation in this range. We have confirmed this effect, but have also found that if the ribosomes are dissociated into their 60S and 40S sub-units by brief dialysis against a magnesium-free buffer (0·1 molar sodium chloride, 1 mmolar tris), precipitation is eliminated and complete melting profiles are obtained. A low scattering level is always observed in the ribosome solutions and correction for this was made by a logarithmic extrapolation of scattering outside the absorption band. The scattering correction at 258 mu amounted in our instrument to no more than an absorbance of 0.03 at 20° C, rising to 0.05 at 95° C, for an initial total absorbance of the order of 0.7. Fig. 1 shows temperature melting curves for the dissociated yeast ribosomes and for the extracted RNA in the same solvent. The protein absorption is assumed to remain unchanged with increasing temperature; a correction for

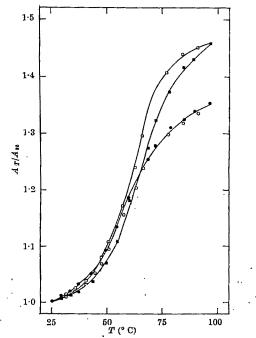


Fig. 1. Temperature-absorbance profiles of yeast ribosomal RNA and ribosomes dissociated into 60S and 40S sub-units. Absorbances of ribosomes are corrected for protein absorption. Open symbols refer to ribosomes, filled symbols to extracted RNA. $\bullet \bigcirc$, 257 m μ ; $\blacksquare \square$, 282 m μ .

the protein absorption is applied from molar absorptivities of the RNA and extracted proteins. This correction affects only the value of the absolute hyperchromic absorbance increase and not the position or shape of the curve; it is small at 260 mµ but appreciable at 282 mµ.

It can be seen, as briefly mentioned earlier 14, that at 258 m μ the melting curves of ribosomes and RNA almost coincide. At 282 m μ , where the accuracy is less (lower absorbance) and interference from protein absorption is greater, the curve for the ribosomes seems to be slightly displaced to lower temperatures. In both cases, the corrected hypochromicities are the same for RNA in the ribosome and the free state within the limits of error.

Fig. 2 shows temperature difference spectra at various temperature intervals for the ribosomal RNA. A comparison with standard difference spectra for poly $(A+U)^{16}$ and poly $(G+C)^{16}$ shows (as do the curves of Fig. 1) that the regions melting out as the temperature increases become progressively richer in (G+C) content. The top curve of Fig. 2 gives the composition of the total RNA helix in terms of these standards and within limitations to be discussed as about equimolar in the four bases.

The interaction of acridine orange with ribosomal RNA, extracted protein and intact ribosomes has been studied. The dye binds to ribosomes as workers previously noted 17,18 but not at all to proteins. Fig. 3 shows spectrophotometric titrations of acridine orange with ribosomes and extracted proteins. Determination of the number of sites available to the dye in the intact ribosomes presents considerable difficulties. To maintain the integrity of the structure an appreciable concentration of magnesium ions is required and magnesium greatly weakens the affinity of the binding sites for the dye (presumably by competition). Consequently, titrations of dye with ribosomes or RNA in these conditions show curvature (as theoretically predictable19) which precludes the accurate determination of end In the conditions used by Furano et al.18, especially with the more magnesium-dependent ribosomes from E. coli, the structure must be presumed to be wholly or partly disrupted20,21 and we therefore suppose that in such conditions a limiting value is obtained for the number of binding sites. The situation is further complicated by the possibility that the curvature of the binding isotherms may be due, at least partly, to a heterogeneity of the binding sites, rather than to weak binding alone. Such an effect has indeed been suggested for binding of acridine dyes on DNA^{22,23}. Our value of about 90 per cent (Fig. 3) for the proportion of phosphate sites available must thus be seen as an upper limit as far as the intact ribosome is concerned. It is in good agreement with that of Furano et al.18 for E. coli ribosomes and qualitatively similar to the result of Morgan and Rhoads¹⁷. If one takes the curvature to reflect heterogeneity of binding, an end point corresponding to as little as 60 per cent of the total phosphate groups can be derived. We have made one further attempt to overcome this problem: it has been shown²⁴ that ribosomes can be internally cross-linked by formaldehyde and will then maintain their native morphology even in the absence of magnesium. Ribosomes treated as described by Spirin et al.24 were indeed shown to sediment at 80S, even in the presence of EDTA. The accidine orange binding curve (Fig. 3) shows the availability of about 80 per cent of the phosphates. We therefore take this as an effective upper limit. It may be noted that ribosomes saturated with dye remain intact, and sediment in the ultracentrifuge as an orange boundary.

Fig. 2 shows that the helical portion of extracted yeast ribosomal RNA is made up of a heterogeneous population of helices of varying composition which melt over a wide temperature range. On the basis of data for synthetic polynucleotides^{15,18}, the total helix content is estimated to be about 60 per cent. This is probably a lower limit, as the full hypochromicity of short helices does not seem to be realized until a substantial helix length has been reached^{25,26}. It is also assumed that the hypochromicity arises entirely from Watson-Crick double helices, and no

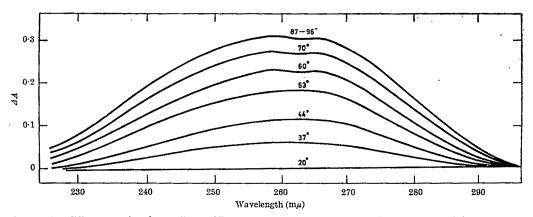


Fig. 2. Temperature difference spectra of yeast ribosomal RNA. The curves show absorbance differences (uncorrected for thermal expansion) of RNA at the indicated temperature. RNA at 20° C is used as reference.

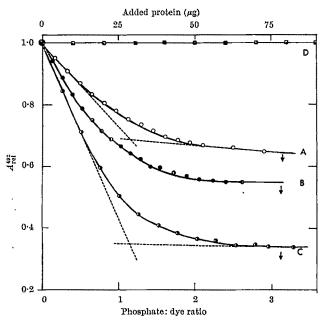


Fig. 3. Spectrophotometric titrations of aeridine orange with yeast ribosomes and extracted soluble ribosomal proteins. A. Ribosomes in the presence of 1 mmole magnesium ion; B, ribosomes in 2 mmoles magnesium; C, ribosomes treated with formaldehyde, no magnesium ions; D, extracted ribosomal proteins. The top scale refers to micrograms of added protein, the bottom scale is the phosphate: dye ratio for all ribosome titrations. The buffer was 1 mmole cacodylate, pH 7.2.

account is taken of any single strand stacked structures^{27,28}, although some such structure must in fact be present.

We can deduce little, quantitatively, about the lengths of helical regions, although a minimum length can be tentatively set in terms of data on oligomer-polymer complexes^{28,31}. At the same time, a comparison of the temperature absorbance profiles of Fig. 1 with those of two stranded viral RNA^{32,33} shows that the helices in ribosomal RNA are not "long". Taking into consideration the formation of crystalline helical fragments with average chain lengths of about forty nucleotides*, by controlled degradation of yeast ribosomes³⁴ and ribosomal RNA (Spencer et al., to be published), we conclude that the helical portions in the native state are unlikely to be longer than this. The assumption that ribosomal RNA is made up of rod-like double helical "hairpin" segments, joined by single stranded links¹⁻⁵, seems then to be entirely compatible with our results on the yeast system.

Fig. 1 also suggests that the conformation of the RNA in the ribosome is similar to that of free RNA, and indeed the value of the hypochromicity of the ribosome seems to bear this out further (compare similar evidence from hypochromicity in *E. coli* ribosomes³⁸). This conclusion conflicts with that of Furano et al. 18, based on measurements of stacking coefficients for binding of acridine orange to *E. coli* ribosomes at low magnesium concentrations. The stacking coefficient 39,40 is a measure of the tendency of an incoming dye molecule to bind at a site adjacent to one already occupied by another dye molecule. Nonrandom binding in these terms is thought to depend on the flexibility of the polymer chain, the stacking coefficient

being high for polynucleotides possessing little or no base pairing. From the values of their stacking coefficient for the ribosome-dye interaction, Furano et al. conclude that ribosomal RNA in the ribosome has a conformation quite different from that in the free state and is in fact single stranded, with no base pairing.

To account for this apparent discrepancy in our results, we offer the following arguments. (1) The interpretation of stacking coefficients in terms of conformation implies that the polynucleotide chain is flexible in the ribosome and can freely distort to allow stacking of the dye molecules. If the ribosome is a rigid structure, then such a deformation would presumably be impossible and the magnitude of the stacking coefficient would then be uninterpretable. (2) The close similarity of the optical rotatory dispersion curves of RNA in the free state and in the ribosome (correction being made for the protein contribution) has been noted by several groups of This suggests that the conformations are in fact similar. If the RNA were single stranded, a different curve would be expected. Fig. 4 shows some results: ORD curves for single stranded ribosomal RNA, based on the dinucleotide data, as applied by Cantor et al.43, have been computed. The curve in Fig. 4, corresponding to a random sequence of the correct base composition, is shown to be very different from the observed curve. By working at very low ionic strength, the RNA may in fact be obtained in the fully single stranded form (compare TMV RNA43). The ORD curve of this form is shown in Fig. 4, and is seen to be in agreement with the calculated curve (based on pairwise interaction of nucleotides). On addition of salt, a large change occurs, the curve moving in the direction predicted for the introduction of substantial base pairing. The introduction of base pairing into a calculation requires more drastic approximations and is less quantitatively reliable. Even here, however, the experimental curve is seen to be quite close to the theoretical, and, for the composition of the double helical portion deduced from our thermal difference

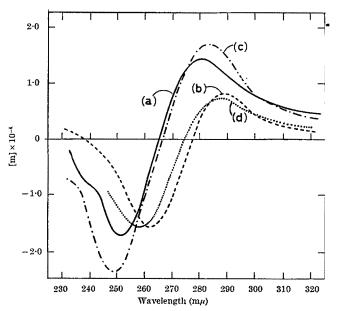


Fig. 4. Calculated and observed optical rotatory dispersion curves for yeast ribosomal RNA. (a) Observed curve for yeast ribosomal RNA (ionic strength 0·1); (b) observed curve for ribosomal RNA in 10·4 molar EDTA (no other salts present); (c) calculated ORD curve for 60 per cent base pairing. The composition of the base paired part was derived from thermal difference spectra. The curve was calculated using standard dinucleotide data⁴³ for the unpaired part, assuming a random sequence of the appropriate base composition, and data for the double-stranded part were obtained directly from Sarkar and Yang^{56,58} for poly (A+U) and poly (G+C). (The difference curves of Cantor et al.⁴³ do not give the correct result for such a system, because they are based implicitly on single strand states of homopolymers.) (d) Calculated ORD curve for a single stranded chain with the base composition of ribosomal RNA, assuming a random sequence.

^{*} The fragments used for the X-ray studies 36,36 have been characterized hydrodynamically (Gratzer, unpublished work) with the following results: partial specific volume 0·506 (determined by the sinker method), $s^0_{20,w} = 2\cdot5S$, intrinsic viscosity 0·050 dl./g, extrapolated weight–average molecular weight (corrected for charge effects) from sedimentation equilibrium 14,800, z-average molecular weight about 21,000. This gives $M_z/M_w \sim 1.4$. The sample was found by acrylamide gel electrophoresis to contain some 12 per cent sRNA. Taking this into account it appears that the fragments which can be crystalized have $M_w \sim 13,000$, and a weight–average length of some forty nucleotides, athough, as hypochromicity and optical rotatory dispersion studies indicate, a considerable amount of non-crystalline amorphous material is present.

spectra, our estimate of 60 per cent base pairing produces the best fit. From these results we are prompted to argue strongly against a single stranded conformation in the ribosome. (3) Finally, we may mention that when acridine orange is bound to RNA it becomes optically active with Cotton effects in the visible region. These are strongly dependent on conformation and can even have different signs in different polynucleotides. We have examined complexes with RNA and ribosomes at saturation and find Cotton effects of identical shape, although the amplitude in the ribosome complex is somewhat greater. We shall therefore proceed on the assumption that our picture of RNA conformation is valid in relation to the ribosome.

The close coincidence of the melting curves of RNA and ribosomes (Fig. 1) leads to a further important conclusion. It is well known that when a basic polymer, such as a ribosomal protein, binds to a nucleic acid, the double helical state of the latter is strongly stabilized, and the melting temperature is raised by some 15°-20° C at ionic strengths such as those used here44-46. Indeed, Bach and Miller46 have shown that substantial increases in melting temperature are brought about by binding of polyampholytes containing only a minimal or zero excess of basic over acidic groups. The absence of any substantial stabilization effect in Fig. 1 can be interpreted only as indicating that the protein is not bound to the helical

regions of the RNA.

The weight-average molecular weight of the yeast ribosomal proteins has been given as about 15,00010 and these proteins, which are partly a-helical and evidently globular in character in situ8, will have an average diameter, based on their partial specific volume of the order of 30 Å. Such a sphere could be accommodated, taking the extreme case, in a loop of RNA a minimum of some 50 Å long (half the circumference of the sphere), which would correspond to some seven nucleotides if the chain were fully extended (taking 7.2 Å as the repeat distance for a fully extended polynucleotide chain). Because the yeast ribosome contains 57 per cent by weight of protein^{10,47}, that is, about 150 molecules, the total minimum length of loop corresponds to somewhat more than one thousand nucleotides but is likely to be substantially greater. On the basis of 5,400 nucleotides in the ribosome, it follows that the greater part of the non-helical nucleotides (more in any case than a minimum of one-half of the minimum non-helical proportion of 40 per cent) would be involved in fixation of the proteins. A possibility which may be considered is that the proteins may be to some extent associated with each other, as in typical sub-unit situations. The total protein-RNA contacts in such a situation and thus the proportion of RNA involved would be considerably diminished. This picture receives some support from the high degree of association between the extracted proteins48.

The temperature-absorbance profiles at 282 m μ (corresponding chiefly to the melting of G-C pairs) actually show a small apparent destabilization of the RNA in the ribosome. The readings at this wavelength are, however, smaller and subject to a considerable correction for protein absorption, which probably changes slightly itself in accordance with the familiar denaturation blue-shift phenomenon48 and we are therefore disinclined to regard this small effect as significant*.

The dye binding experiments indicate that a large proportion of the RNA is available on the surface of the ribosome. We cannot rule out the possibility that the dye penetrates the ribosome and binds in its interior, but it seems inconceivable that anything approaching such a large number of dye molecules could enter the structure. Furano et al.18 argue from their observation that, as acridine orange can be displaced from the ribosomes by high molecular weight polylysine, it must be on the

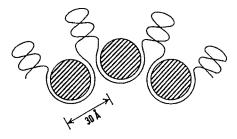
outside. It should be noted that basic polymers, such as this and the ribosomal proteins, must compete with the acridine orange for RNA sites, so that, if the protein is at the centre and shielded, unfolding of the structure will not necessarily expose any large number of unoccupied binding sites. If, however, the protein were on the surface, it would either be displaced by the dye, or the dye-binding capacity would be very small indeed. We have shown that in fact no significant amount of protein is displaced by acridine orange: dye-ribosome complexes of widely varying compositions were pelleted in the ultracentrifuge, and protein in the supernatant was assayed by hydrolysis and spectrophotometric ninhydrin determination⁵¹. An upper limit of about 5 per cent of the total ribosomal protein only can be displaced by the dye.

The conclusion thus seems almost inescapable that the exterior of the ribosome consists substantially of RNA rather than protein. If, moreover, it is accepted that the proteins are associated with the non-helical, rather than helical segments, the exposed RNA should be essentially helical. This is supported by the remarkable resistance of the intact ribosome to ribonuclease52.

Electron microscope examination of yeast ribosomes¹⁰ shows roughly spherical particles with a radius of about 100 Å. The hydrodynamic properties, however, indicate that the effective hydrated particle in solution is larger. The partial specific volume (anhydrous) leads to a volume of 4.3 × 104 Å3, and a radius for a spherical particle of 100 Å; this coincides with the electron microscope value, and suggests that the desiccated particle thus observed is in its most compact state. The frictional coefficient calculated from our sedimentation data is 2.7×10^{-7} and the corresponding frictional ratio is 1.41. For a spherical particle, this leads to a hydrodynamic radius of 140 Å. (Effective hydration 1.2 g/g; it cannot be determined to what extent this hydration represents an external hydration shell, rather than a swollen particle.)

We are now able to construct a tentative model of the yeast ribosome. The surface of the dry particle can accommodate a maximum of 3,400 helical nucleotides. if the helices lie closely packed on the surface. accounts for total number of helical nucleotides deduced to be present. In the hydrated, swollen, particle these helices would presumably be less closely packed. Alternatively, one may consider the RNA helices to project from the surface, as shown in Fig. 5. In this case, the surface area is sufficient to accommodate very easily a sufficient number of helices, no matter how short the average helix. In this model, the accessibility of all helical sites to the dye molecules becomes less problematical. In either case. the amorphous loops are envisaged as projecting into the centre of the ribosome, where they are interleaved with protein molecules. The simple model of Fig. 5 seems to satisfy all the observations. Its relation to the surface patterns observed in electron micrographs of shadowed E. coli ribosomes⁵³ is not obvious, although no incompatibility necessarily exists. Indeed, the lattice of crystal-

SURFACE



INTERIOR

Fig. 5. Suggested model (schematic) of ribosome structure. The diagram shows part of an idealized section of a yeast ribosome, the proteins being shown as shaded spheres attached to the non-helical parts of the RNA in the interior of the particle.

^{*} A recent report of melting curves of intact $E.\ coli$ ribosomes shows the presence of a small phase of lower melting temperature. We have not observed this effect in yeast ribosomes under our conditions.

line RNA 36 does show a periodicity of 40 Å, which is similar to the surface period observed by Hart; a 45 Å X-ray reflexion has also been reported in ribosome gels⁵⁴.

Our principal tentative conclusions then are: (a) the conformation of the RNA in the ribosome is similar to that in the free state, and contains about 60 per cent of paired bases in short helical segments; (b) the proteins are not associated with the double helical parts of the RNA, and may be packed into the non-helical loops and/or associated with each other in some form of quaternary structure; (c) the surface of the ribosome consists chiefly of RNA and not protein; and (d) this surface RNA is, by inference, largely double helical, and the helices probably project outwards from the surface.

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Prostaglandins: Their Disappearance from and Release into the Circulation

by . S. H. FERREIRA* J. R. VANE

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Prostaglandins are released into the splenic venous blood when the spleen contracts. Whatever the significance of this release, the liver and lungs provide an efficient protective mechanism to remove almost all the prostaglandin before it reaches the arterial circulation.

Prostaglandins are widely distributed in animal tissues and are found in particularly high concentrations in seminal fluid. They have potent actions on smooth muscle, including contraction of intestinal muscle and vasodilatation. More than a dozen different prostaglandins have been identified, and recent experiments suggest that some may be released into the circulating blood. For example, stimulation of perfused adrenal glands with acetylcholine liberates a prostaglandin², and stimulation of the splenic nerve in the dog causes large amounts of prostaglandin E_2 to be released into the splenic venous blood. The actions of prostaglandins on the arterial resistance vessels. make it important to know whether a

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prostaglandin released into the venous side of the circulation is able to reach the arterial side in effective concentrations.

Methods of assaying prostaglandins have until now depended on their purification by selective extraction procedures followed by bioassay, usually on strips of isolated intestinal smooth muscle such as the rabbit jejunum, the hamster or rat colon or the rat stomach strip. We have used the blood-bathed organ technique, to estimate continuously the concentrations of prostaglandin in the circulation, to determine their stability in the blood and their disappearance in different vascular beds. The release of prostaglandin into splenic venous blood after splenic nerve stimulation, or after injections of catecholamines, has been demonstrated with the same technique.

Animals of either sex were used. Cats weighing 2-4 kg were anaesthetized with ethyl chloride and ether from a mask; anaesthesia was then maintained with chloralose (80 mg/kg given intravenously). Dogs weighing 5-15 kg were anaesthetized with halothane delivered from a Goldman vaporizer; anaesthesia was then maintained with chloralose (100 mg/kg given intravenously) and supplemented when necessary with pentobarbitone (5-10 mg/kg, given either intramuscularly or intravenously). Rabbits weighing 2-5 kg were anaesthetized by injecting pentobarbitone (20-40 mg/kg) into a marginal ear vein. The anaesthesia was supplemented when necessary by further doses (5-10 mg/kg) given intramuscularly.

In each experiment, the trachea was cannulated and the lungs were ventilated mechanically. Polyethylene cannulae were tied into a femoral or carotid artery and a femoral or jugular vein for removal and replacement of blood. Mean arterial blood pressure was recorded on a Beckman Offner 8' channel dynograph with a Statham P23 Db pressure transducer attached to the side arm of the arterial cannula. Heparin (1,000 IU/kg) was injected intravenously and the assay organs were then superiused with blood delivered by a roller pump at 10 ml./min. After passing over the assay organs, the blood collected in a reservoir and was returned to the venous cannula. either by gravity or by a second channel in the roller pump.

Three strips of intestinal smooth muscle were used as assay organs. They were suspended in polypropylene chambers and superfuseds in series, first with Krebs solution while the animal was being prepared, and then with blood from the animal. The movements of the smooth muscles were detected with 'Ether' strain gauges attached to auxotonic levers9 and recorded on the dynograph. The initial load on the tissues was 1-3 g. The assay system was calibrated by infusions of prostaglandins into the stream of blood after it had left the animal, and

by intravenous or intra-arterial infusions.

Choice of Assay Organs

The rat stomach strip¹⁰ which is contracted by prostaglandins was used for their quantitative estimation in blood. The strip is also contracted by other substances, such as 5-hydroxytryptamine, which may be present in blood. Specificity was increased by the inclusion of other tissues in the assay system. Several different isolated organs were tried; the best combination was found to be a rat stomach strip with a rat colon¹¹ and a chicken rectum¹². All these three isolated organs contracted to low concentrations (1-10 ng/ml.) of prostaglandins; no other substance in concentrations likely to be found in the blood stream gave a similar pattern of contractions.

Part of an experiment, in which a rat stomach strip, a rat colon and a chicken rectum were bathed in blood from a dog, is shown in Fig. 1. The contractions of the assay tissues produced by prostaglandin E_1 were compared with the effects induced by slow reacting substance in anaphylaxis (SRS-A), substance P, angiotensin, histamine, oxytocin and vasopressin. A pattern of contractions, similar to that produced by prostaglandin E_1 , was produced only by vasopressin but with a concentration many times higher than that found in blood. Bradykinin and 5-hydroxytryptamine gave different patterns, inducing large contractions of the rat stomach strip with little or no effect on the rat colon and chicken rectum¹³. In all

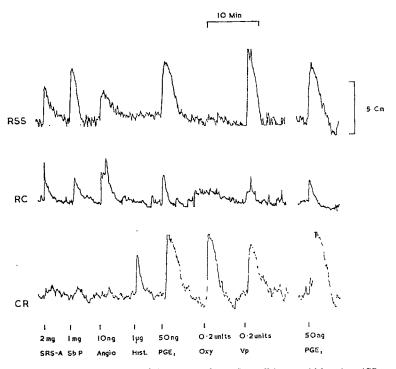


Fig. 1. A rat stomach strip (RSS, top), a rat colon (RC, middle) and a chick rectum (CR, bottom) were superfused with blood from a dog. Injections were made into the blood bathing the assay organs. Slow reacting substance in anaphylaxis (2 mg SRS-A), substance P (1 mg SbP) and angiotensin (10 ng Angio) had no effect on CR but contracted RSS and RC. Prostaglandin E_1 (50 ng PGE₁) contracted all three tissues, oxytocin (0.2 units oxy) contracted only the CR and vasopressin (0.2 units Vp) contracted all three. The dose of vasopressin to produce this effect was, however, much greater than would usually be found in blood. Time, 10 min; vertical scale, 5 cm.

experiments to be described these three blood-bathed organs were used to assay prostaglandins.

Stability of Prostaglandins in Blood

The stability of prostaglandins was measured in blood by passing it through a length of silicone tubing of 3 mm internal bore and of 30 ml. capacity, kept at 37° C by a water bath. The blood from this incubating circuit¹⁴ was then superfused over the assay organs. Prostaglandins were infused at different points in the circuit, so that they were incubated with the blood for various times before being assayed. Fig. 2 shows sections of tracings from an experiment on a dog in which the effects of infusions of prostaglandins E_1 , $\bar{E_2}$ and $F_{2\alpha}$ into the incubating circuit were compared with those produced by infusions close to the assay tissues. None of the three prostaglandins lost activity when incubated with the blood for 2 min. Similar results were obtained in three other experiments on dogs. It was not possible to use such a large incubating circuit in cats because too much blood would have been needed to fill the external tubing; nevertheless, there was no loss of activity when the three prostaglandins were incubated with cat blood for 1 min (Table 1). The finding15 that bovine plasma albumin completely suppressed the contractor activity of irin (which contains a mixture of prostaglandins 18) on isolated smooth muscle suggested that prostaglandins were bound to some plasma protein. Our results show either that this does not happen in the dog and cat or that, if it does, the resultant complex is still pharmacologically active.

Disappearance of Prostaglandins during one Circulation through Vascular Beds

When a prostaglandin is infused into the blood supplying a vascular bed, some of it disappears from the blood and the rest escapes into the venous effluent and mixes with other venous blood. The prostaglandin which escapes can be assayed by comparing the contractions of the assay

ug/min

Table 1. STABILITY OF PROSTAGLANDINS IN BLOOD AND THEIR DISAPPEARANCE

	10	SUME VASUE	Per cent	
	Exp.	Animal	Prostaglandin	disappearance
Blood	1	Cat	E_2 (5 ng/ml.)	0
(Incubation time,	$\frac{1}{2}$	Cat	E_z (8 ng/ml.)	0
1 min)		_		
(Incubation time,	3	\mathbf{Dog}	F_2a (25 ng/ml.)	Õ
2 min)	4	\mathbf{Dog}	F_{sa} (20 ng/ml.)	0
•	4	Dog	E_1 (2 ng/ml.)	0
	4 5 5 5	Dog	F_1 (5 ng/ml.)	0
	5	Dog	E_2 (10 ng/ml.)	0
	5	Dog	F_2 d (40 ng/ml.)	0
	6	Cat	E_2 (<1 μ g/min)	> 95
-	7	Cat	$E_2 \ (< 0.5 \ \mu g/min)$	> 95
Lungs	8	Rabbit	$E_1 (0.5 \mu \text{g/min})$	90
	8	Rabbit	$E_1 (0.5 \mu \text{g/min})$	90
	1Õ	Dog	$E_1 (1 \mu g/min)$	95
Liver	6	Cat	E_2 (0.5 μ g/min)	70
	11	Cat	$E_2 (0.7 \ \mu \mathrm{g/min})$	93
Hindquarters	2	Cat	E_2 (4 μ g/min)	50
	$1\overline{2}$	Cat	E_1 (2 $\mu g/min$)	66

The figures in parentheses indicate the blood concentration for the incubation experiments and the rate of infusion into the effluent blood for the experiments in which comparisons were made across different vascular beds

organs bathed in mixed venous blood with those produced by infusions made directly into the blood leaving the vascular bed. In our experiments, sufficient time was allowed for equilibrium conditions to be established, as shown by the contraction of the assay organs reaching a plateau. The disappearance of prostaglandins was measured in the lungs, the liver and the hindquarters.

To study the disappearance of prostaglandins in the lungs, a catheter was inserted retrogradely through the right carotid artery so that its tip lay in the left ventricle, as determined by the pulse pressure registered by a pressure transducer. The tip was then withdrawn until the change in pulse pressure showed that it lay in the ascending acrta just above the acrtic valves. The position of the catheter was verified at the end of the experiment.

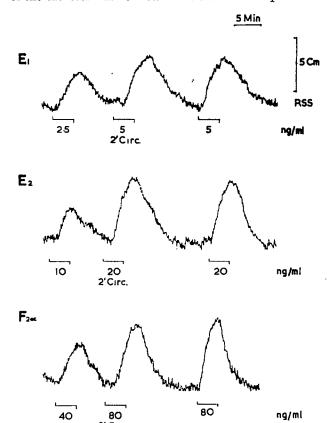
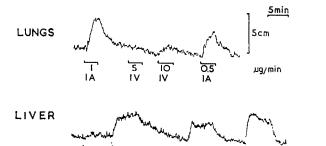


Fig. 2. Each section of tracing is from a different experiment in which a rat stomach strip was superfused with blood from a dog. The first tracing shows that incubation of prostaglandin E_1 (5 ng/ml.) for 2 min with blood did not reduce its activity. The other two tracings show similar results with prostaglandin E_2 and $F_{2\alpha}$. Time, 5 min; vertical scale, 5 cm.



5

ΙP

10

Fig. 3. The top tracing is of a rat stomach strip superfused with arterial blood from a cat. Infusions of prostaglandin E_2 (1 μg and 0.5 $\mu g/min$) were given into the ascending aorta (IA) and their effects compared with intravenous infusions (IV) of 5 and 10 $\mu g/min$. None of the 5 $\mu g/min$ intravenous Infusion could be detected in arterial blood and less than 0.5 $\mu g/min$ after the 10 $\mu g/min$. The pulmonary circulation therefore removed more than 95 per cent of the intravenous infusion. The lower tracing is from an experiment in another cat in which the rat stomach strip was bathed in mixed venous blood and infusions of PGE₂ made either intravenously or into the portal vein (IP). From an infusion of 10 $\mu g/min$ IP, more than 1 μg and less than 2 $\mu g/min$ were detected in the venous blood. Time, 5 min; vertical scale, 5 cm.

Infusions of prostaglandins were made either through this catheter (intra-arterially) or into the superior vena cava (intravenously). Thus, by comparing the effects of these infusions on assay organs bathed in femoral arterial blood, the disappearance of prostaglandins in the pulmonary circulation could be estimated. The results of an experiment in a cat are illustrated in Fig. 3. Infusions of prostaglandin E2 (0.5 µg and 1 µg/min) intra-arterially gave graded contractions of the rat stomach strip. In between these two, intravenous infusions were given. No prostaglandin was detected in the arterial blood after 5 µg/min intravenously and less than 0.5 μg/min after the intravenous infusion of 10 μg/min. Thus more than 95 per cent of the infused prostaglandin was removed in one circulation through the pulmonary vascular bed. similar result (>95 per cent removal) was obtained in another cat. With these rates of infusion, there was no change of blood pressure. In two other species (rabbit, two experiments; dog, one experiment), the disappearance of E_1 in the pulmonary circulation was found to be 90 per cent in each experiment. Thus in all three species, the lungs removed almost all of the infused prostaglandins presented to them. The results are summarized in Table 1.

To study the liver, the abdomen was opened and the spleen removed. A catheter was inserted into the splenic vein and advanced so that its tip lay in the portal vein close to the liver. The assay organs were bathed in mixed venous blood taken from the right atrium and infusions of prostaglandin into the portal circulation were compared for their effects on the assay organs with infusions into the superior vena cava. In one experiment in a cat (Fig. 3), an infusion of 10 μ g/min prostaglandin E_2 into the portal circulation gave a contraction of the rat stomach strip which was intermediate to those produced by intravenous infusions of 1 µg/min and 2 µg/min. Thus 70 per cent of the infused prostaglandin disappeared in one passage through the portal circulation. In another cat, 93 per cent of the prostaglandin disappeared in the portal circulation (Table 1).

For the hindquarters, a catheter was inserted retrogradely through a ligated femoral artery so that its tip lay just above the bifurcation of the aorta. The venous catheter was introduced into the femoral vein on the same side. Mixed venous blood taken from the right atrium was used for assay. In one cat, 50 per cent of prostaglandin E_1 disappeared in the hindquarters and, in another, 66 per cent of prostaglandin E_2 (Table 1).

We can conclude that although the prostaglandins we have tested are stable in circulating blood, they are rapidly removed from the circulation by the liver, the lungs

and to a lesser extent by the hindquarters. Should one of them be released into the portal blood, for example, from the intestine, more than 70 per cent will be removed in the liver and, of that which is left to reach the lungs, more than 90 per cent will be removed. Thus, in the few seconds that it takes for portal blood to reach the aorta, the concentration of prostaglandin in it will be reduced to less than 1 per cent. Because splenic contraction (see later) leads to prostaglandins being released into the portal vein, the avid removal of these substances by the liver and lungs may be an important protective mechanism to prevent them from reaching the arterial circulation. In the ewe¹⁷ as well as in dogs18, cats and rabbits, the hypotensive effects of prostaglandins are greater with intra-arterial than with intravenous injections; thus the mechanism for removing prostaglandins in the pulmonary circulation exists in several species. In this context, it is interesting that after administration to rats19 and mice20 of prostaglandin E_1 labelled with tritium the radioactivity was found mostly in the lungs and that lungs from many species have an enzymatic system which metabolizes prostaglandins21,22.

In our experiments, we have used infusions of prostaglandins, rather than injections, in an attempt to mimic possible release of prostaglandins into the circulation. Because intravenous injections of prostaglandins have pronounced cardio-vascular effects, it may be that the high concentration achieved by a sudden injection swamps the inactivation mechanism in the lungs, thereby allowing passage of a greater proportion of an injection than of an infusion.

Release of Prostaglandin-like Substance from the Spleen

The abdomen of the anaesthetized dog was opened down the mid-line. The vessels connecting the spleen to the stomach were tied and the spleen was freed from mesenteric tissue so that it could be exteriorized. The vascular pedicle was carefully dissected to separate lengths of splenic vein and artery from the sheath of splenic nerves surrounding the artery. A catheter was made from polyethylene tubing of 3-5 mm external diameter with one end doubled around on itself for a length of about 2 cm. This was tied into the splenic vein so that the catheter drained the splenic venous blood. After cannulation, which usually took 15-30 sec, the catheter was allowed to empty into the venous reservoir through which the blood from the assay organs returned to the animal. The assay organs were then bathed in splenic venous blood taken at 10 ml./min from a side arm in the catheter, the surplus effluent from the spleen draining into the venous reservoir. Electrodes were placed on the sheath of splenic nerves and the whole organ was then inserted into an oncometer, the volume changes of which were detected by a Palmer float recorder. Changes in spleen volume were recorded on the dynograph by attaching an 'Ether' strain gauge to the pivot of the float recorder, so that the strain gauge transduced the movements of the float recorder.

In some experiments, splenic venous blood was pumped over two separate banks of assay organs, allowing registration of the responses of six assay organs. In a few experiments, a fine polyethylene catheter was pushed into one of the ligatured tributaries of the splenic artery so that its tip lay in the main splenic artery. This catheter was used for intra-arterial injections to the spleen.

Part of an experiment, in which six assay organs were bathed in splenic venous blood, is shown in Fig. 4. Stimulation of the splenic nerve at 30 shocks per min for 2 min (1 msec duration, 30 V strength) led to a small contraction of the spleen and this was followed by an output of a material into the splenic venous blood which contracted the rat stomach strips. A higher rate of splenic nerve stimulation (1 shock per sec for 2 min) gave a larger contraction of the spleen, followed by a contraction of all the assay organs. With stimulation at 2 shocks per sec for 2 min there was an even greater contraction of the spleen

and of the assay organs. The effects of the released substance on the assay organs could be almost matched by infusing prostaglandin E_2 into the splenic venous blood to give a concentration of 30 ng/ml. The pattern of contraction of the assay organs indicated that the released material resembled prostaglandin. The lack of exact correspondence between the contractions of the individual organs induced by the material and those induced by prostaglandin E_2 may have resulted from the release of more than one prostaglandin, or from the concomitant release of a catecholamine which, by an inhibitory effect on some of the assay organs, may have changed their heights of contraction in relation to the others.

This experiment showed that when the splenic nerve was stimulated, a prostaglandin-like material was liberated in high concentrations into the splenic venous blood, confirming the work of Davies, Horton and Witherington³. Furthermore, the output of the prostaglandin-like activity depended on the frequency of nerve stimulation (five experiments). Whenever the splenic nerve was stimulated sufficiently strongly to induce a contraction of the spleen there was a release of prostaglandin-like activity into the splenic venous blood.

Identification of Prostaglandin-like Material

Samples of splenic venous blood obtained before, during and after splenic nerve stimulation were kindly extracted

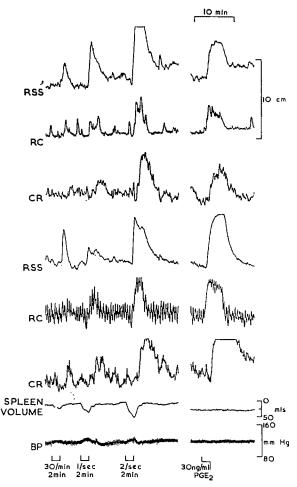


Fig. 4. Splenic venous effluent was superfused over two banks of assay organs (10 ml./min for each channel). Stimulation of the splenic nerves at 30 shocks per min for 2 min caused a small contraction of the spleen which was followed by an output of a material in the venous blood which contracted the rat stomach strips (RSS). Stimulation at 1 shock per sec for 2 min induced a bigger contraction of the spleen and a contraction of all the assay tissues. Stimulation at 2 shocks per sec for 2 min induced a still bigger effect on spleen volume and on the assay organs. An infusion of prostaglandin E₂ to give a blood concentration of 30 ng/ml. induced a similar contraction of the assay organs. Time, 10 min: vertical scales, 10 cm, change in spleen volume (ml.) and mm mercury.

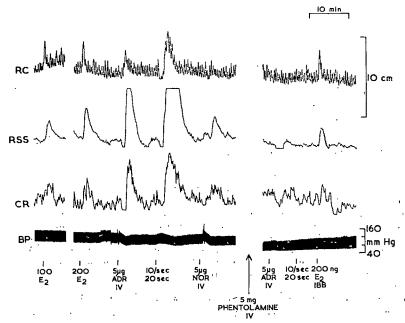


Fig. 5. A rat colon (RC, top), a rat stomach strip (RSS, upper middle) and a chick rectum (CR, lower middle) were superfused with splenic venous blood from a 12.5 kg dog. The blood pressure is the bottom tracing. Prostaglandin E_2 (100 ng and 200 ng E_2) contracted the assay organs when injected directly into the blood bathing them. An intravenous injection of adrenaline (δ μ g ADR IV) produced an output of prostaglandin into the splenic venous blood, as did stimulation of the splenic nerves at 10 shocks per sec for 20 sec. The output after intravenous noradrenaline (δ μ g NOR IV) was much smaller. Phentolamine (δ mg IV) was then given and, 20 min later, neither the adrenaline injection nor the nerve stimulation induced prostaglandin release. Time, 10 mln; vertical scales, 10 cm and mm mercury.

and assayed for us by Dr J. H. Wyllie. The blood was centrifuged and the plasma was acidified by adding one twentieth of its volume of 5.75 normal hydrochloric acid. The acidified plasma was extracted twice with 1.5 times its volume of ethyl acetate. This extraction procedure eliminates all other substances known to contract the rat stomach strip leaving only acidic lipids; it gives an 80–90 per cent recovery of added prostaglandin from blood. The ethyl acetate was evaporated to dryness; the residue was extracted with Krebs solution and its activity assayed on the rat stomach strip.

In three experiments in which the assay by this method was compared with assay by the blood bathed organ technique, there was always an increase in the prostaglandin content of the blood after splenic nerve stimulation as measured by both methods. The concentrations varied from 10-200 ng/ml. when assayed as prostaglandin E_2 , and are similar to those obtained by Davies, Horton and Witherington³, who identified the chief component as prostaglandin E_2 . For the rest of this article, we shall assume that the material in splenic venous blood which contracts the blood bathed organs is principally if not all prostaglandin E_2 .

is principally, if not all, prostaglandin E_2 . The prostaglandin E_2 may have been released from nervous tissue on excitation, from the smooth muscle of the spleen as it contracted, or it may have been present in the blood ejected from the splenic sinusoids by the contraction. To test whether the prostaglandin came from the nerve or was consequent on contraction of the spleen, drugs which cause splenic contraction were injected intra-arterially. Adrenaline (four experiments) was the most potent, followed by noradrenaline (two experiments) and histamine (three experiments). They all induced con-

tractions of the spleen which were closely followed by release of prostaglandin into the splenic venous blood. The output of prostaglandin was greater when the contraction of the spleen was greater. Thus the prostaglandin which is released follows contraction of the spleen and not excitation of nervous tissue per se.

The release of prostaglandin E_2 induced by nerve stimulation is abolished by phenoxybenzamine3, an α-receptor blocking agent. We have confirmed this observation using a different α-receptor blocking agent, and in addition have shown that the output of prostaglandin produced by adrenaline and noradrenaline is also prevented in the presence of α-blockade. Fig. 5 shows contractions of the assay organs induced by prostaglandin E_2 (100 ng and 200 ng) given directly into the splenic venous blood bathing the assay organs. Next, adrenaline (5 μ g) was injected intravenously into the dog. After a small relaxation of the assay organs, presumably because some of the adrenaline reached them, there was a contraction caused by prostaglandin release. Stimulation of the splenic nerve at 10 shocks per sec for 20 sec also released prostaglandin, as did an intravenous injection of noradrenaline (5 μ g). An α -receptor blocking agent (5 mg phentolamine) was then injected intravenously into the dog and 20 min allowed for α-receptor blockade to develop. When the injection of adrenaline

and the nerve stimulation were repeated, there was now no output of prostaglandin, although there was still some relaxation of the assay organs when the catecholamine reached them. This experiment shows that when the contraction of the spleen is abolished, whether it is induced by adrenaline or by nerve stimulation, the output of prostaglandin is also abolished.

We next attempted to find out whether the prostaglandin was released from activated smooth muscle cells or whether it was present in the stores of blood which were ejected during the initial phases of the contraction. Part

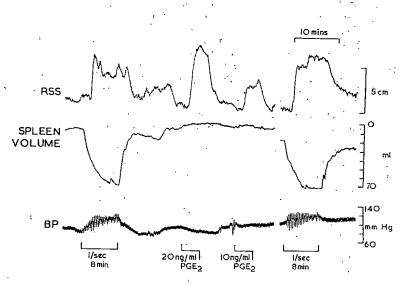


Fig. 6. A rat stomach strip (RSS, top) was superfused with splenic venous blood from a dog. Changes in the spleen volume (middle) and the blood pressure (bottom) were also recorded. The first stimulation (1 shock per sec for 8 min) induced a contraction of the spleen which was maintained, as was the output of prostaglandin. Calibrating infusions of prostaglandin E_a showed that the output was equivalent to about 15 ng/ml. A second stimulation at 1 shock per sec for 8 min gave a similar output. Time, 10 min; vertical scales, 5 cm, change in spleen volume (ml.) and mm mercury.

of an experiment, in which the splenic nerve was stimulated at I shock per sec for a period long enough (8 min) to give a maintained contraction of the spleen, is shown in Fig. 6. During the whole of this period there was an output of prostaglandin equivalent to a concentration of E_2 of about 15 ng/ml. A second stimulation at the same rate 35 min later induced a similar output, suggesting that the output of prostaglandin was associated with active muscle contraction. In many of these experiments, however, there was an output of prostaglandin when the spleen was handled, suggesting that it could occur without an active contraction of the spleen being induced by drugs or by electrical stimulation of the nerve. Experiments in which the spleen is stimulated at different frequencies for long periods of time should help to distinguish between release of prostaglandin from muscle cells and release from the blood stored in the sinusoids. Perfusion of the spleen with an artificial salt solution may also help to elucidate this point.

Our experiments have confirmed that prostaglandins are released into splenic venous blood when the spleen contracts. It is not yet known whether contraction of any smooth muscle will lead to an output of prostaglandin into the venous effluent, although it has been shown that contractions of the rat stomach lead to a release of prostaglandin (see succeeding article). If these potent substances are released from intestinal muscle as well as from the spleen, it seems clear that there is a highly efficient protective mechanism, both in the liver and in the lungs, which will remove almost all the prostaglandin before it reaches the arterial circulation. Thus the prostaglandins are unlikely to be important as circulating hormones, unless they can also be released from the lungs into the pulmonary vein, or unless the mechanism for their removal is disturbed.

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Release of Prostaglandin E_1 from the Rat Stomach

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Prostaglandin is released from the rat stomach during electrical stimulation of nerves in its wall. Sensitivity of gastric muscle to prostaglandins suggests that they have a role in the control of gastric motility.

SEVERAL pharmacologically active substances are released from the isolated stomach during gastric motor activity. Paton and Vane¹ found that acetylcholine appeared in the bathing fluid when gastric nerve endings were stimulated and that the release of 5-hydroxytryptamine (5-HT) and histamine sometimes increased during nerve stimulation or during muscle contraction induced by drugs. It was suggested that these amines are released through mechanical deformation of amine-containing cells rather than through nervous activity per se. Bennett, Bucknell and Dean² found that 5-HT as well as an uncharacterized material were released into the lumen of the rat stomach We now describe further stimulated transmurally. experiments on the release of this material and on its identification as prostaglandin E_1 .

Wistar rats of both sexes were used. They were fed MRC diet B (as modified by Oxo Ltd.). The drugs used were acetylcholine perchlorate, atropine sulphate, bromolysergic acid diethylamide, chymotrypsin, hexamethonium bromide, 5-hydroxytryptamine creatinine sulphate,

(-)hyoscine hydrobromide, mepyramine maleate, methysergide bimaleate, prostaglandins E_1 , E_2 , F_{1a} , F_{2a} and tetrodotoxin. Concentrations of salts are expressed in terms of the base.

Perfusion of Isolated Stomach through its Lumen

The procedure was similar to that previously described^{1,3}. Rats were deprived of food overnight but were allowed drinking water. They were stunned and their throats were cut. The stomach and duodenum were gently removed in one piece and the oesophagus was tied off. A small hole was cut in the fundus and the lumen was irrigated with Krebs solution to remove any stomach contents. A cannula with a platinum wire electrode through its centre was tied into the hole in the fundus so that the stomach could be perfused intraluminally. The preparation was transferred to a bath containing Krebs solution at 37° C gassed with 5 per cent carbon dioxide in oxygen and a second platinum electrode. The duodenum was tied to an outlet cannula

so that the intragastric fluid could be withdrawn at intervals by a syringe. In some experiments the fluid from the stomach was superfused continuously over isolated A perforated polyethylene tube was inserted through a hole in the fundus and out through the duodenum to ensure that the rate of flow of fluid through the stomach was not slowed by occlusion. Each stomach was kept at an intraluminal pressure of 0 cm of water for 10-20 min before the start of the experiment. The pressure was then raised by up to 10 cm water and sometimes there was concurrent transmural or vagal electrical stimulation (2 pulses/sec, 1 msec duration). For transmural stimulation 30-50 V were used; for vagal stimulation, pulses of 5-10 V were passed through two platinum electrodes looped round the oesophagus and the attached vagi. The periods of increased pressure and electrical stimulation were varied according to the type of experiment. In some instances they lasted 1-5 min and alternated with periods of rest at 0 cm water for 5-8 min; in others the stomachs were stimulated continuously at 2-4 cm water until the peristalsis became weak (usually about 30 min).

Pharmacological Assay of the Effluent

The pharmacological activity in the fluid from the lumen of the rat stomach was measured by its contractor effect on the rat stomach strip preparation⁴. This was either suspended in an isolated organ bath in Krebs solution at 37° C gassed with 5 per cent carbon dioxide in oxygen, or superfused directly by the effluent from the stomach at 9–12 ml./min in an apparatus similar to that described for blood-bathed organs. Other rat tissues and rabbit duodenum were sometimes used as well. The responses of the tissues were recorded on a kymograph by isotonic or auxotonic frontal writing levers (magnification, 8–16). The initial load on the tissues was 0-5–1-5 g.

Release of Active Substances

Samples of perfusion (a) Whole isolated stomach. fluid from the lumen of the non-stimulated rat stomach contracted the rat fundus strip prepara-Except on one occasion when spontaneous peristalsis occurred, methysergide or bromolysergic acid diethylamide $(2 \times 10^{-7} \text{ g/ml.})$ almost or completely abolished the contractor effect of the effluent obtained at intragastric pressures of 2-4 cm water (five experiments). Thus, at low pressure, 5-HT was chiefly released. At higher intragastric pressures (10 cm water, two experiments) additional activity which was not antagonized by methysergide appeared in the effluent. During transmural or vagal stimulation at any pressure, activity appeared in the effluent which contracted the rat stomach strip in the presence of methysergide (2 × 10⁻⁷ g/ml.). Fig. 1 demonstrates that only 5-HT was detected in the effluent from the lumen of a non-stimulated rat stomach at an intragastric pressure of 2 cm water, whereas additional activity, not blocked by methysergide, was detected after transmural stimulation. The concentration of this activity declined when stimulation was stopped, but increased again when stimulation was resumed (see later).

(b) Strips of isolated stomach. Strips of rat fundus or of rat gastric body and antrum were cut as described by Vane⁴. One end of the strip was fixed and the other was attached to a short lever so that the tissue could be stretched by the addition of weights. The strips were superfused with Krebs solution at 37° C and the superfusing fluid was then passed in turn over two rat fundus strips. The effect of 5-HT on the lower strip was prevented by the infusion of methysergide into the bathing fluid to give a final concentration of 2×10^{-7} g/ml. When the strips of antrum and body were stretched by weights (4–20 g tension) both of the superfused test strips contracted (four experiments). The strip superfused by methysergide contracted less in relation to the other, suggesting that 5-HT and at least one other substance were

released. No increase in activity in the superfusion fluid was detected when strips of fundus were stretched (4–12 g tension, two experiments).

(c) Release in vivo. Rats which had been deprived of food overnight were either anaesthetized with pentobarbitone (50 mg/kg injected intraperitoneally; four experiments) or were decerebrated under temporary ether anaesthesia (two experiments). The oesophagus and vagi were tied approximately 1 cm above the stomach. Krebs solution at 37° C was passed into the stomach through a tube tied into the fundus and was removed at intervals through a tube tied into the duodenum. Two platinum electrodes were looped round the oesophagus near to the stomach and the vagi were stimulated at 2 pulses/sec (1 msec duration and 10 V) for periods of 5 min. Peristalsis was poor in the rats anaesthetized with pentobarbitone. This was probably the result of the anaesthetic because pentobarbitone (4.3×10^{-6} g/ml.; two experiments) greatly reduced peristalsis caused by vagal stimulation in vitro. Peristalsis was greater in the decerebrate rats, and the fluid from inside the stomach contained uncharacterized material similar to that obtained in vitro. Release of this material was therefore not the result of an artefact caused by isolating the stomach.

Identification of the Active Substance

(a) Pharmacological. As already indicated, the unidentified material was not 5-HT, for its effect on the rat fundus strip preparation was not blocked

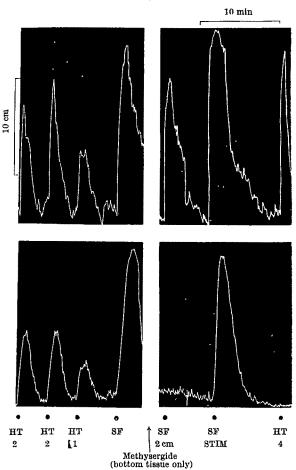


Fig. 1. Two rat fundus strips (top and bottom traces) superfused with the same stream of Krebs solution or with the effluent from a rat stomach perfused intraluminally at 2 cm of water pressure. 5-HT (HT, doses in ng) and the effluent from the non-stimulated stomach (SF) contracted both strips; methysergide (10-7 g/ml., bottom tissue only) prevented these effects. The fluid from the transmurally stimulated stomach (2 pulses/sec, I msec and 50 V for I min; SF STIM) contracted the strips even in the presence of methysergide, showing that another substance was also present.

by methysergide or bromolysergic acid diethylamide. It was not acetylcholine or histamine because its effect was unaltered by hyoscine or mepyramine $(2\times 10^{-7} \text{ g/ml.})$. The substance was not a peptide such as gastrin, bradykinin, vasopressin, oxytocin or substance P because the activity of the effluent was unaltered by incubation with chymotrypsin (10^{-4} g/ml.) for 30 min at pH 8). The substance had no effect on the rat uterus or on the circular or longitudinal muscle of the rat gastric body or antrum, but the rat duodenum, ileum and colon and the rabbit duodenum responded with small contractions. The contraction induced in the rat duodenum further distinguished the substance from bradykinin which causes a relaxation.

(b) Solubility. The Krebs solution containing the unidentified material was shaken with ether. The substance was extracted with ether when the aqueous phase was acid but not when it was neutral or alkaline. This suggested that the substance was an acidic lipid, such as has been extracted from human stomach^{8,9}, and the following procedure was therefore adopted. The ether was first shaken with an aqueous solution of sodium metabisulphate to remove peroxides, and was then washed five times with distilled water. The effluent from the stomach was acidified to pH 2-3 with normal hydrochloric acid and extracted three times with ether, leaving basic substances such as 5-HT in the aqueous phase. The ether extracts were combined and evaporated to dryness at not more than 30° C under a stream of nitrogen. In other experiments, the stomach wall was extracted after cutting the tissue into small pieces with scissors and grinding with distilled water in a Potter-Elvehjem glass homogenizer. resultant suspension was then acidified and extracted in the way described for the effluent. The residue obtained when the ether was evaporated was shaken with 1 ml. of Krebs solution and assayed for contractor activity on the rat stomach strip preparation. The amount of activity recovered from the ether extracts was 26-48 per cent of the total present in the aqueous extracts. Similar incomplete extraction and recovery occurred with pure prostaglandin E_1 .

(c) Spectrophotometry. Because of the relatively large amounts of material required for this technique, the effluents from many stimulated rat stomachs were extracted (eight to twenty-eight in different experiments). The ether extract was dried with anhydrous sodium sulphate and then evaporated under vacuum in the presence of 0.5 g of potassium bromide ('Pronalys', May and Baker). The residue was made into a disk and examined in a Unicam SP 100 spectrophotometer. Pure samples of prostaglandin E_1 and prostaglandin F_{1a} were pulverized with potassium bromide before fusion into disks.

Table 1. INFRARED ABSORPTION DATA FROM THE C=0 STRETCHING REGION

Substance	Frequency (cm ⁻¹) carboxyl	Frequency (cm ⁻¹) carbonyl	Absorbance carboxyl/ absorbance carbonyl
PGE_1	1,710	1,736	0.78
Extract	1,710	1,730	1.06
$PGF_{1\alpha}$	1,695		-

The uncharacterized substance resembles $\mathrm{PG}E_1$ but not $\mathrm{PG}F_{1\alpha}$. The higher proportion of carboxyl absorbance in the extract compared with that of $\mathrm{PG}E_1$ suggests that more than one substance is present.

The infrared spectrum (Table 1, three experiments) in the C=O stretching region showed that both carbonyl and carboxyl groups were present in the extracted material. The wavelengths of these absorptions corresponded to the carbonyl and carboxyl absorptions in prostaglandin E_1 . The higher proportion of carboxyl absorbance in the extracted material (last column, Table 1) compared with that of prostaglandin E_1 suggests that more than one substance is present. If the excess carboxyl absorbance were due entirely to prostaglandin F_{1a} , this compound would comprise about 15 per cent of the total absorbing material in the extract.

(d) Chromatography. In four experiments the material extracted from the effluents of four to eight stimulated

stomachs or the extract from the ground stomach wall was dissolved in 50 per cent methanol. It was chromatographed using the AII system of Gréen and Samuelsson¹⁰ on thin layer plates of silica gel G (Merck) containing 3 per cent of silver nitrate. Pure samples of prostaglandin E_1 , E_2 , F_{1a} and F_{2a} were treated similarly and chromatographed concurrently. Centimetre bands of the plates were then extracted with 1 ml. of 50 per cent chloroform in methanol. After separation from solid matter, the solvent was evaporated at a temperature below 30° C and the residue was shaken with 1 ml. of Krebs solution for biological assay.

The material obtained from effluent or from the stomach wall was detected on the rat stomach strip at the same R_F (0·79) as pure prostaglandin E_1 (Fig. 2). It was thereby differentiated from PGE_2 , PGF_{1a} and PGF_{2a} which had different R_F values. Thus prostaglandin E_1 was present in the stomach wall and was liberated into the gastric lumen during electrical stimulation.

(e) Parallel pharmacological assays. The results from infrared spectroscopy suggested that some F prostaglandin might be present, whereas only E_1 was detected on the rat stomach strip. This preparation is, however, relatively insensitive to F prostaglandins. Parallel assays of ether extracts of stomach wall were therefore performed using both the rabbit duodenum (which is more sensitive to F than E compounds¹¹) and the rat stomach strip. Compared with E_1 the extracts were

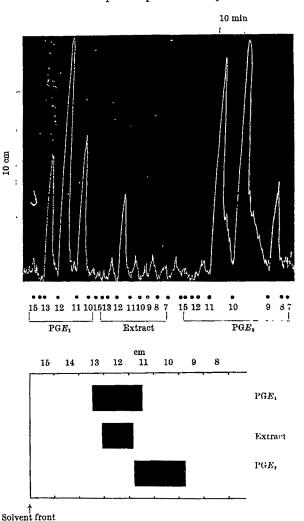


Fig. 2. Parallel chromatograms of rat stomach prostaglandin, PGE_1 and PGE_2 run with the AII solvent system of Gréen and Samuelsson¹6 on silica gel G containing 3 per cent silver nitrate. Consecutive 1 cm bands (as indicated by numbers) were extracted and tested on a rat fundus strip (top). The estimated positions on the chromatogram (bottom) confirm that rat stomach contains PGE_1 .

more potent on the rabbit duodenum than on the rat fundus. The same dose of E_1 plus 10-20 per cent of F_{2a} was as effective as the extract on the rabbit intestine. Thus in addition to E_1 there appeared to be a little F prostaglandin in the stomach wall. Recently, Wolfe, Coceani and Pace-Asciak¹² reported that rat stomach contains prostaglandins (chiefly E_2 and F_{2a}) which are released from the serosal surface during electrical stimulation. Because the only E prostaglandin we found was E_1 , the type of prostaglandin may vary with the strain of rat.

Release of Prostaglandin from Stomach Wall

Effluents and aqueous homogenates of stomach were assayed for prostaglandin on the rat fundus strip treated with methysergide, hyoscine and mepyramine (all at 2×10^{-7} g/ml.). In thirteen experiments the aqueous extracts of stomach contained activity equivalent to $0.35-7~\mu g~E_1/g$ of moist tissue (mean $3.2, S.E. \pm 0.63$). In two experiments the amount in the mucosa was 10.5 and 19·4 μg/g which was four to six times higher than that in the muscle. The release into the effluent in seven experiments during 3 or 5 min periods of stimulation varied from 3-35 ng E_1/\min (mean 11.8, $S.E. \pm 3.8$) of stimulation, and in four experiments 5-25 per cent of the total activity was liberated during stimulation for approximately 30 min. The release of active material into the stomach lumen during periods of 3 or 5 min at 4 cm water pressure either with or without electrical stimulation was determined by assay on the rat fundus treated with methysergide, hyoscine and mepyramine (all at 2×10^{-7} g/ml.). Each period of raised pressure was alternated with a period of rest at a pressure of 0 cm water. Changes in the release of prostaglandin caused by cessation of stimulation or by hexamethonium or tetrodotoxin in the bathing fluid were measured in each sample as changes in the degree of contraction of the assay preparation and not in actual amounts of prostaglandin. In each experiment, when stimulation was stopped the release of active material declined by approximately 25-75 per cent compared with the preceding stimulation period. The amount increased when stimulation was resumed (Fig. 3).

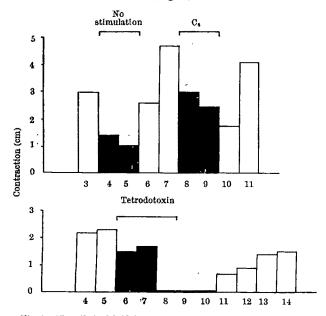


Fig. 3. The effect of fluid from two perfused rat stomachs (intragastric pressure 4 cm of water) on a rat fundus strip blocked with methysergide, hyoscine and mepyramine (all 2×10^{-7} g/ml.). The white columns are the contractions caused by one fifth of the fluid obtained during transmural stimulation for 3 min. The black columns are responses to fluid from the non-stimulated stomach or from the stomach stimulated in the presence of hexamethonium (C_s ; 2×10^{-8} g/ml., top trace) or tetrodotoxin (10^{-7} g/ml. bottom trace). Output of activity falls when stimulation is stopped or when hexamethonium or tetrodotoxin is present and increases again when the drugs are washed out of the bath.

Hexamethonium $(2 \times 10^{-5} \text{ g/ml.})$ added to the bath fluid almost abolished muscular activity in response to electrical stimulation and the stomach became dilated. In each of six experiments the output of prostaglandin fell by 20-60 per cent (average 39 per cent) compared with one or with the mean of the two previous periods without hexamethonium (Fig. 3) but increased again when the drug was washed out and peristalsis had returned. Although the effect of hexamethonium on peristalsis was almost immediate, the output of prostaglandin into the lumen fell only gradually (Fig. 3), as after stopping stimulation.

Tetrodotoxin (10-7 g/ml.) added to the bath fluid nearly abolished the motor response to stimulation. The reduction in the release of prostaglandin ranged from 0-30 per cent in the first 5 min stimulation period after the addition of tetrodotoxin (average 22 per cent; four experiments), but the reduction was 60-100 per cent (average 86 per cent) in the third period while tetrodotoxin was in the bath fluid (Fig. 3). Antagonism of muscle responses therefore preceded the reduction in the release of prostaglandin into the lumen. Output increased again with the return of peristalsis when tetrodotoxin was washed out.

These results show that prostaglandin is released from the rat stomach when nerves in its wall are stimulated. A similar occurrence has been found in the cat spleen (ref. 13 and the preceding article by Ferreira and Vane). Prostaglandin release from the stomach declines when stimulation is stopped or when the nerves are blocked with It has not yet been established whether the prostaglandin comes from nerve, muscle or from mucosa: although both hexamethonium and tetrodotoxin reduce or abolish the excitation of postganglionic nerves, they also prevent muscle contraction induced by electrical stimulation. Prostaglandin-like activity, however, was released from strips of gastric body and antrum stretched by weights, suggesting that it can be liberated by simple distortion of cells.

The greater release of prostaglandin into the lumen of the stimulated stomach compared with the release from the serosal surface12 indicates that the liberation of prostaglandin from the mucosa is greater than from the muscle. This corresponds with the finding that the amount extractable from the mucosa is higher than from muscle and from many other tissues 14. Prostaglandin E_1 and E_2 contract the rat fundus in low concentrations. Their actions on human gastric muscle are more complex because circular muscle is inhibited and some longitudinal muscle is contracted. The sensitivity of gastric muscle to prostaglandins, coupled with their presence in the stomach wall, suggest the possibility that they have a role in the control of gastric motility.

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LETTERS TO THE EDITOR

ASTRONOMY

Re-examination of the Source Counts for the 3C Revised Catalogue

On the basis of optical identifications by Veron¹ and Wyndham², the 254 extragalactic sources of the 3CRevised Catalogue³ have been divided into the following classes: radio galaxies (RG), quasi-stellar sources (QSS) empty fields (E) and unidentified (?). Log $N-\log S$ curves for all the 254 extragalactic sources, for the RG + RG? (certain and possible), for the QSS+QSS? and for the QSS+QSS? + E + E? +?, have been derived 1,4 , with exponents of -1.85 ± 0.05 , -1.55 \pm 0.05, -2.2 ± 0.1 and -2.2 (no quoted error), respectively. The conclusion was then drawn that the $-2.2 \pm$ 0.1 exponent for the quasars indicated an evolutionary effect which was not shown by the radio galaxies with an exponent of -1.55 ± 0.05 . Furthermore, that the quasars plus the empty fields and the unidentified fields showed the same exponent as the quasars alone was taken as an indication that the empty fields and unidentified fields were quasars.

Because of the importance attached by cosmologists to the number-flux curves for various classes of radio source, a re-examination of the data from the optical identifications of the 3C Revised Catalogue has been undertaken.

In a number-flux analysis of this type, it is important that the individual exponents should be determined in a reliable manner and that good estimates should be obtained for the uncertainties in the exponents. Only in this way can reliable conclusions be drawn from the available data. Although the estimation procedure used by Veron^{1,4} is not stated, his published log N-log S plots indicate that he has fitted theoretical N-S integral curves of the form

$$N(>S) = KS^{-\beta} \tag{1}$$

to the experimentally observed numbers greater than chosen flux levels.

Such a procedure is not directly applicable because the usual fitting methods require that the numbers at each flux level should be statistically independent of each other, otherwise these methods are not applicable. This particular objection to the fitting of integral data was the reason for this re-examination of the published 3C Revised Catalogue identifications.

In the experimental determination of an integral spectrum (that is, the number of sources greater than chosen flux levels) those sources greater than the largest level are included in every other level, those at the next highest are included in all but one, and so on. The result is that most of the sources are counted many times. Because of the large numbers at the lower flux levels, there is the illusion that the range of exponents fitting the data is small. Also, as the numbers of sources are propagated from level to level, neither the numbers at each level nor the uncertainty in these numbers are independent.

The lack of independence of nearby points also affects the estimate of the reliability of the fitted N-S curve. Direct application of a chi-squared test for goodness of fit in the integral spectrum results in an over-estimate of the number of degrees of freedom and consequently in an over-estimate of the reliability of the fit. The propagation of numbers from level to level smooths out any fluctuations within the levels, which is of particular importance when examining any N-S data for a change in exponent.

The exponent, deduced from the fitting to integral data, is thus not necessarily a reliable estimate, and, furthermore, the apparent uncertainty in the exponent is usually somewhat under-estimated. These failings are serious when both the value of the estimated exponent and the reliability of this estimate are to be used to differentiate between various physical theories.

In order to derive a reliable estimate of the exponent in the number-flux curve, it thus seems preferable to fit the differential spectrum rather than the integral spectrum to the experimental results. In this way, the numbers in each flux interval are independent, allowing an application of the usual fitting methods.

The differential form corresponding to equation (1) is

$$N(S)dS = kS^{-a}dS \tag{2}$$

where $\alpha = \beta + 1$. The problem is to estimate k and α given the observed numbers M_i in the various flux intervals. In this respect, the method of maximum likelihood is particularly useful. This approach to the estimation of parameters from experimental data was developed by R. A. Fisher⁵, based on the work of Thomas Bayes, and yields an estimate with a number of important properties⁶.

In order to estimate k and α it is necessary to maximize the likelihood of k and α , given the observed counts M_i in each ith flux interval. This reduces to maximizing the product over all values of i of Poissonian terms of the form

$$[E_i^{Mi} \exp(-E_i)]/(M_i!) \tag{3}$$

where E_i is the expected number in the *i*th interval and is given by

$$E_i = \int_i k S^{-a} dS \tag{4}$$

It is usually more convenient to maximize the logarithm of this product as a function of k and α .

This yields an estimate k^* for k of

$$k^* = \frac{\sum M_i}{\int S^{-\alpha} dS}$$
 (5)

where the integration extends over the range of S covered by the data. The estimate α^* of α is given by that value of α which maximizes the expression

$$\log k^* \sum_{i} M_i + \sum_{i} \log \left\{ \int_{i} S^{-\alpha} dS \right\}$$
 (6)

where the integral is carried out over each ith flux interval. This expression is evaluated numerically.

Notice that if instead of the Poissonian form of expression (3) a normal distribution has been assumed, then expression (6) reduces to the usual least squares equation. Thus least squares fitting in this case is applicable only in the sense that the normal distribution function is an approximation of the Poissonian distribution. In particular, when the numbers in each interval are small, as in the present case, the maximum likelihood estimate is preferable to the least squares estimate.

For the 3C Revised Catalogue, expression (6) was evaluated for $\alpha=2(0\cdot01)4$, determining k^* for each α from equation (5). The most probable estimates of α for the four source classes analysed by Veron are given in Table 1. together with their uncertainties. These uncertainties correspond to those values of α where the expression (6) has dropped by one half from the maximum value. Because the distribution of the likelihood estimate is asymptotically normal, this corresponds to the one

Table	1	
Source class	Previous exponent	Present exponent
All extragalactic	$2 \cdot 85 \pm 0 \cdot 05$	$2.79 \begin{array}{l} +0.12 \\ -0.11 \end{array}$
Radio galaxies RG+RG?	$2\!\cdot\!55\pm0\!\cdot\!05$	$2.58 \begin{array}{l} +0.14 \\ -0.13 \end{array}$
Quasars plus empty fields plus unidentified fields QSS+QSS?+E+E?+?	3·2 (No quoted error)	$8.17 \begin{array}{l} +0.21 \\ -0.21 \end{array}$
Quasars Q85+Q88?	3.2 ± 0.1	$3.00 \begin{array}{l} -0.30 \\ -0.27 \end{array}$

Table 2						
Source class	Present	exponent				
Radio galaxies RG plus empty fields +	+RG? E+E?	2.73	+0·14 -0·13			
Ĉertain radio galaxies	$\mathbf{R}\mathbf{G}$	2.26	+0·13 -0·13			
Certain quasars	QSS	2.56	+0.27 -0.25			
Empty fields	E+E?	3.50	+0.46 -0.48			

standard deviation points of the normal distribution. Column 2 lists the exponents and their errors given previously1,4. Furthermore, Table 2 shows the same analysis applied to a number of other classifications. For comparison, both the present and previous exponents are given as differential exponents.

From Table 1 it can be seen that the present results differ somewhat from those in column 2. In the first place the uncertainty associated with each exponent is considerably larger than the previous value, as is to be expected. Furthermore, the present exponents for each category are not always the same as the previous values.

Regarding the interpretation of the data, the difference between the exponents for the QSS+QSS? and the RG+RG? is now no longer well established, because of the increased uncertainties. The latter value of

$$2.58^{f +0.14}_{f -0.13}$$

is well within twice the uncertainty of the QSS+QSS? value of

$$3.00^{+0.30}_{-0.27}$$

Again from Table 1 it can be seen that the only statistically established difference in exponents is between the radio galaxies (RG+RG?) and the quasars plus empty fields (QSS + QSS? + E + E? +?). The exponents for the quasars and the quasars plus empty fields are no longer the same, and so the arguments classifying the empty fields with the quasars are now not nearly as strong, particularly as the empty fields themselves have an exponent of 3.50 from Table 2. In particular, if the empty fields are classified as radio galaxies, then this classification yields an exponent of 2.73 from Table 2. Both this exponent and that for the quasars (QSS+QSS?) alone are then entirely consistent with the overall exponent of

$$2.79^{+0.12}_{-0.11}$$

Table 2 also shows the effects of the doubtful identifications on the exponents. In all cases, as with the empty fields, they are responsible for a considerable steepening of the counts in each classification; 2.56 to 3.00 for the inclusion of the doubtful quasars and 2.26 to 2.58 for the doubtful galaxies.

It is clear then that no definite interpretation regarding the cosmological significance of the difference between exponents can be drawn from the present 3C Revised Catalogue identifications. In the first place, the difference between the exponents of the quasars and the radio galaxies given in Table 1 is not statistically significant. In the second place, the statistical uncertainties quoted in Table 1 are not a real indication of the actual uncertainties. These are due to the numbers of doubtful identifications, and to the assignment of the empty fields, as well as the statistical uncertainties. In this respect, it seems that there are at present insufficient identification data for the 3C Revised Catalogue to derive any definite conclusions regarding differences between the N-S curves for radio galaxies and quasi-stellar sources. Before any firm conclusions can be drawn, (1) the uncertain identifications should be confirmed, and (2) the empty fields should be further investigated in order to determine with which classification they should be grouped.

At this point it should be noted that the arguments refer only to the differentiation between source groups

on the basis of their number flux curves, and not to the determination of any cosmological model from the observed number flux exponents. This is a separate problem, for it has been assumed in the analysis given here that the radio source flux measurements are exact. This is not the case, for it is known that the errors in the fluxes, particularly at the lower flux levels, are by no means negligible. Moreover, I have shown (unpublished results) that the measured flux values are biased towards an over-estimation of the flux. Once again this bias is greatest at the lower flux levels.

I have carried out a preliminary investigation of the effects of this over-estimate for the extragalactic sources in the 3C Revised Catalogue (unpublished results). The results indicate that the counts corrected for the bias are in good agreement with a differential exponent of 2.5 and therefore the overall counts do not seem to reflect any cosmological effects.

I thank Dr Cyril Hazard for his criticism of the manuscript.

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Pre-main-sequence Evolution

In is several years since the first discovery of stellar clusters which appeared to be so young that a number of stars in them were still in the process of contracting onto the main sequence¹⁻⁴. The first theoretical model covering the evolution of stars, on the assumption that their self gravitational energy is the only energy source available, was produced by Henyey, LeLevier and Levée. The results of these computations have been used to produce a theoretical Hertzsprung-Russell diagram for young stellar clusters^{6,7}. In this theory, the evolution of the stars is governed by the equations

$$L = R^2 T^4 \tag{1}$$

$$L\mathrm{d}\tau = -\frac{M^2 \,\mathrm{d}R}{R^2} \tag{2}$$

$$L = M^{\alpha}/R^{\beta} \tag{3}$$

where L, R, T and M denote the luminosity, radius, effective temperature and mass, respectively, all in solar units, and in which T denotes the age in units of the Helmholtz-Kelvin contraction time. α and β are constants with approximate values given by Huang⁶ as $\alpha = 5.4$ and $\beta = 0.79$.

A type of evolution governed by simple equations such as these is mathematically convenient, for it makes it possible to evaluate the mass, radius and age of a star on the basis of the observed luminosity and temperature. Unfortunately, however, it does not correspond with the observed HR diagrams. A large discrepancy between the age of the cluster predicted by this model and the age as deduced from the main sequence turn-off point was pointed out by Huangs, while Williams showed that there is also a discrepancy between the theoretical and observational distribution of stars. The reason for the breakdown

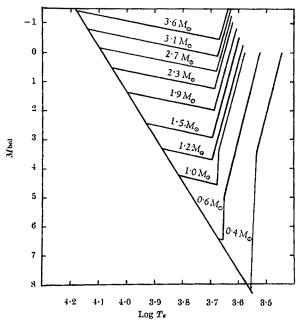


Fig. 1. The evolutionary tracks given by equations (4) and (5) for the stellar masses used by Hayashi².

of these theoretical models was given by Hayashi⁹, who showed that contracting stars would in general possess a convective zone. Consequently, there exists a locus in the HR diagram for every mass, to the right of which no quasi-static solutions can exist. Hayashi therefore proposed a model in which the stars evolve along this locus until it intersects the track of Henyey et al.5 after which they evolve along that track. (He used the values $\alpha = 4.5$, β=0.5, corresponding to an unmodified Kramer opacity for the evolutionary tracks of Henyey et al.) In this way Hayashi produced evolutionary tracks for specified masses and, using these, constructed constant time loci which are in agreement with the observational data. Similar physical models^{10,11} support this argument, and there now seems little doubt that such a model gives essentially a correct description of stellar evolution before the main sequence.

By the very nature of the model proposed, however, it is not possible to represent the evolution by a simple set

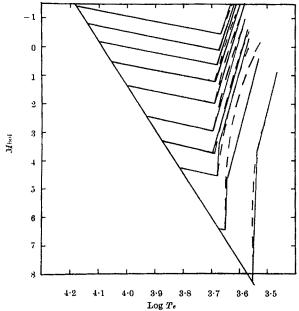


Fig. 2. The evolutionary tracks given by equations (4) and (5) (solid lines) compared with Hayashi's tracks (broken lines), which are superimposed.

		Tal	ole 1		
(M_{\odot})	Log (age)	Log (age) Hayashi	Mass	Log (age)	Log (age) Hayashi
3·6 3·1 2·7	4·65 4·90	4·7 4·9	1·5 1·2	5.95 6.20	6·3
2·3 1·9	5·10 5·30 5·60	5·1 5·4 5·7	0.6 0.4	6·57 7·26 7·80	6·6 7·3 7·7

of equations. Equations (1) and (2) remain valid but equation (3) does not. This means that fresh calculations have to be performed for each stellar mass, which is particularly inconvenient in any discussion of stellar clusters. For this reason we propose an approximation to the Hayashi evolutionary track which can be represented by simple equations. The part of the Hayashi track which differs from that of Henyey et al. is represented by two intersecting straight lines, given by

$$0.21 \log L = 0.46 \log M - 3.16 \log T - \frac{0.195}{1 + 1.36 \log M}$$
 (4)

for high luminosity and changing to

$$0.05 \log L = 2.16 \log M - 3.8 \log T - \frac{0.323}{1 + 30 \log M}$$
 (5)

at the intersection of the two lines. This locus changes to that of Henyey et al. where the two tracks join.

The evolutionary tracks given by equations (4) and (5). for the stellar masses used by Hayashi, are shown in Fig. 1. The tracks of Henyey et al. and the main sequence have been taken from Hayashi's paper. Fig. 2 shows the same evolutionary tracks, but with Hayashi's tracks superimposed, the Hayashi tracks being the broken lines. The discrepancy between the two sets of tracks is very small.

Using equations (1) and (2), it is easily seen that equation (4) corresponds to replacing equation (3) by the

$$L = \frac{M^{\alpha}}{R^{\beta}} 10^{-\frac{0.195}{1 + 1.36 \log M}} \text{ with } \alpha = 0.46 \text{ and } \beta = -1.58$$

Equation (5) requires replacing equation (3) by
$$L = \frac{M^{\alpha}}{R^{\beta}} 10^{-\frac{0.323}{1+30 \log M}} \text{ this time with } \alpha = 2.16$$
 and $\beta = -1.9$

The evolutionary tracks we have proposed can therefore be represented in a form mathematically similar to equation (1) to (3) and calculations can be carried out for this stage of evolution.

For most purposes, the equations can be simplified further. In general, the two lines we have proposed do not cross in a region which is of interest as can be seen from Fig. 1. The evolution is thus governed by one or other of these lines. A good approximation is obtained by taking equation (4) for masses greater than 0.6 Mo and equation (5) for the other masses.

Before these equations can be regarded as a fair approximation to Hayashi's results, the time taken to evolve to the intersection with the track of Henyey et al. must correspond in the two models. It is easy to demonstrate, using the equations given here, that the time taken to evolve to the stage when the luminosity is L_0 and the temperature is T_0 is

$$\tau_0 = \frac{M^2 T_0^2}{(1-\beta) L_0^{3/2}} \tag{6}$$

Taking L_0 and T_0 to refer to the values at the point of intersection with the track of Henyey et al., we find the evolution times (in years) as shown in the second column of Table 1, one HK unit being taken as 107.2 yr. In the third column the corresponding time found by Hayashi is given. Agreement between the two sets of values is again very good.

There are no major differences between the evolution given by our equations and that described by the mathematically more complex evolution of Havashi. In evolutionary work where extreme accuracy is not required, it may therefore be advantageous to use this simple model.

One of us, A. W. C., would like to thank the Science Research Council for a research studentship during the tenure of which this work was carried out.

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PLANETARY SCIENCE

Upper Atmosphere Density in 1966-67: the Dominance of a Semi-annual Variation at Heights near 200 km

ANALYSES of the changes in satellite orbits have established that the principal variations in upper atmosphere density at heights between 180 km and 1,000 km are attributable to solar activity and differences between day and night. A further effect, usually smaller, is the semiannual variation, with maximum densities in April and October and minima in January and July. These results are embodied in the Cospar International Reference Atmosphere and other models of the upper atmosphere.

Solar activity has been greater and more variable during 1967 than during 1966, and might have been expected to exercise a dominating influence over upperatmosphere density during 1967. This expectation has not been fulfilled, however, particularly at heights near 200 km. A recent analysis of the orbit of the satellite Secor 6 (1966-51B) has yielded 154 values of air density at a height of 191 km at dates between June 14, 1966, and July 5, 1967. These results, which will be reported in detail elsewhere, show that solar activity, as represented by the radiation energy at a wavelength of 10.7 cm, did not exercise appreciable influence over the air density. Nearly all the nineteen large geomagnetic disturbances occurring during the life of the satellite were accompanied by sharp increases in air density for a few days; but, when these disturbed days are eliminated, the remaining variations in density are apparently quite unrelated to the wide variations in the 10.7 cm radiation energy, which shows a strong 27-day recurrence over much of the satellite's life in orbit.

The values of air density obtained from the orbit of Secor 6 at a height of 191 km are shown in Fig. 1, after removing geomagnetically disturbed days and making small corrections for the long-term increase in solar activity and for day-to-night variations in density.

(Values between August 23 and September 17, 1966, are regarded as doubtful, because of lack of data and also because several points at times of geomagnetic disturbance had to be removed.) The chief feature of Fig. 1 is a semiannual variation, with maxima of about 5.0 × 10-10 kg/m³ in October 1966 and April 1967, and minima (excluding the doubtful September values) in July 1966 and January 1967 of about 3.7 and 3.2×10^{-16} kg/m³, respectively. These values are averages over about 30 days, rather than extreme maxima or minima. This semi-annual variation, with the maximum density exceeding the minimum by a factor of 1.45, has been in many respects the most important variation in upper-atmosphere density during 1967 at heights near 200 km, and has significantly affected the accuracy of satellite lifetime estimates.

The semi-annual variation in air density, found from the orbit of Echo 2 by Cook and Scott² at a height of 1,130 km at dates up to January 1967, is almost identical in form to the left-hand half of Fig. 1, although the numerical values of density are lower by a factor of more than 105 This confirms that the density at 1,130 km height. variations apparent in Fig. 1 are dependent on date.

The apparent failure of solar activity to influence upper-atmosphere density at heights near 200 km, except through the short-lived solar outbursts which give rise to geomagnetic storms, suggests that the radiation responsible for controlling upper-atmosphere density during the declining years of the last sunspot cycle may not be as strong during the rising part of the current cycle.

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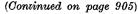
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Differences of Composition among Australian Iron Meteorites

THE discovery of iron meteorite fragments with the structure of medium octahedrites in the neighbourhood of the Wolf Creek meteorite crater compounded a curiosity. The Boxhole and Henbury craters were already known to be associated with meteoritic debris which has the structure of medium octahedrites and although such structures are relatively common among iron meteorites-Hey's2 catalogue shows them to represent 36 per cent of all classified irons—coincidence of structures has caused speculation that two or more of these cratering events

were produced by fragments of the same meteoroid.

The Boxhole and Henbury craters lie about 300 km apart along the N.E.-S.W. line in central Australia (Northern Territory). Wolf Creek is located in western Australia about 850 km N.W. of this area, and thus the three objects do not lie along an arc of the Earth's surface. Although such distances are small by Australian standards, they are much greater than the linear dimensions of any known meteoritic strewn field, as recently discussed by



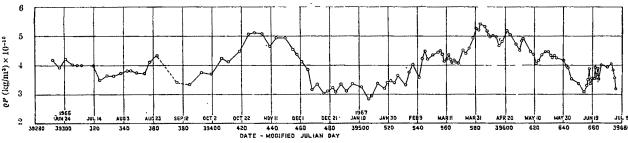


Fig. 1. Values of density, or at a height of 191 km, averaged between day and night, with geomagnetic storms excluded.

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Where should Books be Kept?

CONTINUING uncertainty about the future of the British Museum does at least have the advantage of provoking thought about the functions of national libraries, and by now, no doubt, Mr Patrick Gordon Walker's friends are probably saying that this is what he intended, all along, when he brought the planning of the museum trustees abruptly to a halt four weeks ago. However that may be, Mr Gordon Walker has done a good deal to make up for his heavy-handedness by his decision to have Dr F. S. Dainton as the chairman of the committee which is to re-examine the difficult issues which have tended to be glossed over in the past twenty years. For one thing, Dr Dainton is a scientist. For another, he has played an active part, in the past few years, in fostering experiment in information retrieval. The gibe that the literature is too important to be left to the librarians is, of course, unjust, for librarians have done more than scientists in recent years to ensure that the literature of scholarship shall be managed in an orderly fashion, but it will be a great boon if the planning of new national libraries in Britain can now be seen to be in the charge of one who is sympathetic to the problems of the librarians but who is also demonstrably a user as well.

But what should the Dainton committee push for? And how can it hope to complete so huge a task in less than a year, as the Secretary of State for Education and Science has promised? The difficulty is that the planning of a national central library system should properly depend on much more information about the detailed needs of potential users than is at present available. There is, for example, the question of whether a central reference library in London can adequately cater for the needs of people living and working in other British cities. And what should be the relationship between university libraries and the rest of the community? How feasible is it to separate science libraries from other kinds of libraries, especially now that there is a boom in bridge subjects such as economics and architecture? And even if this is feasible, can it be sensible? There are also questions to be answered about the character of library readership. What value is there in bibliographies, produced by machine or otherwise? What criteria should be adopted for distinguishing between material which is readily accessible and material which is more remotely stored? What policies should libraries follow in the collection of foreign-language material? Who or what should foot the bills?

Dr Dainton's only comfort in this forbidding list of questions is that urgency and the pressure of work may

serve enormously to clarify the mind of his committee. This, so it is said, is the effect of impending execution. And it should not be hard to establish a few simple principles which should quickly help to create a framework within which a new national library service could The starting point should be a forthright recognition that a national library system is so urgent that the link between the British Museum and the library now on the same premises, however valuable, is comparatively unimportant. The two functions need to be divorced. It is also plain that if there is to be a Central Reference Library worthy of the name, it should span all branches of scholarship, science and otherwise. It follows that the Dainton committee should work for a single building to house everything on some easily accessible site in central London. There is also a case for asking that the result should be integrated administratively-not physically-with the central lending libraries. One of the absurdities of the present arrangements is that the overflow from the British Museum library cannot be used by the National Lending Library for Science and Technology, for example. But there is an even stronger case for bringing university libraries more firmly within the national system. It would be better—and cheaper—for everyone if the principle were formally acknowledged that the academic libraries are also regional libraries of great importance.

There are also technical matters which cry out for a firm and, if necessary, authoritarian decision. The time, for example, has come to deprive librarians of the right to develop independent cataloguing systems in the misguided notion that they thus serve the interests of their readers. No cataloguing system can be ideal, but the advantages of a common system override the virtues of any one of them, especially now that computers have arrived in libraries. Indeed, the advantages of uniformity are now so great that it may even be worthwhile to establish international computer facilities for the cataloguing of material and the compilation of bibliographies. The Library of Congress and the British Museum have in the recent past done much to stimulate each other. Has the time now come when they should merge? And should there now be arrangements for pooling the collections of nearby countries in Europe? British libraries are notoriously deficient in material from France, Germany and Eastern Europe. And, finally, there are the computers. Some means must be found for fitting these new machines into the library system in such a way as to leave people with some sense of freedom. Dr Dainton will have a busy year ahead of him.

General Books

ARTS AND SCIENCE

Arts v. Science

A Collection of Essays. Edited by Alan S. C. Ross. Pp. x+158. (London: Methuen and Co., Ltd., 1967.) 30s. net.

THE publication of the Dainton Report is likely to underline once again the damage done to our whole educational system, and now particularly to science education, by the premature choice and excessive degree of specialization in the arts and science sides of our sixth forms. It is depressing evidence of our lack of any effective machinery of change that in spite of almost universal agreement, clutches of conferences, reams of reports and at least two elaborately considered proposals for reform (one from the Vice-Chancellors, the other from the Schools Council) the structure of specialization into arts and science sides, basically recruited at the age of fourteen, has remained unchanged during the last ten years. Only some of the pupils have, in defiance both of Crowther and of one of Mr Lunt's theses in this book, demonstrated their resistance to the dichotomy in spite of all the contrary pressures. Combined A level courses in arts and science have been one of the two growth points in the sixth form over the last decade: and pure science courses, the most heavily specialized option, have declined.

The issue is one of the most important in the whole field of education, if only because of its by-product, the shortage of scientists and technologists entering the universities. Not unnaturally many eminent men have therefore contributed to the debate and Professor Ross has done us a service, not only in his introduction, which is one of the best sections of the book, but in preserving these occasional essays and giving them wider distribution. That, at least, is what he seems to have done; for the diversity of the contributions makes it seem unlikely that, except for the Birmingham group, they were commissioned for this publication, or that the authors were chosen on any particular plan or writing to any common brief.

Books of this kind have one advantage, which is that each author usually contributes because he has had something valuable to say, though the balance of value may be very unevenly distributed. In a book of only 157 pages, for instance, it seems hardly worth devoting twelve to Mr Lunt's analysis of the views of so small and unrepresentative a sample as the sixth form of one direct grant grammar school. Another variation seems to be in the date at which the essays were written. Mr Henn's, for example, clearly pre-dates the realization of the sixth form swing away from science; Mr Lunt's shows the first signs of alarm in quoting the shortfall of science entrants in 1964. It is left to Professor Roy Pascal, in one of the best essays in the book, to accept categorically the need for more science entrants and to suggest once more the remedy which has seemed obvious for the last ten years "that the number of applicants might be increased if it could be brought about that, by taking science, students do not have to abandon all arts subjects".

Mr Kenneth Richmond writes well and makes the useful point that the results of his own rather more extensive cross-cultural tests gave no support either to the Crowther or the Snow thesis, but revealed what most psychologists would have expected, that students of very high IQ were less and not more likely to be "subject-minded" in

Crowther's exclusive sense, or limited to one side or other of the Snow line.

Two of the essays develop unusual interests peripheral to the main discussion. Professor Whitfield discusses the Italian futurists of the 1920s in relation to the distinction between humanism and scientific humanism: Professor Hilton of Cornell discusses power politics in American universities in reference to a general thesis that, at the level of scholarly research, there is no essential difference between the disciplines of science, mathematics and history or literature, except that the application of scientific method to the "arts" subjects is much more difficult and therefore as yet less advanced.

A. D. C. PETERSON

TEACHING PHYSICS

Nuffield Foundation Science Teaching Project

Physics. Guide to Experiments I. Pp. xvi+248. 17s. 6d. Teachers Guide I. Pp. xiv+306. 15s. Teachers Guide II. Pp. x+156. 12s. 6d. Teachers Guide III. Pp. viii+356. 15s. Teachers Guide IV. Pp. viii+404. 17s. 6d. Questions Book I. Pp. vi+86. 5s. Questions Book II. Pp. vi+72. 5s. Questions Book III. Pp. vi+134. 5s. Questions Book IV. Pp. vi+114. 5s. (London: Longmans, Green and Co., Ltd.; Harmondsworth, Middx.: Penguin Books. Ltd., 1967. Published for the Nuffield Foundation.)

THE Nuffield Foundation Science Teaching Projects have now for some years made increasing impact on teaching within the schools and on thought about science teaching over a much wider area. The books under review form a substantial part of the material prepared for teaching up to the Ordinary level of GCE in physics. They represent a degree of coherent and well discussed planning in the subject far beyond anything which has previously been attempted. For anybody not in course of operating Nuffield Physics, it is the teachers guides which bring out most clearly the spirit and intention of the operation, and few who have grown up with the difficulties and weaknesses of teaching in the subject will not admire and agree with a great deal of what is set out there.

The questions which come to mind in studying this material do not relate to the production of the books, which can only be characterized as quite excellent, nor are they of an entirely critical character. Beyond question, the Nuffield venture has already done good far beyond the strict boundaries of the experiment, and it seems certain to do a great deal more. But the scheme as set out here does bring a quite novel situation into teaching at the levels linked with major public examinations. Until now the examining boards have been scrupulous to describe their syllabuses as examining syllabuses, and to insist that these need only have minimal effect on the teaching syllabus, which should reflect the particular requirements of groups of children and the aptitudes and enthusiasms of those who teach them. This view has not commanded the assent of all teachers, for a large proportion submit that these syllabuses are so wide and so detailed as to determine teaching syllabuses as well (and there is a not negligible group who positively welcome this situation). Criticism is sometimes linked with the mistaken view that these syllabuses represent the productions of people not actively concerned with teaching at the relevant level.

and this view has persisted in spite of the clearest demonstrations, in reports of the examining bodies, of the undoubted dominance of teacher representation at all relevant stages of syllabus development. If nobody else can be stigmatized as "them", then it is those teachers who take a leading part in the formulation of examining syllabuses who, by this very act, have cut themselves off from many of their colleagues! One is bound to ask whether this separation of "us" from "them" can be avoided in Nuffield Physics.

The programme set out in the Nuffield Physics books is in fact a most detailed physics scheme, and it has been possible to contemplate so detailed a formulation mainly because the examining boards have been prepared to examine for the time being on the detailed scheme as it has been set out and as it has been required of those taking part. There is thus the danger that this precisely laid out scheme of teaching could become an orthodoxy far more rigid than anything we have yet known. Those who have worked on the Nuffield Projects are well aware of this danger and are determined, as far as they are able, to prevent it developing. They would claim that so great are the changes which they have envisaged and which are incorporated in this detailed programme that it is necessary to bring everybody to a common starting point in the scheme. As time goes on they would hope to see changes within the whole operation of teaching derived from the project, exhibiting those features of difference which must develop if the scheme is to adapt to the needs of widely different groups of children and of teachers of a variety of outlook. It may well be that some of the most cherished sections of the small group who developed the programme will before long be removed completely

In the hands of the abler teachers the future of the project is most promising. Such teachers are not only those whose whole training has brought them to a position where their sympathy with the project is almost assured, but also a vast number, however trained, whose liveliness and sympathy of mind are drawn out by the stimulating discussion of the Nuffield books. It remains to ask what will be the effect on weak teachers. Here conclusions are bound to be speculative but it can well be argued that, as long as the effort is made, the teaching of a great many and probably of most of those not by nature gifted physics teachers ought to be much improved. For such people the books, and the teachers guides in particular, should be matters of deliberate and painstaking, but nonetheless illuminating, study.

One is bound to await the future development of the first generation of Nuffield pupils with interest. their experience have inclined them towards or against further studies in the physical sciences? generalize as easily as others, and when the time comes when substantial reliance on book learning becomes inevitable how will they react to it? These problems are pressing, for the extent to which the Nuffield methods supersede or become incorporated in general practice is a matter of urgency.

On the limited scale of the Nuffield experiment, schools undertaking the work have been equipped for particular subjects in a manner which no one could describe as lavish but which nonetheless has involved expenditures a good deal greater than those normal for groups at this level. If Nuffield procedures become the "norm" local authorities, however willing, may find it difficult to equip on this scale and it is to be hoped that those concerned with the finance of school equipment are already sharply aware of this It is unfortunate that it arises at a time of financial stringency, but, in a community where relatively complex pieces of apparatus of all sorts are becoming a commonplace of normal life, the material with which the average boy or girl has learned the systematics of the subject has not been expanded to anything like the same extent. At a time when the systematic study of science of any sort is falling short of the apparent needs of the country, if Nuffield were not the stimulus for re-equipping some other would no doubt have to be sought.

AN ILLUSTRIOUS NAME

The Electrical Researches of the Honourable Henry Cavendish

Edited by J. Clerk Maxwell. Pp. lxvi+454. (London: Frank Cass and Co., Ltd., 1967.) First published 1879. 1268.

"His name will be an object of more veneration in future ages than at the present moment. Though it was unknown in the busy scenes of life, or in the popular discussions of the day, it will remain illustrious in the annals of science. which are as imperishable as that nature to which they belong, and it will be an immortal honour to his house, to his age, and to his country." In this way, Humphry Davy spoke of the Hon. Henry Cavendish in 1810, the year of Cavendish's death. This is an ironical prediction, for it was all too literally true. What scientist does not know of the legendary Cavendish laboratory in Cambridge named after Henry Cavendish? Yet its prestige deservedly rests on the achievements and distinctions of such holders of the Cavendish chair as Maxwell, Rayleigh, J. J. Thomson, Rutherford and others. But Henry Cavendish himself is shamefully neglected.

Davy's prediction is, in fact, doubly ironical, for although Cavendish established for himself a considerable reputation on the strength of his published work, it was only well after Davy's lecture that his unpublished manuscripts came to light with startling record of independent discovery and anticipation of results which

appeared up to sixty years later.

Although Cavendish's researches covered a remarkable number of fields, for example, the chemistry of gases. heat and thermometry, meteorology, and gravity, his electrical studies most potently indicate his remarkable ability in quantitative experiment and his persistent conceptual insight. In his lifetime, Cavendish published only two of his papers on electricity. The first, "An Attempt to Explain Some of the Principal Phenomena of Electricity by Means of an Elastic Fluid', published in the *Philosophical Transactions* of 1771, supposed that electrification was the disequilibrium of the "electric fluid the particles of which repel each other and attract the particles of all other matter with a force inversely as some less power of the distance than the cube". This impressive paper, in which Cavendish established the consequences of that assumption, left the scientific community with the impression that this was as far as the already distinguished experimenter could get with the topic. Before two years were up, however, he had demonstrated the inverse power to be "between that of the 2+1/50 and that of the 2-1/50th". This conclusion and the experiments behind them he never published. The second published paper. "An Account of Some Attempts to Imitate the Effects of the Torpedo by Electricity, which appeared in the Philosophical Transactions of 1776, is deceptive in its title, for in the course of it he reveals a clear distinction between the "intensity of charge" and the "quantity of charge". "The idea" (of intensity as used by Cavendish). Maxwell tells us, "is precisely that of potential". Further. he argues for the law of divided current, and in support of this brings quantitative results of experiments on the resistances of sea water, iron and so on. These understated comments appear now, of course, rather like the tops of icebergs. For behind them lay a wealth of experimentation. He studied the distribution of electricity on conductors and was led to a clear conception of electrostatic capacity. He compared the charges in "electric substances coated in the manner of Leyden phials", which work led him to recognize specific inductive capacity more than sixty years before Faraday's independent discovery in 1837. His experiments on the resistances of solutions enabled him to deliver an anticipation of Ohm's law for currents.

Cavendish's researches provide brilliant examples of the way in which theory and experiment progress hand in hand. For Cavendish did not owe discoveries to the development of new electrometers or other technical devices. His advances were based on the application of a brilliant theoretical mind to the devising of simple but subtle experiments. His distinction of "intensity" from "quantity" of electricity enabled him to derive exact quantitative results with apparatus no more sophisticated materially than that used by earlier qualitative researchers such as Franklin. The most striking example of this is in his use of his own body as a "galvanometer". In comparing the resistances of solutions he discharged a Leyden jar through his body through a length of solution in a tube, which he varied by altering the separation of the electrodes inserted at each end. When he felt that the shock was indistinguishable from that from the same Leyden jar through an invariant "control" solution, he varied the length of the test solution until the shock was just noticeably greater than that through the control. He repeated this variation until the shock was just less and took the mean of the two readings as the length of test solution which had the same resistance as the control. Maxwell pointed out that these results were obtained "more than forty years before the invention of the galvanometer, the only instrument by which anyone else has ever been able to compare electric resistances". Indeed, "Cavendish was his own galvanometer".

Cavendish's electrical researches have previously had two editions. This third impression is extremely valuable, as the older volumes are not easily obtained. Clerk Maxwell provided an extensive introduction and account of the researches, together with characteristically analytical notes. The index, part due to Cavendish himself and part to Maxwell, is highly detailed and a valuable guide. Almost a dozen facsimiles of Cavendish's hand-drawn figures are included. This new edition will, hopefully, inspire for Cavendish himself "more veneration in future ages than at the present moment".

J. M. NICHOLAS

BACKGROUND TO NEWTON

Theories of Light

From Descartes to Newton. By A. I. Sabra. (Oldbourne History of Science Library.) Pp. 363. (London: Oldbourne Book Co., Ltd., 1967.) 70s. net.

DURING the seventeenth century, a few crucial fields of scientific enquiry became fully matured; among these were mechanics, pneumatics and optics. In studying the work then done in those fields, the historian is not forced to apologize for the chances missed, and the apparent blunders committed, by the great men. He can scrutinize the work done, and judge it by the criteria of adequacy of modern physical science; and after making allowance for the state of knowledge and technique of the time, he can exhibit it as genuine science of a high order of excellence.

The perceptive historian will, however, observe that the style of that scientific work was not quite the same as our own; the greatest men were, in name and in commitment, "natural philosophers". And so their technical work was influenced by their deepest views on method and metaphysics. The influence was not a simple one; for no piece of concrete scientific research can be merely the application of the preassigned method, or the simple illustration of an ontological scheme. The inevitable tensions between principle and practice are further complicated by the social dimension of scientific work; what an author says to his audience will be strongly conditioned

by his assessment of the terms of the dialogue and the point he wishes to establish.

In this study of one line of development of optics, Dr Sabra has done this sort of history supremely well. With a thorough mastery of the historical sources, he has analysed the arguments of the protagonists of the great debates on the properties of reflected and refracted light, and the phenomena of colours. In reconstructing Descartes's theories, and exhibiting the ambiguity of the crucial term "determination", he is able to explain the controversy with Fermat, and also to settle the vexed question of Descartes's originality. His study of Newton's early work in optics appears after a spate of recent research by others, and does not bear the same excitement as the earlier sections. But here, too, he illuminates the history by a well documented demonstration of a thesis: that Newton's interpretation of his "crucial" experiments was governed by his prior commitment to the corpuscularity of light, and to the nature of colours as unchanging qualities of these corpuscles. The work of Fermat and Huyghens and Hooke is also studied closely, as befits their role in the development of optics between Descartes and Newton.

Throughout the narrative we have a cluster of empirical questions (on reflection, refraction and colour), each giving rise to mathematical formulations and physical interpretations, but these latter being conditioned by methodological and philosophical principles. The statements of methodology are exhibited at length, analysed and related to what was actually done. Also, the empirical questions move in a natural sequence, following the points of significant advance: reflection and refraction dominating the earlier part, and colours the later.

This choice of topics indicates the boundaries imposed by the author on his study. For we have, in effect, the history of those particular problems in the matured science of physical optics, the investigation of which culminated in the published researches of Newton. history is genuine, and will appear entirely natural to a modern scientifically trained reader. But this approach applies equally well to the nineteenth century, when the deepest advances in natural science were still conditioned by philosophical commitments. In the seventeenth century, when a field such as optics was just achieving full maturity, it was still in the process of identifying its soluble problems, and putting the others to one side. The concentration on "light" to the exclusion of "sight" was effected only after Kepler and Descartes; and the philosophical problem of the nature of colours had a far more complex and important history in the period before Newton than the author indicates. Moreover, the study of light as a physical agency, not merely causing but also organizing changes in matter, was of great importance in that century; and of this we hear nothing.

The lack of specification of topics such as these which have been excluded from this study may give the reader the impression that this work is "the" history of theories of light in the seventeenth century. The author explicitly disclaims any comprehensiveness for his study; but he is responsible for his judgments of historical significance. In his restriction to the successful, "real" scientific problems in optics, he has lost some of the special atmosphere of that heroic century of the creation of modern physical science.

J. R. RAVETZ

ONE MAN'S ETHNOGRAPHY

Kwakiutl Ethnography

By Franz Boas. Edited by Helen Codere. Pp. xxxvii + 439. (Chicago and London: University of Chicago Press, 1966.) \$12.50; 90s.

THERE are anthropologists who have a preference for armchair anthropology: "theory at all costs" is the motto

of the extremists in this category. In contrast are the ethnographers for whom facts inhibit general comparison and a wider understanding. The marrying of fact and theory, the utilization of one to investigate the other in experimental field research, has unfortunately been a rarer accomplishment. Franz Boas, one of the most famous of anthropologists and master of many brilliant scholars, must be included in the category of born ethnographers.

Boas began as a physicist-geographer whose first contact with primitive culture was in the Arctic, where he went in 1883 to make maps and observe the effects of climate upon the Eskimos of Baffinland. Fascinated by his experience, he decided to change to the study of human He emigrated from Germany to the United States, took up an academic career and made his second field of study the Indians of the Northwest Coast, notably the Kwakiutl of Vancouver Island and the adjacent

Altogether Boas spent two and a half years in this area, making thirteen field trips between 1886 and 1931. The total time spent in the field was not excessive by modern standards and his visits were more widely spaced than usual. In between he worked on the language and on texts, conducting a long correspondence with George Hunt, a Kwakiutl informant of outstanding talent. He also brought informants to his home. The result was an enormous output of articles. Codere in a useful bibliography lists 174 items.

The nature of Boas's writings, of which his last work, Kwakiutl Ethnography, is typical, stems from his contention that: "It is our serious purpose to understand the thoughts of a people, the whole analysis of experience must be based on their concepts, not ours" (xviii, Introd.

Kwakiutl Ethnography).

Boas tried to achieve this aim partly through scrupulous field work and partly through the collection of texts. In field work he set stringent standards for himself at a time when anthropological field methods left much to be desired. He was in this respect a pioneer. He also originated the use of phonetically written texts for linguistic and ethnographic purposes. More than 3,000 pages of Kwakiutl texts have been published and he turned himself into a competent linguist in order to give a word for word translation. By the use of verbatim reports he sought to eliminate the distortion which he considered unavoidable in descriptions of culture by field workers of an alien culture. His aim was to get down to basics, for he considered that texts were never out of date although theories might be.

The aim of making the most massive collection of reliable data, the extreme concern to eliminate cultural bias and the sense of the overwhelming complexity of culture, all contributed to inhibit the development of a theoretical or comparative approach. Although theoretical formulations and a coherent description of Kwakiutl society and culture might have been his ultimate goal, Boas placed too many obstacles in the way of its achievement. Consequently, we are left with a body of verbatim reports and translations with a minimum of explanation, interpretation or analysis. Boas never reached the point at which he felt able to synthesize or interpret his rich

Kwakiutl Ethnography presents more unprocessed material, derived from informants' statements rather than from Boas's own observations. Although it amplifies our knowledge of Kwakiutl traditional culture it still does not enlighten us on important institutions such as the potlatch or the localized kin groups. Indeed, references to the latter are contradictory and show that Boas never understood them. To the end he remained the great ethnographer, never coming to terms with the theoretical possibilities which anthropology was offering, especially during the latter part of his career.



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Boas was working on Kwakiutl Ethnography when he died in 1942. The incomplete manuscript was eventually passed to Helen Godere, herself a well known Northwest Coast scholar, for editing as a posthumous publication. Codere has written a useful introduction in which she discusses Boas's theory of ethnography and gives details of his field research. In addition, she has tried to make the manuscript more complete.

Chapters are prefaced by editorial notes referring to other Boas publications on the same subject- an invaluable device for those wishing quickly to consult everything of importance on that particular topic. In addition. she has included sections from various other works by Boas and combined them with the chapters of the manuscript in order to fill the gaps. She has clearly stated her editing methods and wisely confined her additions to selections from Boas's later publications which yield his most experienced and considered results. The eleven chapters relate to technology, social organization, the potlatch, war, religion, winter ceremonial, mythology, the arts and the life cycle. There are various appendices, a AUDREY BUTT bibliography and an index.

1 Rohner, R. P., Science, 158, 362 (1967).

DEVELOPMENT IN THE PAST

Essays in the History of Embryology and Biology By Jane M. Oppenheimer. Pp. ix+374. (Cambridge. Mass., and London: The M.I.T. Press, 1967.) 100s.

This valuable book is a collection of essays dealing principally, and I wish it had been totally, with the history of embryology, a subject on which Professor Oppenheimer is admirably qualified to write. If she makes, as she certainly does, considerable lapses in dealing with the eighteenth and earlier centuries, this can be excused by the extreme unreliability of most of the extant secondary sources.

She can scarcely be expected to know how fanciful is the notion that St Augustine's theories of simultaneous creation and of rationes seminales constitute "a theory of emboîtement"; nor can she be severely consured for failing to discover that Aromatari's preformationism has been read into a text which gives it no serious support. Fortunately, the scholarly work of Adelmann has made it possible for her to condemn the absurd accusation against Malpighi that he was able "to see one thing and believe another" (Cole, F. J., Early Theories of Sexual Generation, 48; Oxford, 1930).

I could wish that she had given some attention to Swammerdam's excellent Historia insectorum generalis; though I hesitate to say that so knowledgeable a person does not know the work, it seems to me probable that she was misled by the title, which gives no indication that the book is entirely devoted to observations on the development of insects.

Even when studying the biology of the nineteenth century, the temptation to rely to some extent on secondary sources is difficult to resist, the field being so large: but it is unfortunate that Professor Oppenheimer has been led to rely on Rádl's Geschichte der biologischen Theorien, of which the two volumes appeared respectively in 1905 and 1909.

Rádl's treatment of Nägeli, Weismann, Roux and Mendel now seems both inadequate in length and totally mistaken in emphasis. What he says about Weismann, indeed, seems explicable only on the assumption of some personal animosity.

It is much to be regretted that Professor Oppenheimer appears to have been persuaded, whether by Rádl or otherwise, that it was not worth while to devote attentive study to Weismann's work. It may be a consequence of this neglect that she has been led to attribute to Roux

an adhesion to the Roux-Weismann hypothesis, which Roux himself strenuously repudiated. Unless I am grossly mistaken, his contribution to this hypothesis was limited to the idea expressed in his brochure *Ueber die Bedeutung der Kerntheilungsfiguren*, of 1883; an idea which, it is true, when rigidly developed led to the hypothesis, but which was so developed only by Weismann.

In reading Roux's embryological work it has seemed to me particularly striking that from first to last there is no evidence that he ever believed that development must necessarily follow the rigid mosaic form entailed by the Roux-Weismann hypothesis. If I have dwelt on this matter, it is because Professor Oppenheimer has a great admiration for Roux, who well deserves it; and I should like to convince her that she needs not to attribute to him this particular foible.

A short review must necessarily appear over-critical, for it is impossible to characterize in a few words the general merit of a book. I can only say that this book should be read by anyone engaged in the study of the history of embryology. Among many other advantages, it possesses that of calling attention to the admirable work of Boveri, and to the great merits of Driesch's Analytische Theorie der organischen Entwicklung, of 1894. Among the persons who failed to appreciate this work, the one who might most greatly have profited by it was Driesch himself.

Professor Oppenheimer's book implicitly calls attention to the impossibility of dealing with the more recent history of biology without an extensive knowledge of the German language. This, it is abundantly clear, Professor Oppenheimer possesses, though there are a few oddities in her translations.

J. S. WILKIE

PLAINS INDIAN RELIGION

O-kee-pa: a Religious Ceremony and Other Customs of the Mandans

By George Catlin. Edited with an introduction by John C. Ewers. Centennial edition. Pp. vi+106+13 plates. (New Haven, Conn., and London: Yale University Press, 1967.) 90s.

In 1832, George Catlin spent about three weeks among the Mandan Indians on the upper Missouri River, during one of his trips to record in paint the rapidly changing life of Indians on the American frontier. Despite the brevity of his visit, he had the good fortune to attend the major Mandan religious ceremony in the company of capable interpreters. This volume reprints his fourth published description of what he saw, written many years later. The credibility of Catlin's earlier accounts had been attacked, because of his frank descriptions of ceremonial selftorture and ritual sexual intercourse; because he had blamed the fur traders for the introduction of smallpox among the Mandan in 1837 and exaggerated (although only slightly) its devastating effects; and because he related his ethnographic materials to a bizarre theory of early Welsh influence on Mandan customs, language and physical type. In 1867, Catlin answered his critics by presenting a more complete description and by printing testimonials to his accuracy. As Ewers makes clear, Catlin's general reliability is confirmed by the briefer accounts of earlier and later eyewitnesses, and also by the much more complete and comprehensible material provided by A. W. Bowers, an anthropologist who interviewed aged Mandan in 1930-31, forty years after the last performance of the Okeepa.

Comparison of the coloured plates in this edition with the original photolithographs shows some loss in sharpness of definition and in colour tones, but in only one case (plate 12) is any significant ethnographic detail obscured in the reproduction. But Catlin's paintings and engravings are notoriously variable in reliability, partly because of his practice of reworking the same material for many years after his original field work. Thus the thirteen lithographs in this work should be compared critically with the four paintings of the Okeepa which Catlin made while he was still in the Mandan village, and with his other surviving Mandan paintings. It is unfortunate that Ewers has not attempted this here, for his own previous studies of other paintings of Plains Indian subjects provide the best demonstration of the methods which must be applied in evaluating illustrations as historical ethnographic documents. The text, also, must eventually be compared in detail with Catlin's three earlier accounts of the ceremony.

Catlin in 1867 held back some of his descriptions for a "folium reservatum" printed on tinted paper for the use of "scientific men". By 1950, when Bowers published in the University of Chicago's Social Anthropological Series, scientific women were also interested, so he turned to his wife for help in "toning down the accounts of the buffalo-calling rites with their sex licenses to make them readable without embarrassment to mixed audiences". In 1967, Ewers removes some of the sensationalism from this striking feature of Mandan ceremonialism (and indeed of Mandan trading behaviour) by explaining it in terms of general Plains Indian concepts of supernatural "power" and its transmission. The self-torture so vividly described and illustrated by Catlin was shared by other Plains tribes, among whom it was a central element of the widespread Sun dance. Other features which Bowers adds to the Okeepa as described by Catlin also fit it into a Plains context and make it appear to be a deviant form of the Sun dance: the prominence of sacred bundles, the quest for visions, ceremonial distribution of property, sponsoring of the ceremony by a single individual, the importance of clans, and other characteristics. Seen thus, the Okeepa may be remarkable in its complexity and the explicitness of its symbolism, but it is by no means exotic among Plains Indian rituals. Even Bowers's minimally theoretical ethnography maintained that Mandan "ceremonies were native dramatizations of the sacred myths", of which he collected several examples relating to the Okeepa, and so this would be an excellent place to test the utility of modern structural analysis of myths, ritual and symbolism as a tool for evaluating fragmentary and partly contradictory historical evidence, while reconstructing a consistent patterned description of the ceremony. This new edition of the basic eyewitness account, with the editor's helpful introduction and annotation, should promote the attempt.

WILLIAM C. STURTEVANT

MORE ABOUT STONEHENGE

Stonehenge of the Kings

A People Appear. By Patrick Crampton. Pp. 171. (London: John Baker, 1967.) 45s. net.

The author of this book asks "Palace? Temple? Fort? Computer? Calendar? What is Stonehenge itself, the building?" He himself sees it as a place where inauguration feasts of local chieftains took place. "It is in this sort of ceremony and in the seasonal festivals presided over by the king that I see the function of Stonehenge... nothing so far points to Stonehenge itself being a utilitarian building, and I think that in its heyday it was the focus in the Stonehenge capital at which the people of southern Britain convened and through a seasonal peaceful assembly expressed overlordship and power... I see it as the fit setting for a king to perform his practical and magical ceremonial functions."

All this is possible; no one in his archaeological senses has ever denied that Stonehenge was a place of assembly, and to guess whether that assembly was religious, magical, or secular is to waste time. We do not know, and in the present state of our knowledge of prehistory we cannot

know. That it was a computer or a calendar has not been proved by Gerald Hawkins or any others to the satisfaction of prehistorians. It is clearly not a house or a tomb. Stonehenge is very probably a ritual assembly place as has been said since John Aubrey in the seventeenth century thought that the people who ritually assembled there were the Druids. I certainly do not find it, as Crampton says, "the most enigmatic building in the world". It is a tour de force in the tradition of the stone and wood henge monuments which were a part of the life of the society of third millennium B.C. England.

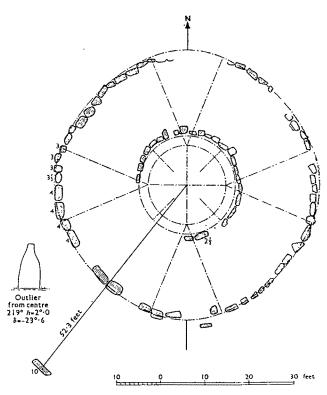
Mr Crampton is obsessed by the excavation of Clickhimin and the excavator, John Hamilton, will blush when he reads that his dig was "probably the most illuminating excavation ever carried out on a structure of barbarian northern Europe". The broch of Clickhimin in the Shetlands is now claimed by Crampton as something which "may prove to be the 'Mycenae' of northern. barbarian Europe". The excavator uncovered the internal structure of an earlier stone-built fort dating from the fourth to the second centuries B.C., and Mr Hamilton wrote of it, "For the first time, the domestic arrangements inside such an early Celtic fortress were completely revealed". Of this modest and correct statement, Mr Crampton writes: "His expansion of this humdrum statement is perhaps one of the most important advances in British archaeology—I am inclined to think the most This Clickhiminomania dominates the argument of this book. What was proved to exist in the immediately pre-Roman centuries in the north of Scotland was, we are told, the rule for all prehistoric Britain: "the illumination shed by Hamilton's excavation on barbarian Europe will be no less than that Schliemann shed on early Greece . . . a historical discovery difficult to over-assess in its importance will have been made at the prehistoric fort at Clickhimin".

Seeing the prehistoric world of Britain with Clickhiminstained spectacles, Crampton assumes that Woodhenge and our other henge monuments were two-storeyed dwellings inhabited by a royal, tribal household similar to that of Tara, but adds that this pretence may seem "wild". Admittedly, he was writing before Dr Wainwright's 1967 excavations at Durrington Walls-but then he might have turned those temples into two-storey dwellings and Durrington into a prehistoric city. He has a vision of second millennium Britain. "I see Wessex as an area of wealth and power dominated by five heroic communities. I see five kingdoms ruled from nucleated capitals at Lambourn, Avebury, Stonehenge, Oakley-Knowlton, and Dorchester . . . I see the populations of the tribal capitals living in drum-tower, timber forts, two or more storeys high, some of the henges and barrows being the surviving remains of these. I see these structures housing a hundred up to considerably greater numbers in each."

The author is entitled to his private visions, but he must not expect those of us who spend our lives studying the third and second millennia B.C. in Britain to substitute his personal view for the archaeological facts. This is a very unfortunate and unworthy addition to the long list of books written about Stonehenge. The general reader should not be taken in by its good production and many well-done plates and figures. It is full of errors: plate V, for example, is one of the well-known kerb-stones at Newgrange with interlocking spirals and lozenges and chevrons. We are told that similar motifs occur "in large numbers" in the tombs of Ireland, Brittany and Spain. I, who spend a large part of my life constantly studying mural megalithic art, would be glad to know of one example—apart from Gavrinnis—in Brittany or Spain. The "large numbers" are fictitious, and so, alas, is much of the book. Surely we are, in prehistoric archaeology, past the stage when personal guesses can be elegantly dolled up in books costing more than two pounds. We can all guess at the past, but we should present our guesses—however wild and at first sight improbable—with moderation, scholarship, and an understanding of what is at present known about prehistory. This Mr Crampton does not do.

GLYN DANIEL

MEGALITHIC SITES



Stone rings at Easter Delfour, from Megalithic Sites in Britain, by A. Thom (Oxford University Press; 63s.). The maximum outer diameter of the rings is 22 megalithic yards. In the book the megalithic yard, which measures 2.72 ft., is established as the basic unit of measurement in megalithic Britain, and is used in an astronomical interpretation of the function of these sites.

ENGLISH MEDIEVAL MEDICINE

Medicine in Medieval England

By C. H. Talbot. (Oldbourne History of Science Library.) Pp. 222+8 plates. (London: Oldbourne Book Co., Ltd., 1967.) 35s. net.

DR C. H. TALBOT, Research Fellow at the Wellcome Historical Medical Museum and Library, is well known for his studies of Anglo-Saxon and medieval medicine in England. In this book he writes on these subjects.

In the first chapter on Anglo-Saxon medicine he notes that after the conversion of the Anglo-Saxons to Christianity (A.D. 597-670) important intellectual links were forged with Ireland and Gaul. Teachers and books were imported; schools, including those in which medicine was taught, were established, and the cultural level of the Continental seats of learning served as a standard for the English people. An expert bibliophile, he describes the books and writings on medicine from Greek, Latin and Arabic sources which, probably, were studied by Anglo-Saxon leeches and their students. Some of these were translated into Anglo-Saxon, and then the Anglo-Saxons wrote treatises of their own, like the papers collected in the Leech Book of Bald which has survived. The Anglo-Saxon libraries, especially that of York, were consulted and extolled by continental scholars; but they were pillaged

and burnt by the Scandinavian invaders, or much more would be known about Anglo-Saxon medicine. There was a partial revival of medical manuscripts under King Alfred. They are not numerous and are in the British Museum and at Oxford and Cambridge. "Touching for the King's Evil" and the use of magic, amulets and charms in Anglo-Saxon medicine are discussed.

A chapter on Arab medicine, which was based on Galen's works, and reference to the writings of Rhazes, Averroes and other Arabians follow. The third and fourth chapters describe the Universities of Salerno and Montpellier. Talbot shows that generalizations about the influence of Salerno on English medicine are not valid, for they do not take into account considerable differences which separate the earlier from the later productions of this school. In fact, the earliest use of a Salernitan text in England seems to have been in the twelfth century. He also finds no evidence for the phrase, adopted by many modern writers on historic medicine, Ecclesia abhorret a sanguine, as a reason for the separation of surgery from medicine. It does not appear in the Council of Tours, A.D. 1163, or any Decretal of the Popes. It dates from Quesnay of Paris in 1774. Montpellier, it may be noted, had a fully developed medical faculty by A.D. 1137.

The chief features of medieval medicine occupy the rest of the book. Medical education, preceded by the acquirement of a degree in arts, is the subject of the fifth chapter with special reference to the lengthy course at Oxford. In the next chapter an account is given of the work of Gilbertus Anglicus, one of the first English writers on medicine, and others. Reference is made to Roger Bacon's attack on the physicians of his time. The seventh chapter gives much information on medieval surgery. The rest of the book is devoted to chapters on John Gaddesden, on anatomy and its exponents, the ordinary medieval practitioners, medical ethics and etiquette as then practised, hygiene, epidemics, hospitals, and vernacular texts; the book closes with a chapter entitled "The Final Phase".

This summarized account indicates the wide range and depth of the book and the important contributions which it makes to medical history of the Anglo-Saxon and medieval periods. In a brief preface Talbot states that his book is addressed to the general reader, not to specialists. This is, however, the work of a scholar embodying his researches, and will also appeal to general and medical historians. The bibliography is meagre, and references are omitted purposely, although their inclusion would have been welcome to students of the periods. But Talbot has given us so much that it is perhaps ungracious to ask for more.

ARTHUR MACNALTY

FUEL AND FOOD

The Limits of Man

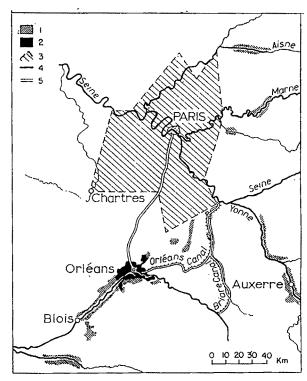
An Enquiry into the Scientific Bases of Human Population. By Hugh Nicol. Pp. 283. (London: Constable and Co., Ltd., 1967.) 35s. net.

Ir there are any people who believe that either in business or agriculture you can get something for nothing, they will probably be disillusioned by the time they have read Professor Nicol's repetitive refutation. Similarly, anyone who thinks that plants contain nothing but C, H and O will have the error thoroughly thumped out of him. These illusions may be widespread, but not, let us hope, in quarters where agricultural policy is established. Yet much of the book seems to be directed towards, or at, people in these places; it is a continuous editorial castigation of their misdirection of national and world agricultural policy with some bits of popular science and scientific history scattered through it.

The principal theme is that present agricultural policy relies too heavily on fossil fuels for the production of machinery and fertilizers, that ultimately these fuels will be exhausted, and that other methods of farming, for example, the use of clovers and other nitrogen-fixing legumes, deserve more attention. These are valid points, and it is good that people should comment on the way in which we are now squandering unrenewable resources, but agriculture is responsible for such a small part of the total waste that it seems hard that it should be singled out for special condemnation. No doubt, however, it is the duty of biologists to try to set engineers and physicists a good example.

The book is enlivened by many quotations—some pertinent and some comic. The most relevant is from R. G. Ingersoll: "In Nature there are neither rewards nor punishments—there are consequences". N. W. Pirie

WINE FOR PARIS



Provisioning of Paris with cheap wine in the seventeenth and eighteenth centuries (after Dion). I, Vineyards producing cheap wine. 2, Parishes in the Orléans district indicated in 1709 as being "all in vineyards". 3, Area around Paris in which wine production for sale to merchants and innkeepers had been prohibited in 1577. 4, Rivers used for navigation. 5, Paris-Orléans road. From An Historical Geography of Western Europe before 1800, by C. T. Smith. (Longmans, Green and Co., Ltd.; 65s.)

GREAT BIRD ARTIST

Thorburn's Birds

Edited with an introduction and new text by James Fisher. Pp. 184 (82 plates). (London: Ebury Press, and Michael Joseph, Ltd., 1967.) 50s.

ARCHIBALD THORBURN (1860–1935) is described in the introduction as having been "the first great bird artist of the twentieth century as well as the last of the nineteenth". In technical terms of book illustration, his work provides a bridge between the hand-coloured lithography of the earlier period and the methods of reproduction

used today. His work combined scientific accuracy with artistic merit, and he made his subjects look alive. With a few exceptions, he restricted himself to the birds (and sometimes mammals) of western Europe: he was in great demand as an illustrator both in Britain and on the continent. He also published four books of his own; and as an artist he was never better than in his British Birds, of which the four quarto volumes first appeared in 1915-16 (with a supplement in 1918). The pictures from that are here used again, and it is excellent that a new generation should have the opportunity of enjoying them.

The artist's original text was undistinguished and is now out of date. James Fisher has provided an entirely new one, in a novel and useful form. For descriptions he lets the plates speak for themselves, while he leaves habits to other works, and this allows him to concentrate on distribution. For each species there are, on the page facing the relevant plate, two paragraphs of compressed information. The first gives the world range, indicates the probable geographical origin, and mentions any seasonal movements. The second gives the status in Great Britain and Ireland—distribution within the area, size of population, changes that have taken place and current trends. The whole constitutes a valuable reference text both for ornithologists and for conservationists, the author's scholarship in these matters being of a high LANDSBOROUGH THOMSON

BRITISH LIBRARIANSHIP

Librarianship in Britain Today Edited by W. L. Saunders. Pp. xviii+173. (London: The Library Association, 1967.) 40s.

DURING the late summer of last year a three week course for librarians from overseas was held, at the invitation of the British Council, by the University of Sheffield Postgraduate School of Librarianship. The course included visits to selected libraries and lectures on topics of significance in the British library scene. The lectures presented during the course form this book.

The aim of the course was to provide a comprehensive

survey of present-day British librarianship: a formidable task even for a three week course. The fourteen lectures, given by leading figures in the profession, have met this requirement admirably. The editor of this book and director of studies for the course is to be congratulated on his selection of topics and the speed with which he has been able to publish. This up to date survey, even when the topicality of the papers has passed, will still have a value in presenting a picture of an exceedingly important stage in library development.

The scope of this book, however, is not as wide as its title implies. The content is governed by factors which determined the form of the course. First, the members of the course were expected to be from public and academic libraries, and second, the contribution made by the many visits cannot be included. The resulting exclusion of any detailed treatment of special librarianship is a deficiency which could have been remedied by the subsequent inclusion of additional papers in the book.

University librarianship receives detailed coverage with four excellent papers on the established and new universities, developments in the new technological universities and library buildings; together they succeed in partially filling an unfortunate gap in the literature.

The last two papers on "British Librarianship in the Next Decade", by civil servants in the Department of Education and Science, Mr P. H. Sewell, library adviser, and Mr B. J. Perry, senior scientific officer, OSTI, are of particular interest. In his contribution, Mr Sewell sees "the pattern of British library services as being more and more geared to identifiable community needs and departing in some respects from the institutional patterns with which we are familiar". Mr Perry also sees the development of "an increasing number of mechanized services ... [which] will be rigorously tested as to their suitability in meeting user needs . . .". Mr Sewell envisages the greater interdependence of libraries within specific areas with even in some cases integration of staffing between local libraries of different types. Mr Perry stresses the developments which should take place in the use and exploitation of mechanized bibliographical services and details the impressive work which OSTI is now undertaking in this field. DEREK JONES

Biological Science

BENEATH THE TUMOUR VIRUSES

Subviral Carcinogenesis

Edited by Yohei Ito. (First International Symposium on Tumor Viruses.) Pp. xvi+441. (Chikusa-ku, Nagoya: Aichi Cancer Center, Research Institute, 1966.) PRE- and post-congress meetings now surround a major international jamboree like satellites, and it must be admitted that they are often more rewarding to the specialists concerned than the main cong ess itself. The first international meeting of tumour virologists was organized at Negoya by Professor Ito and his colleagues after the Ninth International Cancer Congress in Tokyo, and the contributions have now been published under the title Subviral Carcinogenesis. The meaning of this title is a little obscure, but the volume contains a wide ranging series of papers extending from molecules to mice and occasionally to man.

Considerable progress has taken place in the characterization of tumour virus nucleic acids, as is clear from sections devoted to this subject. This particularly applies to the DNA-containing viruses, leading, for example, to the extensive homology studies with adenoviruses and to the detection of viral nucleic acid in "virus free" tumour cells. Even the RNA-containing tumour viruses which have been notoriously difficult to purify are now being characterized, and it is of interest that a variety which have been examined carry large and similar sized RNA molecules. There is a useful paper on the controversial problem of DNA participation in the biosynthesis of Rous sarcoma virus, an RNA virus.

The tumour cell antigens were also thoroughly covered. Some of the papers dealt primarily with the relevance to transformation mechanisms, including an authoritative contribution on the role of the papova virus T antigens. A particularly interesting Russian report concerns a newly discovered agent, whose sole manifestation appears to be the induction of a new skin transplantation antigen.

Several papers dealt with the important topic of defectiveness in tumour viruses and the dependence of one virus on another (in contrast to virus dependence on These concerned the classical Rous sarcoma virus and its helpers, complementation between DNA containing viruses, and the adenovirus SV40 hybrid virus.

In the case of the mouse leukaemia virus, progress has been rather limited, but recent developments, such as the spleen colony technique and the isolation of the associated mouse sarcoma virus, should lead to rapid progress in the coming years. The possible role of viruses in human cancer was only dealt with specifically in two papers, which stress the difficulty of interpreting the meagre findings. Progress obviously awaits a better understanding of the experimental systems. In addition to the papers directly concerned with tumour viruses, there was a scattering of other papers, some of which are particularly relevant to the tumour virus field, for example the account of bacterial conversion.

This book, which is a credit to Professor Ito and his Japanese colleagues, constitutes a useful and up to date collection of findings by leading virologists—or rather subviral oncologists.

MICHAEL STOKER

TUMOUR ANTIGENS

Specific Tumour Antigens

Edited by R. J. C. Harris. (A Symposium organized by the International Union Against Cancer and the USSR Academy of Medical Sciences.) (UICC Monograph Series, Vol. 2.) Pp. 366. (Copenhagen: Munksgaard, 1967.) 126 D. kr.

This report comprises thirty-four articles which in the main are summaries of groups of papers published in detail elsewhere and of which half emanated from laboratories in the United States or Soviet countries. The terminal discussions reflect the general doubt as to whether our available techniques are yet capable of defining completely the antigenic structure of normal tissue and therefore of differentiating tumour specific antigens. Thus Grabar appears convinced that azodye induced hepatomas in rodents are marked by specific antigens of which one component is an embryonal antigen (C.A.), but Deckers, working in the same field, notes the absence of embryonal antigens.

The significance of virus infections in experimental neoplasia and in the subsequent antigenic variation of the infected host cell is considered for a wide range of agents, including those associated with Rous sarcoma, Maloney lymphoma, Graffi and Gross virus-induced leukaemia, $S\bar{V}40$ and the adenoviruses. Melnick and Rapp analyse the complexity of virus transformation of cells by the use of metabolic inhibitors, such as cyclosine arabinoside which permits the synthesis of tumour antigen but arrests the production of SV40 viral antigen in monkey cell cultures. These authors and others report the formation, during cell transformation, of viral hybrids such as adeno-SV40 virus. The concept of immunity with regard to cancer is closely examined from the viewpoint of experimental pathology. Thus Harris, by the prior use of chicken tissue antigens for the induction of acquired immunological tolerance, reversed the normal rejection of Rous sarcoma virus-induced tumours in turkeys.

The sections of the report on human cancer studies include the immunological analysis by Korngold of serum gamma and macroglobulins. Sabin describes an unsuccessful search in the sera of children with early childhood tumours for complement fixing antibodies for antigens extracted from these tumours, but emphasizes the need for similar investigations using cytoplasm-modifying antibodies

Throughout the report doubts are expressed as to the validity of claims based on the use of immunologically heterogeneous systems for tumour specific antigens. Hence the interest which arises in the clinical report by Southam on the homotransplantation of human cell lines, including tumours, in healthy volunteers, the experimental production of hypersensitivity to autologous cell extracts in leukaemia patients and the effect of autologous fleucocytes or plasma on auto-transplants of cancer cells

in patients. Southam concludes that this approach has provided some support for the hypothesis that patients may develop an immunological defence mechanism. Surgeons have not infrequently come to a similar conclusion on clinical grounds, such as the occasional recovery to full health and survival for many years of patients with lesions so gross at operation that further surgical treatment other than a confirmatory biopsy was deemed unjustifiable.

The report conveys successfully the impact which cancer research has produced on the cross-linkage of experimental pathology, immunology, virology, biochemistry and genetics, and provides a valuable reference source. Perhaps of greater importance is the clear and refreshing demonstration that nations of different ideologies cooperate successfully in at least one area of human endeavour. Fitting tribute is paid in the foreword to the work of the Symposium organizer, Professor L. A. Zilber, who died in his laboratory in Moscow shortly after the meeting.

C. A. GREEN

HAEMS AT HOME

Hemes and Hemoproteins

Edited by Britton Chance, Ronald W. Estabrook and Takashi Yonetani. (Proceedings of the Third Colloquium of the Johnson Research Foundation of the University of Pennsylvania, Philadelphia, April 16 and 17, 1966.) Pp. xv+624. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) \$13.75.

THE haemoproteins have been subjected within recent years to very intense study by physico-chemical techniques. They are peculiarly suited to the variety of approaches which are now available and progress in the field is rapid. It is important, therefore, that at suitable intervals the whole corpus of knowledge concerning them and their enzyme properties should be reviewed and related one to the other.

The first colloquium on haems and haemoproteins was convened by Professor M. R. Lemberg and held in Canberra in 1959. It was a great success and set the pattern for future conferences. The second colloquium took place in 1964 at Amherst and now the third has been held at the Johnson Research Foundation of the University of Philadelphia and is recorded in the volume being reviewed. In the words of the editors, "The colloquium was organized to honour M. R. Lemberg, H. Theorell, D. Drabkin and D. Goddard and especially to celebrate two landmarks in the study of haemoproteins—the late David Keilin's book on cell respiration and cytochrome and the fortieth anniversary of the studies by R. Hill and H. F. Holden on the resolution of haemoglobin". There were sixty-five participants, fifty-six presented papers and a number of specifically arranged discussions.

The range of subject matter covered may be gathered from the titles of the topics presided over by individual chairmen: "Structure and Reactions of Haem", "Reactions of Haem with Proteins", "Ligand Binding to Myoglobin and Haemoglobin", "Intermolecular Interactions of Crystalline and Soluble Myoglobin and Haemoglobin", "Structure and Reactivity of Hydroperoxidases", "Physical Properties of Cytochromes c and b_5 ", "Ligand Binding and Reaction Mechanisms of Oxidases", "Kinetics of Electron Transfer Reactions in Cytochrome c", and "Theoretical Interpretations".

Contributions and discussions are at a high specialist level. The emphasis throughout is upon the physicochemical approach except for the three papers and discussion debating the structure of haem a and cytochrome c oxidase. In a characteristically witty and penetrating postscript, entitled "The Forgotten Cell", D. L. Drabkin reminds us that biological systems have properties and present problems which are inherently important and he

warns against myopic preoccupation with purified artefacts!

Despite its price, this is a volume which no one working in the field can afford to be without and which to enzymologists should be of particular value. It is in the haemoproteins that the allosteric transformations of structure associated with enzyme activity can be most readily studied.

The volume is excellently produced, for which both editors and publishers should be congratulated.

C. RIMINGTON

FREEZE-ETCHED SPECIMENS



Freeze-etch view of the nucleus in an onion root tip. nucleus, showing the nuclear pores, is the darker region in the upper part of the picture. From Cell Ultrastructure, by William A. Jensen and Roderic B. Park (Prentice-Hall International; 16s.). The book contains a collection of electron micrographs of a wide variety of animals, plants and bacteria, and summarizes the knowledge of cell structure which the electron microscope has made available. The book is intended to supplement biology textbooks in elementary courses.

CHROMOSOMES FOR STUDENTS

Medical Cytogenetics

By Mihály Bartalos and Theodore A. Baramki. xxiii+419. (Baltimore, Md.: The Williams and Wilkins Company, 1967. Distributed in the U.K. by E. and S. Livingstone, Ltd., Edinburgh.) 100s.

THE advent of this expensive book was widely publicized. From the preface we gather that its authors regard it as a fitting climax to the developments in human cytogenetics since 1956. In the foreword, by a physician from the Johns Hopkins Hospital, the book is described as "a complete and authoritative overview". So the drums roll and the trumpets blare, but after reading the book I am tempted to make my exit at this stage, referring readers to the immortal last line of T. S. Eliot's The Waste Land.

This is not a book for scientists. It is addressed to the student (unspecified but presumably medical) and to the clinician, and also hopefully to the cytogeneticist. It is written by two clinicians, one a paediatrician and the other an obstetrician and gynaecologist, and it is in the classical genre of medical textbooks. The book consists of an uncritical assembly of a mass of statements which will only serve to confuse the medical student and the clinician. who are not in a position to distinguish the serious from the trivial or fact from fancy. Nor, unfortunately, do the authors provide much guidance.

There is little point in the inclusion in a book for nonspecialists of detailed minutiae, much irrelevant, relating to techniques. The authors, in fact, betray their unfamiliarity with techniques by advocating, for example, outmoded and clumsy photographic methods for counting and analysing chromosomes. They seem totally unaware that some who contribute much to human cytogenetics do not use them at all nor have ever used them. The statement in the chapter on the effect of radiations that "rings and dicentrics can be scored readily, even by those

with a minimum of training" speaks for itself.

Although there is a mass of detail there is much that is important which is barely mentioned. The classic work of Ford and Hamerton on human male meiotic chromosomes is dismissed in thirteen words, and there is no mention of Chu, Darlington, Makino and McIlree, who have made important contributions on this subject. There is a total failure to understand the significance of the work of Tjio and Whang on the direct bone marrow technique and why this method of preparing cells for analysis was such an important advance on the earlier short term culture technique for marrow. Autosomal structural heterozygosity, the commonest form of detectable aberration, is far from completely dealt with, and the authors betray their discomfort in their description of the possible products of the interaction at breaks produced in G_1 , S or G_2 . Chromosome mosaicism is a topic of considerable importance to the cytogeneticist and to the clinician who has the responsibility of dealing with chromosomally abnormal patients. Yet scant attention is given to chromosome count distributions and to the criteria for establishing the diagnosis of mosaicism, although these have been the subject of public debate since 1960.

This book cannot be commended save as a source book W. M. COURT BROWN of references prior to 1965.

SEX CHROMOSOMES

Sex Chromosomes and Sex-linked Genes

By Susumu Ohno. (Monographs on Endocrinology, Vol. 1.) Pp. 192. (Berlin, Heidelberg and New York: Springer-Verlag, 1967.) \$95.

Dr Ohno's book on sex chromosomes and their genes bears witness to the great deal of work on this topic, to which Dr Ohno himself has valuably contributed. The book is divided into three sections. In the first section it is shown how distinguishable sex chromosomes are thought to have arisen from morphologically identical chromo-This does not, however, necessarily imply a somes. strictly parallel evolution of sexual dimorphism from hermaphroditism. This evolutionary pattern is, of course, built up from comparison of chromosome morphology from present day animals. But the logical barrier in accepting the proposed sequence of past events inferred from present day appearances is swept aside by Olmo's persuasive enthusiasm in unfolding his thesis. Reference to Fig. 3, showing the evolution of snake Z and W chromosomes, will illustrate the type of argument used. Unfortunately, in this instance, no critical evidence is presented to show that the inversion, if it actually is one, is pericentric, even though this is crucial to Ohno's argument as to how chromosomal differentiation is initiated.

Admittedly the critical evidence may be unobtainable; even so Ohno's discussion of chromosomal evolution reveals the strength of this type of approach, yet he neglects its inherent weakness. Evolution of genomes is also dealt with; it would have been helpful if a diagram had been supplied to "fix" the mass of facts presented, relating taxonomic position with DNA value within this cytologically rooted evolutionary tree. The first section ends with a useful comparison of X and Z-linked genes between phylla. From the evidence presented it seems likely that both Adam and the Serpent carried similar genes on their sex chromosomes.

The second section deals with desage compensation and "lyonization" of X chromosomes. This is discussed from a biochemical, morphological and cytological point of view. No mention is made of scepticism of this phenomenon and newcomers to the field may perhaps like to read Grüneberg's views before completely digesting the interpretation.

How do the sex chromosomes determine sex? The last section attempts to answer this question and thereby to justify the book as a "Monograph on Endocrinology". But, as Ohno admits, no answers are forthcoming. This leads to an expectedly sketchy chapter on sex-determining factors. A second chapter on the development of gonads will, no doubt, resuscitate endocrinologists after the previous 160 pages of cytogenetics.

This is an attractively written and produced book and a veritable mine of information. Cytogeneticists will not be disappointed by Ohno's scholarship and authority. The many photographs and diagrams are of high quality although the photographs of Fig. 20 are unnumbered and should be viewed sideways on. There are a few typographical errors (for example, homozygous for homologous; page 102, end of first paragraph) and the potentially libellous phrase "mongolian idiocy" appears for Down's syndrome.

In all, this is a compulsively readable account of a subject hitherto badly in need of Ohno's capable treatment.

P. W. Barlow

NORMAL AND ABNORMAL CELLS

Practical Cytology

By R. G. W. Taylor. Pp. xi+148. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 39s. 6d.; \$7.

In clinical medicine the term cytology has come to have rather a special meaning, namely the examination of cells as an aid to diagnosis with some stress on the detection of malignant and benign growths of the female reproductive tract. It may also be added that the term has a euphemistic value when dealing with patients, and indeed even when giving extra-mural lectures to unsuspecting women's organizations. It is not surprising, therefore, that a book bearing the general title *Practical Cytology* should have a restricted content, being almost entirely concerned with smear techniques in medicine with a marked bias towards their application in gynaecological disorders.

The first chapter deals with the preparation of smears; the theory of fixation on which any rational approach depends is limited to a single sentence: "The principal requisite of a cytological fixative is that it shall precipitate the cell protein, and in particular the nuclear protein in a pattern characteristic of the form during life". The staining, mounting and examining of smears appear in the next chapter. Here the author seems unsure of the background of his readers; the opening page deals en passant with Papanicolaou's solutions, the Jenner-Giemsa method, Feulgen's stain and "it must be remembered that methanol fixation is a pre-requisite for all Romanowsky sequences". A few pages farther on, there are sections entitled "Cleaning Smears", "Loading the Slide Carrier", "Blueing Smears" and so on. There are numerous staining schedules,

with little explanation; but all rigidly and ponderously timed so that "most of the black magic associated with staining disappears".

A diagram of a general cell which marks the beginning of the third chapter is so abysmal as to defy further useful comment; it is also odd to see a detailed table designed so that the greater part of it actually appears upside down (the rest is printed sideways). This chapter, however, heralds the first of four others on normal and abnormal epithelial cells. The descriptions of the cells are clear and informative and there are some good, simple diagrams. The need for numerous photomicrographs has been recognized, but unfortunately these are generally of such mediocre quality that they do not help; none are in colour.

The last two chapters deal with documentation and ancillary techniques. There is a useful appendix and a brief bibliography.

The book really adds little to a subject which is itself fascinating and work which is rewarding.

D. LACY

MOLECULAR PHARMACOLOGY

Drugs Affecting the Peripheral Nervous System Vol. 1. Edited by Alfred Burger. (Medicinal Research: A Series of Monographs.) Pp. xxiii+620. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1967.) 220s. net.

IT is fashionable nowadays to add the adjective molecular to many of the normal subdivisions of biology, and it is a nice semantic exercise to consider what is meant. by this qualification in each of its various applications. Pharmacology is no exception and the expression "molecular pharmacology" is enjoying a current vogue although, rather more than in most cases, it is a tautology. Pharmacology has been faced with the problem, in its most acute form, of relating the biological and the molecular properties of drugs since its emergence as a separate scientific discipline in the middle of the nineteenth century. By using purely empirical procedures a great array of potent and useful drugs has been assembled, but our understanding of how these drugs act at the molecular level is still very slight, and has depended on collateral advances in other related subjects. This multi-author work collects together a great deal of experimental evidence and attempts a survey of the ideas and theories currently deployed. Attention is restricted to the peripheral nervous system—a further volume dealing with the central nervous system is promised—and consequently covers much the same ground as D. J. Triggle attempted single-handed in his Chemical Aspects of the Autonomic Nervous System. Although it appears more than a year later the literature survey is not much more up to date, but this is a much more substantial work and the overall coverage is more exhaustive.

The intention of the editor was to concentrate on the relationship between biological effect and chemical structure, but because of variations in the amount of material available and the personal preference of the various authors the balance of individual chapters varies within wide limits. The first chapter, by S. Ehrenpreis on cholinergic mechanisms, consists of a critical review of Nachmansohn's theory of the role of acetylcholine in nervous transmission followed by an interesting, if highly speculative, defence of the proposition that cholinesterase and the choline gic receptor are one and the same. At the other extreme is a very long chapter by L. Gyermek on ganglion stimulant and depressant drugs, which consists of an exhaustive review of the many structure—action studies carried out with ganglion preparations with virtually no theoretical discussion.

There are two rather cursory chapters on cholinesterase, but the remainir g articles on muscarinic and cholinolytic drugs, drugs acting on the skeletal neuromuscular junction, sympathomimetic and sympatholytic agents and drugs acting on sensory systems achieve a more even balance between the raw data of structure-action correlations and theoretical interpretation.

Most of the people working on these various areas of chemical pharmacology will be familiar with the general patterns of chemical structure variation that have been explored. What is most useful in this book is that the various theories which have been proposed are all collected together and extensively discussed. This survey shows that there is no shortage of ideas about the way in which chemical structure influences biological activity, but also clearly reveals the basic problem of chemical pharmacology—that it is extremely difficult to devise critical experiments to support or refute these ideas. Many intriguing problems are discussed and this collection of articles should prove very useful to anyone interested in this difficult problem of the relationship between chemical structure and physiological effect.

This volume is handsomely produced and printed; it is prefaced by an embarrassing piece of American mortuary prose that would have been better omitted; it is also very expensive.

E. W. Gill

ANTIBIOTICS GIVEN TREATMENT

Antibiotics

Origin, Nature and Properties. By Tadeusz Korzybski, Zuzanna Kowszyk-Gindıfor and Włodzimierz Kurylowicz. Translated by Edwin Paryski. Vol. 1: Pp. xix+1-1144. Vol. 2: Pp. xviii+1145-1651. (Oxford, London and New York: Pergamon Press, Ltd.; Warszawa: PWN-Polish Scientific Publishers, 1967.) 294s. net per set of two volumes.

WITH the explosive proliferation of published data in the field of antibiotics during the past decade, the need for an authoritative English language reference work, embracing combined knowledge of the many aspects of the biology and chemistry of those diverse compounds, has been keenly felt. These two volumes represent a most ambitious attempt to fill this gap, with the very large number of individual antibiotics listed providing its own tribute to the diligent application of the authors to such a herculean task.

The presentation, in view of the virtual impossibility of achieving an ideal classification of the antibiotics, is excellent, with the work being divided into seven main sections: antibiotics obtained from (a) the order Eubacteriales; (b) the order Actinomycetales, (c) Fungi imperfecti, (d) Basidiomycetes and Ascomycetes, (e) lichens and algae, (f) higher plants, (g) animal sources, of which the first two sections constitute the first volume and the others constitute the second volume. Further subdivisions are then made according to genus and on the basis of chemical structure, antimicrobial spectra and so on, as best suited. The presentation of many of the data in clear tabular form is a great advantage to the reader. While the inclusion of compounds derived from higher plants and animals as antibiotics is open to debate, it again underlines the wide coverage of the work.

The biological topics, which include accounts of the origin, culture conditions, isolation techniques, antimicrobial spectra, and clinical implications of the various antibiotics, are magnificently handled, as are considerations of stability, physical properties and so on. The copious cross-referencing within the various sub-sections, each of which has its own bibliography, is a most commendable feature. In view of this, it is all the more unfortunate that on the chemical side the work falls short of what might have been achieved.

The authors have largely ignored biogenetic aspects, as evidenced by the controversial statement in the introduction that the biogenesis of the antibiotics remains unclarified, and by the brief dismissal of the biogenesis of various specific compounds such as stipitatic acid and patulin in terms of statements to the effect that this has been studied. The failure to indicate the results of these biogenetic studies is most surprising, especially in view of the intense current interest in the topic as evidenced by the many published papers and by the appearance of several books devoted to this aspect alone.

An obvious attempt to update the original Polish version of the work has been made. This has led to some unfortunate situations, such as the clumsy insertion of the correct formulae for verrucarol and its close relatives with the retention of erroneous structures for the derived verrucarins and roridins, and the insertion of the correct formulae of the polymyxins with the retention of an erroneous table which complies neither with earlier spurious assignments of structure nor with the correct structural assignments of polymyxins B₁, B₂ and E₁. Even so, there are very few post-1964 references to original literature, amendments to the section on viomycin (where no evidence is presented in support of the revised structure) being a notable exception.

In some instances the chemical data are unreliable, indicating that, although the literature survey would appear to extend through 1964, its breadth in this area is suspect. Specific examples are the incomplete or incorrect structures given for iridomyrmecin (which incidentally, is misspelt), "polyporenic acid", geodin, ordin, solanocapsine, pleuromutilin, pristimerin and grifolin—to mention random examples—where the correct structures were all in the published literature by 1963, in some cases in the 1950s. The time interval occasioned by the translation and publication of the English version, of course, precludes the correct portrayal of the structures of important compounds such as fusidic acid, viridin, marasmic acid and hirsutic acid, and also precludes consideration of the stereochemistry and conformational aspects of the streptomycin molecule, as well as discussion of a number of more recently isolated antibiotics.

Portrayal of absolute stereochemistry by means of stereoformulae appears to have been done in a most arbitrary manner, and for important groups such as the penicillins, cephalosporins and tetracyclines there is no stereo portrayal at all.

Nevertheless, despite the shortcomings of these volumes on the chemical side, and in one or two other areas—as with the bacitracins, where more than the three bacitracins listed are known—workers in the many faceted area of antibiotic research will find it hard to resist their acquisition, for they provide a rapid and convenient source of comprehensive information and references to the original literature up to about three years ago. Purchase of both volumes is mandatory because the comprehensive authorand subject indices for the two are in the second volume.

M. MARTIN-SMITH

MURINE PATHOLOGY

Pathology of Laboratory Rats and Mice

Edited by Ernest Cotchin and Francis J. C. Roe. Pp. xxiii+848. (Oxford and Edinburgh: Blackwell Scientific Publications, 1967.) 155s.

THE Nuffield Foundation has recently become interested in the possible long term effects that food additives may have on human health. The screening of such substances to detect possible toxicity often involves tests on laboratory animals, and so the Foundation held a conference in 1966 devoted entirely to the pathology of laboratory rats and mice.

Two of the participants, E. Cotchin and F. J. C. Roe, have edited the twenty-four papers that were presented by recognized authorities and have produced a most comprehensive and welcome monograph.

Each paper is followed by an extensive bibliography, together with shortened versions of the discussions which succeed each paper. Although these bring the book up to date, they would have been easier to follow if they had been given under headings similar to those in the chapters.

It is appropriate that the first chapter should be devoted to the target organ of so many toxic substances, namely the liver, and it is encouraging to discover chapters covering hitherto neglected subjects such as the fungal infections, parasites and ophthalmic pathology of rats and mice, but it is disappointing to find that there are no chapters on bacterial diseases, cardiovascular diseases of mice and diseases of the lymphatic system. Although pneumonia and virus infections are adequately treated, salmonellosis, which is probably the commonest infection of these animals, is given barely one page, and even then only in passing.

The advantages of using specific pathogen free or disease free animals are amply demonstrated throughout and, in particular, the part that inapparent disease may

play in invalidating experimental results.

Several pleas are also made for the increased use of defined inbred strains or, as one author put it, "inbred mice are as indispensable to the biologist as pure chemicals are to the chemist". Yet, even today, people still ask for white mice!

There is little doubt that this book will be invaluable to both the animal pathologist and the producer of laboratory rats and mice; the former because it may enable him to decide whether any lesions that are found after death are due to the experimental procedures or naturally occurring disease, and the latter because the prompt recognition of disease and its diagnosis saves a great deal of time and money.

Although neither of the editors, both well known in their field, has contributed any of the papers, they feature prominently in the discussions, and must be congratulated for the excellent presentation, the many very good illustrations and the more than adequate index.

It is hoped that a companion volume will soon appear. covering the pathology of other species. JOHN BLEBY

RANGE OF MOLLUSCS

By J. E. Morton. (Biological Sciences.) Fourth, revised edition. Pp. 244. (London: Hutchinson and Co. (Publishers), Ltd., 1967.) 13s. 6d. net.

WHEN I was a student, work on molluses consisted of a largely anatomical survey related to hypothetical ancestral forms caught up in a variety of posturings from which some emerged with coiled shells, some with straight shells, some with none, some with torsion, some without. There was no hint, or hardly any hint, of the rich and exciting results of the work on functional anatomy which were to emerge later, although some of Orton's observations (which began it all) had already been long published and the first fruits of Yonge's magnificent extended study of these animals had been gathered.

Molluses are unusual animals, and, at least for some people, exciting ones. Their chief attraction is the quite extraordinary way in which they retain their characteristics of anatomy-and even of function-no matter how much their bodies become contorted in pursuit of some special adaptation, and it is the curious intimate mixture of anatomy and functioning in relation to adaptation for life in a particular niche that makes them such an absorbing study. It is a never ending study, too, for year after year something unusual is discovered even about the most ubiquitous type. Despite this, and despite the recent resurgence of interest in the phylum, there is at present only one book which has attempted a summary .of modern malacological work at undergraduate level: Morton's Molluscs, first published in 1958 and now appearing in a fourth, revised edition. The text has been extended to incorporate new material-most significantly, information about Neopilina, still not known when the first edition appeared, though Morton had bad luck here in being just too early to include it, and an expanded treatment of Wells' work on cephalopod behaviour. Perhaps the change for which most readers will be grateful is the increased number of illustrations, most in the Morton idiom, though still, I find, small considering the page size, and sometimes (as in Fig. 29, page 137) rather too complex.

There are some things I miss: I would like in particular to see an account of how the molluscan radula—a major feature of the group-works, not just of what it looks like; there is a lot of interesting work done on the behaviour of molluses other than cephalopods though not referred to here; and the account of boring by muricacean neogastropods oddly omits all reference to the most extensive and experimentally critical work on this topic-that of

Carriker.

These, however, are minor carpings aroused in my mind by not finding perfect a book which I should like to be perfect. Morton has made a bold and outstandingly successful attempt to get all the really important facts about the living molluse into very brief compass. My students tell me that they enjoy it and that he has succeeded in adding to his information some of the fascination which he, I know, and most malacologists have found in the study of molluses. But perhaps the credit for this should really go to the mollusc and not to the author. A. GRAHAM

LIFE IN WATER

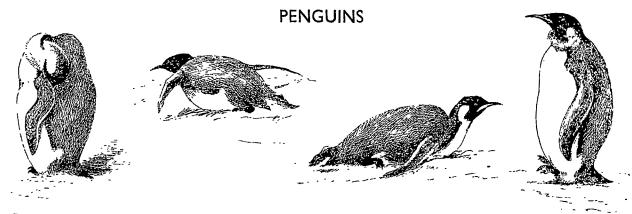
Oceanography and Marine Biology

An Annual Review. Vol. 4. Edited by Harold Barnes. Pp. 505. (London: George Allen and Unwin, Ltd., 1966.) 105s. net.

THE fourth edition of this annual review continues to navigate the hazardous waters surrounding a journal that attempts to serve a number of different disciplines, united by a common interest in the sea. Paradoxically, the biggest problem that it has to overcome is that which presumably it is intended to promote, namely, the exchange of ideas at the research level between the physical and biological sciences.

The first four reviews cover different aspects of physical oceanography. The article by W. Krauss on internal waves in the sea is a mathematical treatment of data collected at sea, and, apart from acknowledging the international reputation of the author in this field, comment is beyond my ability. In contrast, the paper by K. Wyrtki on the oceanography of the eastern Equatorial Pacific is a readable and informative discussion of the hydrography of this ocean, with particular attention paid to the problems raised by the recently This section is discovered Equatorial undercurrent. completed by an account of conditions governing the formation of ice in the Baltic by E. Paluso, and a record of some of the initial difficulties experienced in the organization of the international Indian Ocean expedition by R. Currie.

The origin, identification and distribution of gelbstoff in the sea are discussed by K. Kalle. This non-specific term is applied to a heterogeneous group of yellow compounds derived from the degradation of marine algae and also from humic compounds of terrestrial origin. is supplemented by a review of the extracellular products of algae by G. E. Fogg, dealing with compounds of smaller molecular weight which are more often studied in culture media. The important contribution of micro-organisms to the fertility of the sea is generally realized but little understood, and an interesting account of marine psychro-



Four views of the emperor penguin (Aptenodytes forsteri) from Birds of the Antarctic, by Edward Wilson and edited by Brian B. Roberts (Blandford Press; 105s.). Wilson took part in Scott's two Antarctic expeditions, and this book contains more than three hundred of the paintings and drawings which he made, together with notes about Wilson and his work and extracts from his journals.

philic bacteria by R. Morita describes metabolic studies that have been made on a group that thrive in conditions of temperature such as exist in the deeper parts of the oceans. The review by R. Scagel on the Phaeophyceae catalogues the extensive literature on this group but suffers from the lack of personal interpretation of the material presented.

Two articles have a palaeontological bias; that by R. H. Benson describing recent marine ostracods deals with living representatives of this order which is commonly preserved as fossils. The paper by H. B. Fell on ancient echinoderms in modern seas is a review of the author's own interpretation of echinoderm phylogeny, which, it should be pointed out, has not found universal acceptance among workers in this group.

Four papers dealing with aspects of animal behaviour are by J. A. Allen on rhythms and population dynamics of decapod crustacea; by E. W. Knight Jones and E. Morgan on responses to hydrostatic pressure; and by P. M. J. Woodhead, who examines the behaviour of fish in relation to light in the sea. He shows that in diurnal patterns of behaviour light has an important influence on feeding, sound production, spawning and schooling, and thus on the successful fishing of many commercial species. Lastly, J. Thompson discusses the biology of the grey mullets, and one is bound to comment on the poor quality of his Fig. 2 (page 306), which should not have escaped the vigilance of the editor.

The littoral ecology of tropical West Africa is reviewed by G. W. Lawson, who has wide experience in this field. Following Lewis's classification he has produced a guide to the flora and fauna of the principal ecosystems along the coast. Although his fauna list is by no means complete, it in no way detracts from this valuable synthesis which will stimulate further and more detailed studies.

The book is adequately provided with maps, figures and tables, but the references do not contain the titles of the publications, which are often required when requesting them on inter-library loan schemes.

Derek Dorsett

TEXT ON TAXONOMY

Taxonomy

A Text and Reference Book. By R. E. Blackwelder. Pp. xiv+698. (New York and London: John Wiley and Sons, 1967.) 150s.

NEARLY seven hundred pages about taxonomy cannot be expected to be easy reading, either for the specialist

or for the undergraduate. But Dr R. E. Blackwelder states in the preface to his new book, *Taxonomy*, that "It is specifically planned to be used as a textbook in two courses: a beginning course on the nature and practice of taxonomy and an advanced one, on the theory and technicalities . . ." Then again, also in the preface, he says, "It is a book about taxonomy for taxonomists", while the subtitle of the book claims that it is both a text and a work of reference.

Presenting the intricacies of taxonomy at length to satisfy so diverse a public and to fulfil such diversity of need is a near impossible task and it is not surprising that the attempt has resulted in a relatively expensive work not fully suited to the student purse or mind, or to the taxonomist. This is a pity, for there is a need for a comprehensive book on this subject that would encourage students with a leaning toward taxonomy to enter this field—Dr Blackwelder's book might achieve the reverse. Nevertheless, it contains a very great deal of information not easy to find elsewhere and much opinion and advice arising from his long experience as a leading taxonomist that others will wish to note. For a subject which is heavily determined by its history, however, it is surprising that the historical component receives scant attention in a couple of pages or so of the introduction. In consequence it is not always clear why things are now done the way they are and much of the sense of continuity of taxonomy through time is lost.

It is not easy to follow the theme of this book, for it can be opened and read almost anywhere (albeit with profit) and it does not seem to matter very much what has gone before or what comes after. Moreover, the text is badly interrupted by lists such as universities of the United States offering courses in taxonomy, societies concerned with taxonomy, and museums, which could have been relegated to an appendix. The student thus gains little sense of order and no stimulus to read further, and for this reason the book is unsatisfactory as a student text.

J. E. Webb

SEAWEED POLYSACCHARIDES

Chemistry and Enzymology of Marine Algal Polysaccharides

By Elizabeth Percival and Richard H. McDowell. Pp. xii+219. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 60s.; \$12.

THE general nature of the polysaccharides of seaweeds, forming in the main the cell walls and storage products,

has been known for a long time. Algal starches have long been known, for example, to be very much the same substances as the starches of higher plants and the cell walls of many algae have been known to resemble those of higher plants in being based on cellulose associated with a wide variety of non- or para-crystalline polysaccharides based on sugars other than glucose. During the past decade or so a number of workers in various parts of the world have turned their attention to detailed chemical examination of algal polysaccharides, and this book, written by two authors of whom the senior author particularly is an outstanding authority, presents a comprehensive account of the present state of this study. The stimulus for this sudden expansion in our knowledge of marine algal polysaccharides undoubtedly springs from two sources: the increasing commercial utilization of these products of algal metabolism and an increasing tendency among plant physiologists to use the simpler algae as model systems as an aid to an understanding of higher plants. This book is unavoidable reading from either point of view. It does not pretend to deal exhaustively with many of the topics covered, but the reader is directed copiously to the relevant original literature listed at the end of each chapter. This makes it an easily read book, manageable in size, and yet an almost complete guide into all the current and immediate past literature of importance. It assumes in the reader some previous knowledge of organic and physical chemistry but this is offset to some extent by the second chapter in which detailed coverage is given of the modern methods of chemical structure determination with polysaccharides.

After a preliminary survey in the first chapter and the methodology of the following chapter, six chapters deal in turn with food storage, food reserve and structural polysaccharides, with alginic acid, sulphated polysaccharides and polysaccharides containing uronic acid and ester sulphate groups. A useful last chapter compares algal polysaccharides with both higher plant and animal polysaccharides.

This is a very useful and timely book. It is well presented, with a subject and author index, and I detected exceptionally few printing errors, none of them misleading. It is strongly recommended reading not only for phycologists but for plant physiologists in general and for all those involved in commercial exploitation of seaweeds.

R. D. Preston

AQUATIC MICRO-ORGANISMS

Microbiology of Oceans and Estuaries

By E. J. Ferguson Wood. (Elsevier Oceanography Series.) Pp.xi+319. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 135s.

THERE is undoubtedly a need for a good textbook on marine and estuarine microbiology. The range of topics covered by Professor Wood in his recent book, *Microbiology of Oceans and Estuaries*, is well chosen and his writing is lucid. It is questionable whether the book will serve as a useful textbook, for it falls between two fires, being too general for the specialist and far too discursive for the beginner.

Many chapters and sections are welcome and well written; in particular are those dealing with microbial symbioses, fouling, corrosion, fish spoilage and geomicro-They bring together under one cover a wide variety of information together with many of the personal observations and findings of the author. The parts of the book describing the inter-relationships between pH, redox potential and various chemical, biological and geobiological events are generally interesting and useful. The description of the bacterial groups in the chapter on bacteria and fungi is clear and easily understood and will probably be useful to the student. In sharp contrast, his description of the phytoplankton groups is poor. text of the section on the dinoflagellates is very difficult to understand and the accompanying illustrations give little help. In this chapter species and genera are referred to and even discussed at length, without their features being described to the reader. A person with limited prior knowledge of phytoplankton will gain little from this chapter. The quantitative aspect of marine production is given very curt treatment and the author's personal views on the subject are made very plain.

In summary, the book lacks the orderly presentation of information expected of a textbook and leans rather heavily on the personal observations and opinions of the author. In all fairness to Professor Wood it should be said that it is not easy to write a book on aquatic microbiology and probably impossible to write a good one. The price of the book is surprisingly high considering its size and that it is not lavishly illustrated.

P. J. LE B. WILLIAMS

Physical Science

NEW WAYS WITH ATOMIC LEVELS

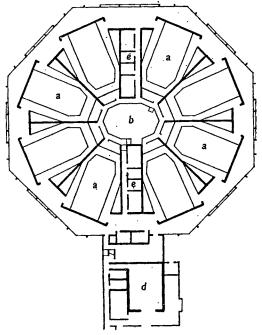
Second Quantization and Atomic Spectroscopy (G. H. Dieke Memorial Lectures.) By Brian R. Judd. Pp. viii+61. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1967.) \$5.95; 48s. net.

This little book is concerned with a different approach from the usual to the question of atomic energy levels. The usual method is to construct a suitable electronic wave function to represent the most important part of the complete wave function, and then to mix in with it wave functions for other "excited" configurations. The analysis is heavy, as anyone will soon admit who has struggled with the appropriate wave functions for rare earth configurations based on f^n . Professor Judd shows that the systematic use of creation and annihilation

operators can greatly reduce the labour. Moreover, because one configuration can be found from another (the basic one) by annihilating one electron and creating another in a different orbital, there is no need actually to set out explicit forms for these other configurational wave functions. The techniques of second quantization have not often been used in this way, but they throw light on the Racah formalism of fractional parentage, and are easily visualized in terms of Feynman-type graphs.

The present book is described as the lecture notes for a series of lectures at the Johns Hopkins University. The eight chapters are clearly set out, though they would be easier to read if they had been a little more extensive. They represent a significant advance in this field. Their only major defect—which rubs them out from all but libraries—is their cost. Forty-eight shillings for fifty-five pages and an index is ridiculous. C. A. Coulson

WHERE TO TEACH SCIENCE



Plan for a classroom and studio building of the University of Miami, Florida. a, Auditoria, each seating three hundred people. b, Back projection area. From The Science Lecture Room, by Jeremy Taylor (Cambridge University Press; 60s.). The book is a planning study which examines the principles of location and design of lecture rooms in the development of university science areas.

LIQUID AND SOLID HELIUM

The Properties of Liquid and Solid Helium By J. Wilks. (The International Series of Monographs on Physics.) Pp. xii+703. (Oxford: Clarendon Press; London: Oxford University Press, 1967.) 150s. net.

THE torrent of scientific papers is such that physicists would be carried away beyond rescue but for the work of those few of their devoted colleagues who, by pausing from time to time to embody their learning in monograph form, construct a series of rocks on which the rest of us may clamber from the flood and attempt to renew our perspectives for a while before diving in again. The first liquid helium rapids of the late 1930s were stemmed in 1942 by Keesom's celebrated work, Helium, from which a whole conspectus of helium, solid, liquid, gascous, atomic, nuclear, scientific, historic and technical, could be taken. The waters continued to rise, with the additional hazard that some navigation in Russian was called for, until Atkins in 1959 regained control of some tributaries by his book Liquid Helium. Now, twenty-five years after Keesom's book, comes Dr Wilks's The Properties of Liquid and Solid Helium.

This work will command not only respect but also gratitude from all those who have fallen under the fascination of the condensed states of helium; the respect will be shown to the author's authority, and the gratitude to the clarity with which he treats both experiment and theory. His lucid style is supported by the excellent printing and diagrams typical of the Clarendon Press. Roughly two-thirds of the book is devoted to liquid helium four, one sixth to liquid helium three, and the remaining sixth to the solid phases. As is proper, the author's particular interest in zero sound is allowed to detain us a little when we read about helium three.

One of the conspicuous features of the book is the gathering together and presentation of introductory

material necessary for an understanding of the theory. Thus the author takes care that the requisite ideas of fluid mechanics, statistical mechanics and quantum mechanics are explicitly laid out ready for use. He also offers a quite invaluable chapter on phonons in crystalline solids, by way of introduction to the excitation theory of liquid helium II.

It is only to be hoped that the rather high price of this book will not prevent it from being available to the many scientists to whom it has so much to offer.

D. V. OSBORNE

RELATIVITY AND SOLUTIONS

Relativistic Hydrodynamics and Magnetohydrodynamics Lectures on the Existence of Solutions. By Andre Lichnerowicz. (The Mathematical Physics Monograph Series.) Pp. ix+196. (New York: W. A. Benjamin, Inc., 1967.) \$9.50.

This book is addressed to a restricted audience; it is concerned with the mathematical study of the equations of relativistic hydrodynamics. Its origin lies in a course of lectures given to graduate students at the Centre of Advanced Study in Dallas at the end of 1965. The book is, in essence, the reproduction of a set of mathematical lecture notes with an almost complete absence of physical interpretation.

Familiarity with relativistic hydrodynamics is assumed and this involves a working knowledge of relativity, hydrodynamics and the theory of partial differential equations.

In a fluid which is hydro- and thermo-dynamically perfect the treatment yields three systems, gravitational waves, hydrodynamic waves and stream lines, and the problem is extended by introducing an electromagnetic field and a fluid which is charged but has a vanishing electrical conductivity. The more difficult case of relativistic magnetohydrodynamics assumes inductive effects on a perfect and infinitely conducting fluid. This leads to Alfven, hydrodynamic and relativistic shock waves.

JOHN PAIN

MASS SPECTROMETRY

Mass Spectrometry of Organic Compounds

By Herbert Budzikiewicz, Carl Djerassi and Dudley H. Williams. (Holden-Day Series in Physical Techniques in Chemistry.) Pp. xv+690. (San Francisco and London: Holden-Day, Inc., 1967.) \$19.75.

This is both a very valuable and an interesting book, for several reasons. The coverage of the organic compounds discussed is very wide, comprising most simple classes of materials possessing functional groups, including the alcohols, aldehydes and ketones, ethers and other oxygen-containing molecules; their sulphur analogues; amines, amides, cyanides and other nitrogenous systems; halides, as well as the parent hydrocarbons; organic phosphorus compounds and even a comprehensive, if brief, account of organo-metallic systems.

Moreover, this book is written from a distinct point of view which is clearly stated at the outset (page 9, first paragraph et seq.); namely, that of charge localization. This is an important unifying concept which is currently of considerable interest. The views of these authors with whom this theory is already associated as a consequence of an earlier work (Interpretation of Mass Spectra of Organic Compounds) are most timely, although the simplicity of the idea is somewhat eroded by specific compounds, notably among oxygen-containing molecules (for example, page 100, line 6; page 133, line 9; page 180, fourth paragraph).

Further, the scale of this book and the speed at which it was achieved are a remarkable tribute to the authors. There seem to be a few errors which are mainly trivial. Those noticed by the reviewer tend to occur towards the end and are more irritating than serious. These include two misspellings, phosphorus (page 648, line 27) and Tabrizi (page 668, ref. 12), and an unfulfilled promise of cross references (page 612, lines 2 and 3).

In a book of this character what is included and omitted must be at the discretion of the authors, although I regret that some guidance was not afforded to future imitators, advising which ions should be considered for the discussion of an unknown structure and which may be discarded as inessential.

A particular criticism which may be made is that in considering "electron book-keeping" (page 1, line 14), the claims of R. M. Silverstein and G. C. Bassler (Spectrometric Identification of Organic Compounds, John Wiley and Sons, Inc., New York, 1963, page 8, column 1—author's note) to have originated this idea should have been overlooked.

Finally, the publishers are to be congratulated on the production of a book of such quality. The format is pleasant, the text legible, and the numerous line diagrams well produced; as indeed are the graphic formulae so often a stumbling block in the production of chemical texts.

Altogether a book to be recommended to those interested in the subject and a must for workers in this field.

ROWLAND I. REED

ORGANIC POLAROGRAPHY

Substituent Effects in Organic Polarography By Petr Zuman. Pp. xvi+384. (New York: Plenum Press, 1967.) \$17.50.

THERE is a tendency to assume that, as far as polarography in organic chemistry is concerned, the method is no more than an analytical tool. Further, because the present writer is the author of a very well known volume entitled Organic Polarographic Analysis, it might be casually assumed that the volume being reviewed is merely an extension of the analytical applications of polarography in organic chemistry. Such assumptions would, however, be far from the truth for, in this monograph, Dr Zuman presents an original attempt to meet a real need, that is, to provide a systematic relationship between the polarographic behaviour of organic substances and their structure.

Experimental evidence is summarized which shows that polarographic data can be treated by the use of coefficients which characterize the effects of substituents quantitatively, giving a clear understanding of structural effects on half-wave potentials.

The book contains ten chapters, beginning with one on the development of the study of structural relations, classification of and techniques used in the polarographic study of structural effects, which should be read by all physical organic chemists.

To establish a correlation between reducibility and structure, a general equation for the shift in half-wave potential caused by substituent effects is derived in the second chapter, which takes into account, among others, conditions for the application of the general equation. In the following chapters, the possibility of application of special forms of the general equation to benzene derivatives, monocyclic compounds, and alicyclic systems, to name only a few, is demonstrated.

Zuman claims that he has two principal aims. The first is to show the organic polarographer the types of problems which can be treated quantitatively: the second is to show physical organic chemists what polarography offers for the solution of fundamental problems. Both aims have been achieved admirably in a very interesting book.

R. J. MAGEE

SYMPOSIUM ON TITANIUM

Titanium and its Alloys

Edited by I. I. Kornilov. (Publication No. 10: Investigation of Titanium Alloys.) Translated from the Russian. Pp. vi+386. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1966.) 117s.

THE book presents the translations of the papers delivered at the Second Conference on the Theoretical and Experimental Investigation of Titanium Alloys which was held at Baikov in March 1962. The papers are divided into three groups under the headings "Reaction of Titanium with Other Metals and the Structure of Titanium Alloys" (thirteen papers), "Interaction of Titanium with Gases and Corrosion Properties of Titanium Alloys" (eleven papers) and "Mechanical and Technological Properties of Titanium Alloys" (twenty-three papers).

The first group of papers opens with a review of the crystal structures and the electronic structure of titanium and its alloying behaviour and, on the basis of these data, seeks to develop an alloy theory for titanium. Several papers on phase equilibria in titanium alloys follow. Partial phase diagrams, variously determined by metallographic studies, X-ray diffraction, electrical resistivity and hardness measurements, are presented for the systems titanium-aluminium, titanium-aluminium-molybdenum, titanium-niobium-chromium and titanium-vanadiumniobium-molybdenum, using alloys prepared from iodide titanium (that is, high purity material) and for the systems titanium-aluminium-chromium-iron-silicon and titanium-aluminium-chromium-iron-silicon-boron, using alloys prepared from commercially produced sponge titanium. Particular attention is paid to certain sections through the last system, because these sections form the constitutional basis for the Russians' AT series of commercial alloys. The paper on the titanium-aluminium system contains a critical review of earlier work on this controversial diagram and presents a new version of the diagram. Despite this revision of the diagram, the papers on the polycomponent systems containing aluminium all assume the simple form of the diagram that was proposed initially. One paper presents data on the crystal structures of ternary intermetallic titanium compounds containing a transition element and an element belonging to one of the groups IIIB, IVB or VB of the periodic table. A systematic variation in structure with the group of the third element is demonstrated. Finally, a paper is devoted to the use of a high vacuum dilatometer, to the study of phase changes in titanium and to the cyclic sintering of isostatically compacted titanium.

Despite the fact that the second group of papers is devoted to the interaction of gases with titanium and the corrosion properties of titanium and its alloys, four other papers on these topics are to be found in the third group of papers: these are considered with those in the second group. Altogether, six papers deal with the elevated temperature oxidation of titanium and certain of its alloys. In all cases, formation of scale and oxygen contaminated layers at the surface was observed and the papers record studies of the kinetics of the oxidation processes, the nature of the scales formed and the influence of the gas contaminated surface layer on room temperature mechanical properties. Two papers are devoted to aspects of hydrogen in titanium and its alloys: one to a study of the evolution of hydrogen from metal heated in vacuum and the second to a study of its influence in producing delayed cracking and embrittlement at low temperatures. Five papers on corrosion report the behaviour of titanium and some of its alloys in various corrosive media, including some encountered in the chemical, the pharmaceutical and the food industries, while a single paper reports the tendency of certain alloys to corrosion and stress-corrosion in synthetic industrial media.

The final group of papers deals with a variety of topics,

including the effects of structure and composition on the elastic moduli of titanium alloys, the effects of composition, cold working, annealing and heat treatment on room temperature mechanical properties of both laboratory prepared and commercially produced titanium alloys, stress-rupture characteristics (described throughout the book as "heat resistance"), creep and thermal stability of various alloys, assessment of the suitability of certain alloys for cold rolling and tube production and aspects of the welding, brazing and soldering of the metal and its alloys. The absence of any work on fatigue behaviour is noteworthy.

Finally, the book concludes with a brief summary of the recommendations of the conference with respect to future investigations of titanium alloys and the coordination of such work between research institutes and industry.

Most of the papers are written in a concise manner. The references to earlier work show a surprising familiarity with papers published in the West. Most papers are of the type produced during the development and evaluation of alloys and are largely factual in character and practical in outlook. In the West, this type of work is usually carried out by private companies which treat the results as confidential and, consequently, such work rarely finds its way into scientific and professional journals. Thus, this book is likely to be of most interest to metallurgists and others employed in the development and evaluation of titanium and its alloys. Metallurgical students, however, may find the book of some interest, not for the detailed contents of the papers, but for the insight that can be gained into the type and volume of work involved in the development of alloys-information which is not readily available elsewhere. Following through the many papers dealing with the AT series of alloys from their phase equilibria to the evaluation of their mechanical behaviour could be an instructive exercise.

A serious omission which detracts appreciably from the value of many papers is the failure to give the analyses of the alloys used in the investigations. Furthermore, in a number of papers alloys are referred to only by their technical designations, the specifications for which are not given in the book. In many instances, typical compositions of alloys may be found by reference to other papers in the book, although in a few instances this procedure fails to yield the desired information. The serious reader would do well to equip himself with the list of compositions corresponding to the various specifications before embarking on a study of the papers.

fications before embarking on a study of the papers.

In general, the quality of the translation of the papers that are largely factual in character is good. The quality of translation of the few papers that are discursive in character is less good and in places they are difficult to follow. More careful editing could have avoided this situation. A few persistent misleading mistranslations occur throughout the book: iodide titanium invariably is described as titanium iodide and melting as smelting. The text and line drawings are printed clearly and the photomicrographs and other half-tone diagrams, on the whole, have reproduced well.

R. Haynes

NIOBIUM AND TANTALUM

The Chemistry of Niobium and Tantalum By F. Fairbrother. (Topics in Inorganic and General Chemistry, Monograph 10.) Pp. viii + 243. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 105s.

THIS book contains a wide ranging and up to date appraisal of knowledge of the chemistry of these two metals. In all, 688 references are cited, some as late as mid-1966, but the author has succeeded in presenting a

vast amount of information in a clear, concise, and readable way throughout the book. Full experimental details are omitted and the chemistries of the two elements are described side by side following the precedent set in earlier monographs.

After a short introduction, the author presents a chemist's account of the separation, extraction metallurgy and physical properties of the elements. This is followed by a detailed treatment of compounds of the elements with oxygen, the halogens, hydrogen, carbon, silicon, boron, the elements of group VB, and the remaining chalcogens. The clarity of description in the section on anhydrous binary halides is particularly illuminating. A comprehensive survey of methods available for quantitative analysis of the elements comprises the final chapter and the book ends with a good subject index.

Most of the numerical data on niobium and tantalum compounds have been obtained from X-ray measurements and studies of phase equilibria. The author has assiduously collated and presented these data in numerous tables, making the text a valuable source-book for X-ray workers in the field. Spectroscopists interested in longer wavelengths are referred to the original papers: this is disappointing in view of the small but growing body of information on electronic, infrared and nuclear magnetic resonance spectroscopy, which has been contributed to by theoretical chemists as well as by preparative chemists. Undergraduates and newcomers to the field should, however, have little trouble in grasping a clear picture of the chemistry of the elements from this monograph.

The book contains no important printing errors and is satisfactorily bound. It is distressing to note that the monograph is the most expensive in terms of price per page as well as the most recent in the series, but for libraries and specialists this book is a must.

E. J. F. Ross

PILLARS OF WISDOM?

Seven Solid States

An Introduction to the Chemistry and Physics of Solids. By Walter J. Moore. (The General Chemistry Monograph) (New York and Amsterdam: Series.) Pp. xii + 224. W. A. Benjamin, Inc., 1967.) \$7 cloth; \$2.95 paperback. This short book provides an introduction to solid state physics and chemistry with undergraduate chemists in mind as the most likely readers. Its approach is attractive and original. Seven specific substances (salt, gold, silicon, steel, nickel oxide, ruby and anthracene) are described. one in each of the seven chapters, and their properties used as illustrations of general topics in the solid state. For instance, the chapter on common salt contains an introduction to crystal structure, X-ray diffraction and lattice vibrations. The chapter on gold includes a general discussion of the properties of electrons in metals, and the chapter on ruby outlines the behaviour of transition metal ions in the presence of crystalline electric and magnetic fields. One or two sections seem especially valuable. For example, there is a very useful simple description of the iron-carbon alloy phase diagram in the chapter on steel. which is not easy to find elsewhere. The chapter on silicon contains an adequate basic description of semiconductor properties, the p-n junction and transistor mechanism. There are several useful sections on crystal growing methods, and the chapter on ruby contains a good introduction to the principles of electron spin resonance and maser action.

A great many topics are dealt with in two hundred pages and the treatment is, in many cases, inevitably lacking in depth. Discussion of some material—for example, specific heats of solids—is so brief as to lack plausibility. One is disappointed to find the description of magnetically ordered systems restricted to only three

pages of text. The book is well produced and the diagrams and photographs are particularly attractive. The style is informal and non-mathematical.

As a first introduction to the subject, this book is likely to appeal much more to chemistry than to physics students. The manner in which the solid state is taught in physics departments implies the use of a book in which a more restricted range of topics is treated in greater detail, and in which use can be made of a basic knowledge of thermodynamics, statistical mechanics, and quantum mechanics, which the student will, one hopes, have acquired at an earlier stage. R. E. MEADS

PHOTOCHEMISTRY CONFERENCE

Reactivity of the Photoexcited Organic Molecule (Proceedings of the Thirteenth Conference on Chemistry at the University of Brussels, October 1965.) Pp. ix + 350. (London and New York: Interscience Publishers, a Division of John Wiley and Sons, 1967.) 126s.

It is a pity that this good book is a "conference proceedings". As such, despite the useful and valuable information it contains, the book will be neglected. It will be misplaced in the library, in that it will not be among the current literature; it will be difficult to refer to and it will be tedious to write out the references to the articles which it contains.

This seems to be the fate of all conference proceedings now, with the exception of those published in the normal literature such as Discussions of the Faraday Society. By being produced sometimes under the name of an editor and sometimes not, they inevitably seem to be filed in the wrong places. Quoting articles from them in papers or in reviews is always difficult: for anyone who does not agree, I suggest that they write out for themselves a short form reference of the article contained in this volume by G. Porter on page 79, working from the full reference to the book shown above. I suggest there will be as many versions as people attempting the problem and many of them will be ambiguous and lengthy. A further point is the price of this book, which is high; the publishers seem to know that it will not be seen by too many people.

One solution to the problem would be for the publishers to obtain contracts in the usual way, but to combine together perhaps with the aid of a learned society to produce series of conferences proceedings in, say, physical chemistry, organic chemistry, fluid dynamics, etc. These, while still being produced by various publishers, would be assigned a volume number by agreement so that for example "Conference Proceedings in Physical Chemistry" formed a sensible series. Then one might refer to the article mentioned above as say G. Porter, Conf. Proc. Org. Chem., Volume 000 (1967), page 79. In this way our librarians would both wish to obtain them and know where to file them; they would be easy to reference. The greater circulation would allow the price to be lower and people would be encouraged to buy individual volumes on topics which interest them.

Once again, this is a good book. There are a series of reports by distinguished workers: Coulson, Daudel, Porter, Hammond, Yang, Dauben, Havinga and Schmidt. These range from the theoretical aspects of the subject through reactivity and energy transfer to reports on the organic photochemistry of selected fields including the solid state. Each forms a useful review and in some cases would make a good introduction to the subject for a new graduate student, or someone interested in seeing what organic photochemists are up to. Following each report there is a discussion: as is the custom of the Solvay conferences all the contributors are notable and the questions are to the point.

There are no real deficiencies in the book: it would have been interesting to read the paper by Schenck which is not published but for which the discussion is printed. It is mildly frustrating to find that the French contributors still retain the language of diplomacy rather than using the language of science as everyone else does.

In short, this is a delicious mixture of primer and review, in which the flavour of the field can be tasted. For this reason I am anxious that it should not disappear from sight in the usual way of conference proceedings.

Peter Borrell

CHEMISTRY OF OCEANS

Equilibrium Concepts in Natural Water Systems (Advances in Chemistry Series, No. 67.) Pp. viii + 344. (Washington, D.C.: American Chemical Society, 1967.)

This book, containing the sixteen papers given at an interdisciplinary symposium on the geochemical development of the Earth's natural waters, illustrates clearly the difficulties both of geochemistry and of interdisciplinary symposia. It emerges with credit, but not distinction, from both encounters.

Studies of heterogeneous equilibria involving oxides, carbonates and silicates in aqueous media (of which several accounts are given here) are often impeded by the slowness with which equilibrium is approached. Moreover, an additional degree of freedom is available to colloidal systems in which surface energies contribute significantly to the total Gibbs free energy. In the organic field, the problems are no less severe: photosynthesis causes the accumulation of thermodynamically unstable carbon compounds which may persist for geological timespans. Complications of a higher order are introduced by the possibilities of biological mediation in many other chemical processes.

It is therefore scarcely surprising (despite the title of the volume) that several authors should express careful reservations concerning the relevance of equilibrium thermodynamics to studies of natural water systems. It is hoped, however, that progress can be made by comparing natural systems with calculated equilibrium states of suitable models (or steady states if open-system models are used). This approach acquired much impetus from the pioneering work of L. G. Sillén, who contributes to this volume an account of a clarified method of representing complex equilibria, and illustrates impressively its value in an application of Gibbs' phase rule to a geochemical balance in an eight component ocean model. Other contributions are largely directed towards the provision of basic data for such studies, and outlining the difficulties besetting their interpretation. Two examples are given of successful comparisons between real systems and appropriate models.

Participants at interdisciplinary conferences are menaced by the danger of mutual unintelligibility. This has been admirably overcome in the present instance, for the contributors have evidently been encouraged to remain within a fairly narrow geochemical context. At the other extreme, such a limitation could detract from the value of interdisciplinary exchanges, transforming what should be exciting adventures in cross-fertilization into a kind of scientific incest. This danger has also been avoided, although more narrowly: one might have expected more scope for controversy to be evident in a developing subject

of such complexity.

This book is clearly intended to stimulate further research: its success will be measured by the brevity of its life-span. Its value as an instantaneous picture of current research would have been enhanced by the incorporation of an editorial epilogue synthesizing the various views expressed.

There is an irritating abundance of minor typographical J. M. HAYNES

Applied Science

STATISTICS HANDBOOK

Handbook of Methods of Applied Statistics

By I. M. Chakravarti, R. G. Laha and J. Roy. Vol. 1: Techniques of Computation, Descriptive Methods, and Statistical Inference. Pp. xiv+460. 98s. Vol. 2: Planning of Surveys and Experiments. Pp. x+160. 68s. (Wiley Series in Probability and Mathematical Statistics.) (New York and London: John Wiley and Sons, 1967.)

In the preface to this handbook, the latest addition to the justly famous Wiley series, the authors state their aim—"to bring together under one title most of the essential techniques of quantitative inference"—and describe their method of achieving this aim—"In each part, every basic statistical method is described in detail with a precise explanation of its theoretical foundation. Then the computational procedure is discussed and illustrated by numerical examples completely worked out. These are followed by a large collection of selected and graded exercises". These are ambitious claims and in the main they are well realized.

This pattern of theoretical discussion, description of method and worked example enables the authors to be very informative about an immense range of topics, some of which require considerable sophistication on the part of the reader. Practically every basic statistical technique is thus presented, the only notable omissions being decision theory methods and spectrum analysis, although some topics such as the method of steepest ascent and quality control and sampling inspection schemes receive a more brief treatment. The organization of the material permits easy reference to particular topics, although bold faced type in place of italics for subsection headings would have facilitated this still more. Except in the second part of the first volume, the theoretical discussions are informative and sound and the methods suggested are varied and appropriate. By and large, one can only complain of relatively trivial errors of omission. Throughout the second part of the first volume, however, population and sample are confused both in the text and in the notation, a fault that in a work of less overall value would in my opinion damn the work completely. Fortunately, the third part, on statistical inference, appears to have been written by a different member of the team of authors and there, for the reader who gets that far, the misconceptions of the second part will be corrected.

The extensive collections of exercises are built on real data and indicate strikingly the many branches of science and technology in which statistical methods are applied. Unfortunately no solutions appear to be available. There are a few proof-reading errors, most of them obvious, although in the first volume the failure to use the bold-faced 'e' for the unit vector on page 412 makes possible its confusion with 'e' which is the number of degrees of freedom on page 417, and there is some misprint in the definition of convergence in probability on page 256. The index is, for a reference work of this type, only just adequate. No tables are given but references are given for most of the standard tables. References at the ends of chapters to books in which could be found formal proofs of the points made in the theoretical discussions would have been a valuable addition to this generally very useful compendium of statistical theory and method.

Despite these minor points and the more serious confusion in the second part, this handbook is a worthy

addition to the Wiley series and a pleasant reminder of the flair for statistics so frequently displayed by Indians.

M. E. Solari

LASER INFORMATION

Laser Systems and Applications

By H. A. Elion, with a foreword by T. H. Maiman. Pp. xi+624. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 147s. net.

Of the 624 pages in this book, 353 are devoted to a NASA special bibliography on lasers classified by subject matter and authors. Only the NASA accession numbers are given and no reference is made to the open literature. Presumably the buyer is paying nearly four guineas for this bibliography. This scarcely seems a good buy even for readers who have easy access to the eleven Federal Regional Technical Report Centres listed on page 607. More useful bibliographies are regularly published in the Journal of the Optical Society of America and the IEEE Journal of Quantum Electronics.

A further fifty pages are devoted to appendix 2 describing simple experiments in physical optics using gas laser beams. While this appendix would be a very useful guide for introducing gas lasers into sixth form teaching laboratories, it seems somewhat out of place in the present volume.

The remainder of the book is a readable, well illustrated account of the state of the art of applying lasers to a variety of problems. These include welding and machining; chemical and biological applications; holography and spectroscopy; ranging, surveying and metrology; communications and optical data processing and display. Little demand is made on specialized technical knowledge and mathematical formulae are kept to a minimum.

The notes on health hazards are sensible if brief. The tables of physical constants and characteristics of various types of lasers contain by far the most useful information in the book.

D. J. Bradley

COMPUTERS ADVANCE

Advances in Computers

Vol. 8. By Franz L. Alt and Morris Rubinoff. Pp. xii + 345. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 116s.

Advances in Computers has now reached the eighth volume. Over the years, the editors have given us a series of timely and authoritative articles on subjects of current interest in the computer field. This volume lives well up to the standard that has been set in the past.

standard that has been set in the past.

The first article, by Thomas N. Pyke, of the National Bureau of Standards, is on time-shared computer systems. The author defines time sharing as the simultaneous access to a computer system by a number of independent users. Pyke has obviously been in a position to follow closely the developments he describes, and the article can be strongly recommended to experts and non-experts alike.

Jean E. Sammet of IBM writes on formula manipulation by computer. She gives a good summary of the various systems that have been developed for doing algebraic manipulation of a formal kind (including dif-

ferentiation and integration) on a computer. A bias towards FORMAC may be forgiven her.

Tom Steel of the System Development Corporation complains, in an article on standards for computers and information processing, that this topic is apt to induce disinterest, boredom and apathy in the reader. He might have added active opposition (have we not heard it said that standardization is not putting right the last fellow's mistakes?). Steel seeks to arouse at least a fleeting fascination for the subject by sketching the history of standardization and its influence on society. He goes on to describe the way in which United States standards are evolved and the various agencies that are concerned. The topic to which he gives most attention is that of standardization of programming languages, a field which he is too modest to point out owes a good deal to his personal efforts.

Dr Naomi Sager of the Institute for Computer Research in the Humanities of New York University contributes an article on the syntactic analysis of natural language. She discusses various methods for grammatical analysis of sentences and gives an example of her own approach to

the problem.

The two final papers are more in the nature of research contributions than expository articles. R. Narasimhan of the Tata Institute of Fundamental Research in Bombay presents a unified metatheory of programming languages and computers. This is a theory of the way in which programming languages may be described, and these turn out to be the same as the ways in which computers themselves can be described. The last paper is by L. A. Lombardi of the University of Rome, and is entitled "Incremental Computation". Lombardi outlines a language which accepts problems whose formulation is still incomplete and for which additional information is supplied in instalments. This paper is to be viewed as a contribution to the new type of thinking that is becoming necessary now that we are beginning to achieve what Licklider (quoted by Pyke in the first article in the volume) has termed mancomputer symbiosis. M. V. WILKES

POLISHING METALS

Metallographic Polishing by Mechanical Methods By L. E. Samuels. (Metallography Series.) Pp. x+195. (London and Melbourne: Sir Isaac Pitman and Sons, Ltd., 1967.) 70s. net.

METALLOGRAPHY is concerned with those crystalline features that manifest themselves in metals at microscopical level, and their significance in respect of the ultimate metallic properties. Conventional metallography primarily utilizes the optical microscope, using the greater resolving power of the electron microscope as a supplement to elucidate where appropriate the finer details of structure.

Metallographic examination of a metal involves, on one hand, the proper preparation of the samples and, on the other, their critical examination under the microscope. Clearly, the success of the latter depends to a large extent on the efficiency of the preparation stage. In recent metallography, two names stand out as having made distinctive contributions to the techniques of preparation. The first is that of the late P. A. Jacquet and the other is that of the author of the present book.

L. E. Samuels cannot aspire to such eminence as Jacquet, who made such unique contributions to electrolytic polishing. Mechanical polishing in one form or another has been with us ever since the science of metallography evolved. Even diamond polishing, with which Samuels's name is associated, was first used by a number of other workers. Samuels's claim to fame is that, from specialized applications, he introduced diamond polishing as the universal technique and, indeed, he took most of the art out of mechanical polishing for optical micro-

scopy. He pioneered the use of a paste carrier rather than a liquid. He studied the variables, and described the optimum conditions. Moreover, he developed confidence both by the superb results obtained in his own work, and by analysing the behaviour of the underlying metal surface during the polishing action. These results have been described in a series of useful papers, but it is fitting and beneficial that he should now incorporate much of his experience into a book.

It might at first be doubted whether the subject of mechanical polishing justifies a book on its own account. Examination of the contents of this volume, however, will soon dispel such doubt, although in my opinion the work would have been better and of wider value had it incorporated at least a general introductory account of etching.

The author deals with sectioning, machining and abrasion, with mounting of specimens, and with their polishing, including the use of fine oxide powders for post-diamond finishing. He analyses the fundamental principles as well as describing the actual practice. Very detailed or subsidiary aspects of preparation are dealt with in a number of appendices. These include such useful items as the manufacture of a conducting plastic, special lap materials, levigation of alumina, diamond paste preparation, factors controlling the resonance of an electromagnetic vibratory polisher, and electroplating methods for edge protection of specimens. In addition, there is a valuable chapter comparing electrolytic with mechanical polishing. The text is illustrated throughout by excellent photomicrographs selected largely from the author's own investigations.

In all, this is a good book, clearly written by an expert, and it will be of great value to all concerned with metallography.

A. R. Bailey

WORK AND HEAT

Engineering Thermodynamics

Work and Heat Transfer (SI Units). By G. F. C. Rogers and Y. R. Mayhew. Second edition. Pp. xii + 662. (London: Longmans, Green and Co., Ltd., 1967.) 60s. net.

The new edition, in SI units, follows the same successful layout as the first edition in Btu. The primary need for this new edition springs from the declared national policy of changing to the metric system. The authors have gone all the way and chosen what is known as a coherent system. That is to say, they have abandoned the general relationship p=kma in favour of the specific relationship p=ma. The fundamental units used are kilogramme, metre, and second, for mass, length, and time, with a derived unit kg.m/s² or newton for force. This is the system that will generally be used in scientific work and one can only hope that industry will follow suit.

The book contains all that was best in the first edition. but the authors have brought the work more up to date by some additions. They have, for instance, introduced the concept of availability in closed and open systems. Also, they have gone further than previously in their discussion of the use of the Gibbs function. Latent enthalpies of fusion and of evaporation are studied in greater detail.

The authors, in the new edition, discuss briefly some of the consequences arising from the application of nuclear energy sources to steam cycles, such as restrictions on the maximum permissible temperature that fuel elements are allowed to reach, and from the uses of a secondary fluid to transfer energy from the elements to the steam.

Combustion theory has been newly set in its correct light and is shown to be a particular class of chemical reactions in general. Into the chapters dealing with the internal combustion engine the Wankel engine has been introduced with illustrations and adequate description. A whole new chapter on direct conversion covers thermionic and

thermoelectric converters, magnetohydrodynamic generators and fuel cells.

In the chapters on heat transfer, the conduction section has been enlarged, and the convection section deals in much more detail with the mechanics of boiling. All these additions are considerable improvements.

The printing of the new edition appears to be slightly smaller and better in this newest edition; the lines are a little farther apart and the pages larger. The whole effect is pleasing and inviting to the reader. The number of

pages has increased from 559 to 601.

The book is the best general textbook for undergraduates learning thermodynamics to degree level and is a very useful reference book for graduates working in other fields. The authors have struck a relationship between the purely scientific and the practical that seems to suit the engineering academic very well.

Russell Hoyle

JOURNAL FOR MATHEMATICIANS

Journal of Engineering Mathematics

Vol. 1, Number 1 (January 1967). Pp. 86. (Groningen: P. Noordhoff, Ltd., 1967.) Subscription per volume \$16.50, postage included.

THE work in applied mathematics in the many outstanding educational and research institutions in the Netherlands is of great importance in both volume and quality, and it is good to see that this work will in future be readily available in a convenient form through the Journal of Engineering Mathematics, being published on a quarterly basis from April 1967 onwards. Although this is almost entirely a Netherlands initiative in respect of publishers, editors and authors of the numbers so far available, papers will be published exclusively in English (preferred). German and French.

It is also good to see the emphasis on application of the mathematics to practical problems in the engineering indus try made both in the journal's title and in the statement of policy on the inside cover, which reads: "The aim of the journal is to promote the application of mathematics to engineering problems and to stress the intrinsic unity of the fundamental problems of different branches of engineering varying from basic principles of physics and mechanics to management and computer science."

These aims are very close to those of the three year old Institute of Mathematics and its Applications in the United Kingdom. In both cases they stem from the need to help the growing body of applied mathematicians in industry and gove.nment (in graduates alone, more than four thousand in the United Kingdom and increasing at 5 per cent per annum), and those in universities and technical colleges, to become more creatively aware of each other's work: especially, it is desirable that the best modern mathematical techniques and principles for applying them be made known to those in industry, and, also, that the researches of those in academic life become closely related to the needs of industry and government, including, of course, their long-term needs.

Journals like the Netherlands Journal of Engineering

Journals like the Netherlands Journal of Engineering Mathematics or the British Journal of the Institute of Mathematics and its Applications can contribute valuably to all this in three ways. First, they can, and should whenever possible, elicit papers from mathematicians working in industry, which will make known characteristic types of mathematical problems with which industry is faced. Secondly, they should encourage applied mathematicians in academic life to submit papers in which the work's practical aims and relevance are clearly set out. Thirdly, they should seek from all appropriate quarters survey papers which will indicate the state of the art in particular (and especially the newer) fields of application of mathematics, or in particular areas of mathematical technique.

There is inevitably a transition while such a journal is getting under way, during which some of the older type of "academic" applied mathematics gets published. Before the journal becomes well known, there are problems of filling up numbers, which militate against rigorous selection from among the papers submitted. Experience with the Journal of the Institute of Mathematics and its Applications shows, however, that provided a substantial number of papers of the right kind are included from the outset, it is increasingly possible, by soliciting both appropriate surveys and also papers from mathematicians in industry and government, to build up a journal that can be more and more of an influence for the creative application of mathematics to the practical problems of industry.

It is evident from the first two numbers of the Journal of Engineering Mathematics, edited by Dr H. W. Hoogstraten of the Technological University of Delft, that the journal will indeed develop along these lines. Problems treated have been chosen not because the mathematics are "elegant", but because they will give the centring force between the rotor and stator coils of an electrical machine at each offset position, or because they will indicate limiting factors on the hydrological performance of water supply systems in one of the Frisian islands, and the results are given in appropriate numerical and graphical detail. There are also good papers from noted Netherlands schools of the mechanics of fluids and of solids.

At the same time, it will in addition be necessary to seek out papers from those who are applying mathematics in industry itself. Wherever possible, it should be made clear, furthermore, that papers from academic institutions should show unambiguously the practical significance of the work submitted. In these conditions, the journal should develop into one of great value to those all over the world engaged in the application of mathematics to the problems of engineering industry.

M. J. LIGHTHILL

OPERATIONS RESEARCH

Introduction to Operations Research

By Frederick S. Hillier and Gerald J. Lieberman. (Holden-Day Series in Industrial Engineering and Management Sciences.) Pp. x+639. (San Francisco and London: Holden-Day, Inc., 1967.) \$13.75.

THE techniques of operations research belong in the main to the mathematical sciences; the practice of these techniques is an experimental science. In some subjects with similar characteristics there is abundant material for laboratory exercises of all grades of difficulty to amplify and illustrate the various stages of the theoretical teaching, and these practical exercises can be presented concurrently with the formal teaching. In fields in which the experimental material is derived from case studies from life the student needs familiarity with, if not mastery of, a sufficient number of techniques to gain full benefit from the practical study. Operations research is predominantly of the latter kind and texts are necessary to present the basic methodology.

Any author of such a text has a wide choice of the amount of mathematical preparation and degree of sophistication he will require of his readers. Professors Hillier and Lieberman have purposely made slight demands in their introductory book; they ask for some familiarity with elementary calculus and for the level of mathematical maturity which usually accompanies such knowledge. In all but a few places (notably in the last section in more advanced topics in mathematical programming) the mathematics is elementary; proofs are often omitted or outlined, there is no insistence on rigour and space is given instead to illustrations of the implications of the results. This approach has much to commend it; the students reading books on operations research are likely to have had little previous contact with the

subject and less experience of it at work. At this stage it is, I believe, more important that the student gains an understanding of the results of the mathematics than that he should be able to derive these results. In the former case, motivation for study of the mathematical details and methods can be established, while in the latter a barrier may be encountered which is never surmounted. A vital part of the understanding of the mathematical results lies in an appreciation of magnitudes when the symbols of the general case give way to the numbers of the particular. Any student can (or should be able to) substitute and evaluate an expression; unfortunately few will do so unless pressed and fewer still will make the immediate approximate calculations which can give the user the "feel" of a formula. The authors do not lose sight of the numerical aspect and many of their examples require calculation as well as algebraic manipulation. Of course, the examples are meant to illustrate the methods; perhaps the numbers are chosen nicely in order to avoid burdening those with less splendid and available automatic computing facilities than at Stanford. Should we not, however, be nearing the time when the embryo scientist can be expected to have a basic knowledge of computer use, ready access to a machine, as well as his grounding in mathematics? When we can assume this—and may it be soon in Britain—the numbers in problems can become realistic and with them the student's approach will become closer to the practitioner's.

Nearly half of the book deals with mathematical programming, introducing some network theory and applications, game theory and dynamic programming, as well as linear, integer and non-linear programming. About the same proportion of the book is devoted to probabilistic models—congestion, inventory, Markov chains and simulation—and a section giving preparation in probability and statistics which can be omitted by those who do not need it. A welcome feature is the repeated questioning whether the model or analysis is appropriate, sometimes with vivid examples (for example, "the result says, for example, that there should only be one rest room, albeit a large one, in the entire Pentagon! So what is wrong with model 3a?"). The mathematics is indubitable and inapplicable.

The book is long; a teacher may have a preference for minor changes in the contents and some will continue to seek satisfactory ways of obtaining and presenting problems in their raw, unformulated state, but the approach to the introductory teaching of operations research techniques will meet with the approval of many.

E. S. Page

SOIL, A LIVING TISSUE

Soil Biochemistry

Edited by A. D. McLaren and G. H. Peterson. Pp. xiii+509. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1967.) 180s. net.

There can be no doubt that the biochemistry of soil, whether of this world or the next, constitutes one of the most important fields of research of today. This book provides an up to date review of the most outstanding aspects of terrestrial soil, its organisms and living constituents and their interactions. It also gives a brief and tantalizing guide for the assessing of possible organic constituents of the surface material of planets. Under the expert editorship of A. D. McLaren and G. H. Peterson, twenty-three international experts, mostly American, have written specialist chapters. The editors themselves provide an interesting introductory chapter of the biochemistry of terrestrial soils indicating the nature of soil constituents and processes and describing the interactions of living species associative and antagonistic in the soil. The book is divided into three parts: (1) the isolation

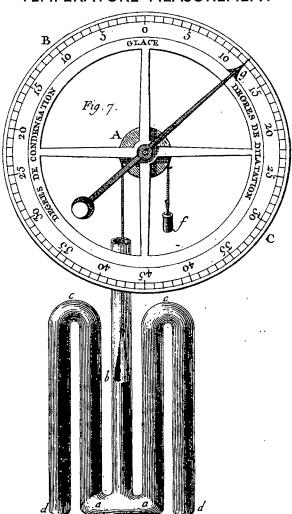
and characterization of soil biochemicals; (2) the metabolism of soil biochemicals; and (3) relationships between soil and microbes. In the first part the organic substances dealt with are general nitrogenous compounds, nucleic acids, carbohydrates, organic acids and free radicals. There is little novelty among the various compounds beyond the fact that they are all clearly constituents of soil. The account of the stable free radicals, such as humic acid in the soil, is timely and interesting. Future studies using electron paramagnetic resonance on the complex soil macro-molecules may throw some light on their complex molecular structure.

The second part covers energy relationships, the biochemistry of the nitrogen cycle, organic sulphates and phosphates, lignin and humic acids and phenolic compounds. Two more important chapters describe the decomposition of herbicides and the degradation of detergents in soils. Concise descriptions are given of various soil enzymes.

The third part discusses the microbiological and biochemical aspects of the rhizosphere, which is the zone of soil in which plant roots exert their influence. All the chapters are written clearly and in great detail, providing a wealth of information with full references. The book will prove of the greatest value to advanced students and to all chemists and biologists.

M. STACEY

TEMPERATURE MEASUREMENT



Berthond's dial thermometer, 1763. From A History of the Thermometer and Its Uses in Meteorology, by W. E. Knowles Middleton. (Johns Hopkins Press; Oxford University Press; 80s.)

(Continued from page 880)

Table 1. CONCENTRATIONS OF NICKEL, GALLIUM, GERMANIUM AND IRIDIUM

Meteorite	Nickel	Gallium	Germanium	Iridium
	(per cent)	(p.p.m.)	(p.p.m.)	(p.p.m.)
Boxhole	7·64	18·1	$\begin{array}{c} 37 \cdot 2 \\ 34 \cdot 2 \\ 37 \cdot 3 \end{array}$	9·1
Henbury	7·44	17·4		15·0
Wolf Creek	9·23	18·4		0·040

Wasson and Goldstein³, so it would have been especially interesting if a relationship were found between two or more of these objects.

Recent work has shown that detailed studies of the chemical composition of iron meteorites can be used to classify these objects into chemical groups, and in addition to define the relationship of the meteorite within a particular group3-5. Studies of the Chilean hexahedrites3 and of the Arizona octahedrites have provided strong evidence that fragments of the same fall tend to be extremely similar in composition. Thus a study of the three Australian irons offered the possibility of testing for compositional indications of a relationship among them.

We have found that most medium octahedrites fall into one or the other of two closely related groups designated IIIA and IIIB. Group IIIA includes irons with 7.4-8.7 per cent nickel, 18-22 p.p.m. gallium, 33-46 p.p.m. germanium, 0.4-17 p.p.m. iridium and structures intermediate between medium and coarse octahedrites. Group IIIB includes objects with 9.2-10.7 per cent nickel, 16-20 p.p.m. gallium, 28-38 p.p.m. germanium, 0.01-0.1 p.p.m. iridium and structures ranging from medium to fine octahedrites. Gallium and germanium are positively correlated with nickel in group IIIA and negatively correlated with nickel in group IIIB. The correlation of iridium with nickel is negative in group IIIA and not yet defined in group IIIB. Nickel has been determined by atomic absorption, and gallium, germanium and iridium by neutron activation.

Table 1 shows the results of duplicate determinations of the four elements in a specimen from Wolf Creek, provided by S. R. Taylor, and in specimens from Boxhole and Henbury, from the Leonard Collection of meteorites at this university. Nickel, gallium and germanium results for the latter two objects are essentially the same as those reported earlier4. Each iron is observed to be unique in composition. There is some overlap in the gallium and germanium contents, but the differences in nickel and iridium concentration are substantially greater than experimental errors. 95 per cent confidence limits are about 1.5 per cent of the reported nickel concentrations, about 10 per cent of the higher iridium values. The Wolf Creek iridium result may be in error by as much as 30 per cent. Boxhole and Henbury are found to be typical low nickel members of group IIIA, whereas Wolf Creek is a typical low nickel member of group IIIB.

This evidence clearly indicates that each of these craters was formed by a unique event. A simple calculation shows that the likelihood of three such events occurring in succession is not unreasonably small. probability of the Earth capturing crater-forming irons of a given structural class is given by the fall statistics cited here for small objects, the probability of three successive cratering events by medium octahedrites is $(0.36)^3$, or a respectable 0.05. The probability is even higher if it is considered that the first event was classestablishing and should be considered separately.

JOHN T. WASSON

Department of Chemistry and Institute of Geophysics and Planetary Physics, University of California, Los Angeles.

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Diffracted S

Gutenberg and Richter¹ commented in 1935 that diffracted S (the S wave diffracted around the Earth's core) was recorded more frequently than diffracted P, but they provided no observational data. To our knowledge, the only reference to observations of S beyond the shadow zone since that time was made by Lehmann², who expressed doubt (on the score of amplitude) whether this or the corresponding P phase were really diffracted waves. Again, no details of arrival times were given. The scant attention paid to diffracted S may be attributed to the fact that it is not listed in the standard travel time tables and consequently is not reported in station bulletins. On the other hand, observations of diffracted P are common, because it appears on seismograms as the first arrival at distances beyond about 100°.

Duwalo and Jacobs3, in a theoretical study of diffraction around a liquid core, have shown that SH waves can be diffracted around the core boundary. At the same time, they pointed out that a diffracted SV wave is not possible, because no shadow zone exists between S and SKS. This provides a criterion for the identification of diffracted S, because core phases such as SKS and SKKS must emerge as SV waves4.

On a number of occasions we have observed, on Canberra records, a phase which satisfies this criterion from earthquakes at distances between 99° and 130°. The phase is detected on long period Press-Ewing horizontal seismometers ($T_s=30$ sec, $T_g=100$ sec) as a single-cycle wave with a period of about 20 sec. It occurs about midway between SKKS and PS, where no other arrival of S type is expected, and can be readily identified in routine analysis of the seismograms.

Table 1 gives details of the earthquakes and the observed travel times, based on information from USCGS PDE cards. The first two events, both from Pakistan, illustrate a method of determining the distance at which S grazes the core-mantle boundary. The particle motion of S from the event at 98.8° distance is unpolarized, while for the other, at 99.6°, it is strongly polarized in the SH direction. This implies that the change from a to unfracted S takes place between these two distances, although further data are needed for verification of the result. No comparable method exists for determining the change from P to diffracted P.

Records of three events included in the table are shown in Fig. 1. The SH nature of the phase can be seen most clearly in the arrivals from Mexico and Panama. Waves from these earthquakes approach Canberra from azimuths which are almost due east. In both cases, the diffracted S arrival appears on the N.-S. seismogram, while the core phases are present on the E.-W. records. Lehmann² likewise noted that two Chilean earthquakes at distances of 106° and 116° and azimuths W.S.W. from Copenhagen gave an S phase visible only on the N.-S. record at Copenhagen. A striking feature of the Panama records is the large amplitude of diffracted S relative to the other phases. We surmise that the fault motion was at right

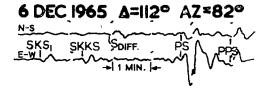
Table 1

Date	Origin time (G.M.T.)	Location	Epi- central distance (deg)	Depth (km)	Magni- tude	tra	yel ne sec
Feb. 7,66	04 26 14	Pakistan	98.8	33	6.0	25	10
Aug. 1,66	21 03 00	Pakistan	99-6	33	6-2	25	18
Dec. 22,65	19 41 23	Kodiak Is.	105-1	50	6.5	26	03
Dec. 6,65	11 34 54	Mexico	111.8	37	6.0	27	06
Mar. 20,66	01 42 50	Uganda	113.6	36	6-1	27	36
Dec. 9,65	06 07 49	Mexico	116.8	57	6.0	27	39
Feb. 15,67	16 11 12	Peru-	121.8	597	6.2	28	34
100. 10,0,	10 11 12	Brazil	1210		V -		٠.
Sept. 17, 65	13 13 56	Ecuador	123-2	190	6.0	28	14
Sept. 9,65	10 02 25	Central	123.3	27	5.9	28	59
p v,		America			• •		
Aug. 19,66	12 22 10	Turkey	123.5	26	6-1	28	40
Dec. 15.65	23 05 21	S. of	125.6	15	6.0	29	19
200. 10,00	20 JU 11	Panama		-0			~~
Feb. 9.67	15 24 47	Colombia	128-1	58	6.3	29	07

angles to the direction of Canberra, so that very little P or SV energy was transmitted in this direction.

In Fig. 2, the Jeffreys-Bullen travel time curves are compared with the observed times of diffracted S, SKS1, SKKS, PS and PPS, after corrections for focal depth have been applied. The scatter in the diffracted S readings is similar to that of SKS_1 and SKKS. The readings tend to be about 10 sec later than the predicted times, except for the higher-amplitude PS phase. This may only mean that in most cases the first half-cycle of motion was too weak to be identified with certainty.

The least-squares regression line from the diffracted Sdata has a slope of 8.61 sec/deg, with a standard error of 0.28 sec/deg. The Jeffreys-Bullen S curve has a constant slope of 8.33 sec/deg between 100° and 107°. Records of the events at a number of stations in line with the epicentre could be used to improve the accuracy of the estimate, and to investigate the variation of amplitude with distance, as Sacks has done for diffracted P (refs. 5





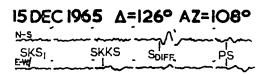


Fig. 1. Seismometer records of three events; in Mexico, Peru-Brazil and Panama.

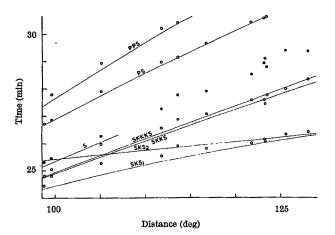


Fig. 2. The Jeffreys-Bullen travel time curves compared with the observed times of diffracted S, SKS, SKKS, PS and PPS, after corrections for focal depth have been applied.

and 6). The spectral analysis techniques described by Alexander and Phinney in their studies of diffracted P (refs. 7 and 8) could be applied to the diffracted S pulse as well, to obtain a more complete picture of the structure at the base of the mantle.

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Pyroclastic Rocks of the White Mountain Magma Series, New Hampshire

SILICIC volcanic rocks (Moat volcanics) of the White Mountain magma series, which crop out in the Moat Mountain and Mount Kearsarge (Pequawket) areas of northern New Hampshire, USA^{1,2}, are dominantly of pyroclastic origin. The comendites² of these areas are chiefly, if not almost entirely, phenocryst-rich, lithic-poor to moderately lithic-rich tuffs. Most of the tuffs seem to have been densely to moderately welded and probably many, if not most, are of ash-flow origin.

The largest and best exposed body of volcanic material of the White Mountain series is located in the Moat Mountain area immediately north-west of Conway, New Hampshire. Our recent field examination of many of the more accessible outcrops on the Moat Mountain block, supplemented by a thin-section study of geographically and lithologically representative specimens, has revealed that the phenocrysts—chiefly sanidine (now unmixed on a microscopic scale) and quartz—in the comendites are highly fractured. The larger (1 to more than 3 mm) crystal grains commonly show one or more crystal faces, and are unquestionably the fractured remnants of originally euhedral phenocrysts. Of subequal total volume, however, are smaller (1 to less than 0.1 mm) angular crystal grains typically bounded on all sides by generally curved fracture surfaces. There seems little question that nearly all of these smaller grains were originally parts of larger (greater than 3 mm) euhedral phenocrysts. This ubiquitous breakage of intratelluric crystals, usually typical of pyroclastic rocks and presumably resulting from the rapidity and violence of eruption and transport, is probably the strongest single line of evidence for a pyroclastic origin.

Usually indistinct, but unquestionable, eutaxitic (collapsed pumice) structure is visible at a number of localities from the south flank of South Moat Mountain to the north flank of North Moat Mountain and has also been observed in Dry Brook at approximately 1,600 ft. elevation. Numerous boulders showing well preserved eutaxitic structure are also present in the channel of Dry Brook.

The thick (greater than 10,000 ft.) sequence of silicic Moat volcanics in the Moat Mountain area shows pronounced and laterally persistent large-scale layering2. This layering suggests more a sequence composed predominantly of pyroclastic rocks, and particularly of ashflow cooling units, than a thick pile of silicic lavas, which typically form non-stratiform domes, plugs and dikes and relatively irregular and areally restricted flows.

Most of the comendites in the Moat Mountain area contain from 1 per cent to more than 5 per cent of lithic fragments, both of apparently cogenetic volcanic material and of country rock. Although suggesting a pyroclastic origin, the lithic-fragment criterion must here be used with caution because lithic fragments are found in certain apparently non-particulate rocks of the White Mountain magma series.

Although certain eutaxitic structure or large-scale layering has not yet been observed in the comendites exposed on the flanks of Bartlett Mountain in the Mount Kearsarge area, the highly fractured nature of the abundant phenocrystic material in these rocks is strongly suggestive of a pyroclastic origin. The lithic-free to lithicpoor comendites are indistinguishable petrographically from the matrix material of the striking lithic-rich Pequawket breccia, which crops out on the east side of Bartlett Mountain and on Mount Kearsarge. Moreover, there seems to be a continuum of lithologies from lithicpoor comendite to very lithic-rich Pequawket breccia, suggesting that the breccia is largely subaerially deposited pyroclastic material rather than event breccia.

The Moat volcanics are closely associated in space and time with the mineralogically and chemically similar ring dike complexes of the White Mountain magma series¹⁻⁶. The chief bodies of Moat volcanics are partially or completely surrounded by ring dikes and are structurally low with respect to pre-White Mountain basement rocks^{1,2}. The field relations can reasonably be explained either by subsidence of blocks of Moat volcanics along arcuate fault planes now more or less reflected by the ring dikes, by deposition of the Moat volcanics within equant topographic depressions produced by earlier subsidence or by some combination of these two mechanisms. Furthermore, this structural association of at least locally very thick volcanic piles of predominantly pyroclastic origin is consistent with the interpretation (for example, refs. 7-13) of at least some ring dike complexes and cauldron subsidence structures being the lower-level manifestations of main vent areas of the collapse caldera type from which immense volumes of pyroclastic material have been erupted. Such an interpretation is very likely applicable to the White Mountain area. If so, the silicic volcanics, now preserved, are, almost assuredly, only an extremely small fraction of the total volume of pyroclastic material erupted during the activity of the White Mountain province.

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PHYSICS

Tracks in Mica caused by Electron

Muons may leave detectable tracks in muscovite mica under suitable conditions such as those found deep underground after the formation of the mica crystals in pegmatites1. The momentum spectrum of the muons, as determined by scattering measurements, indicates the presence of muons of high momentum (>10 GeV/c). It is therefore natural to try to find the products of electromagnetic interactions of the muons, in particular the occurrence of electron showers.

It is well known that high energy electrons show minimum specific ionization and therefore tracks of weaker intensity than those caused by muons are expected. Indeed, there was no prior reason to suppose that electrons could leave detectable tracks in mica. Because ionization occurs principally by delta ray formation, however, it is not unreasonable to assume that at localized regions along the track of an electron the specific ionization could be sufficiently above average to produce a nucleation centre. These infrequent centres would then be decorated by the exolving iron during the sensitive phase of the mica crystal. The resulting track would take the form of a random sequence of decorated nucleation centres showing no obvious correlation other than being approximately rectilinear. For the track of an electron to be detectable. however, there must be a sufficient number of such decorated centres within a radiation length of mica to be noticeable above the background. For high energy muons the distance between nucleation centres is of the order of microns while the radiation length in mica is about 10 cm or 105μ, which suggests that there is considerable latitude in the possible mean spacing of nucleation centres which would result in a recognizable track.

An additional factor in the possible detection of electron tracks is the relatively large extent of multiple scattering. which, together with the known restriction in angle perpendicular to the (001) plane for the recording of tracks, suggests that the track of an electron would be split up into a series of short sections. Whenever the track lies within the detection range in angle and the specific ionization is sufficiently high, then a section of track might be recorded. The combination of these two factors suggests that the spacing between decorated centres might well be several orders of magnitude greater than for muon tracks, possibly about 1 mm or 103 µ.

The existence of a restriction on the angle of a track for it to be recorded out of the (001) plane should not be a limitation in the detection of an electron shower because of the relatively large range of angles of tracks of electrons in a shower as a result of both the opening angle in pair formation and the effect of multiple scattering. Indeed, it might be fortuitous in preventing overcrowding of tracks. In consequence, tracks caused by an electron shower should exhibit a definite correlation in direction throughout an appreciable thickness of mica in a direction perpendicular to both the (001) plane and the axis of the shower. Such behaviour would be in sharp contrast to that of the tracks of muons from neutrino interactions deep underground.

A search was made for evidence of electron showers in a quantity of muscovite mica which showed tracks of muons. The mica, of commercial S.D.B. type, came from both India and Africa. Definite evidence for the occurrence of electron showers was found and is summarized here.

Many rectilinear sequences of decoration centres were found which showed a spread in angle in the (001) plane of a few degrees, were confined closely to the (001) plane and showed correlation of direction. Such arrays were also observed to show correlation of direction throughout appreciable thickness, t, of the blocks examined, as indicated in Fig. 1. Observations were restricted to specimens showing no structural damage and little internal decoration.

In Fig. 2, the length distribution of tracks in several blocks of mica, which extended over a thickness totalling more than 1 cm of crystal, is shown. The median of the distribution is about 6.3 cm. Because the root mean square value of the atomic number for muscovite is about 10.0, the radiation length is roughly 10.6 cm, which implies that the most probable length of track before a radiative collision occurs is about 7.8 cm. Multiple scattering will tend to shorten the observable length of a track and so the observed median is in good agreement with the predicted length.

Measurements have been made on the spacing between decoration centres and yield a median value of 2,200μ. It is instructive to plot the results in order to show how the number of gaps of length equal to or greater than a given length vary with the length, for it has been shown² that in emulsions there is an exponential relationship for a given specific ionization. Because the phenomenon under

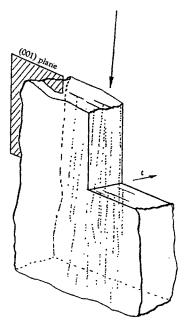


Fig. 1. Diagram showing the penetration of an electron shower into a crystal over a considerable thickness t.

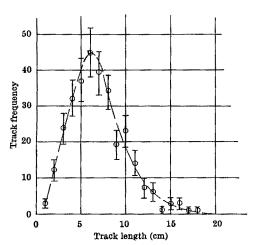


Fig. 2. Length distribution of the electron tracks.

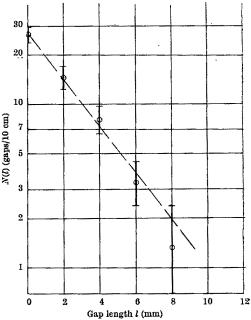


Fig. 3. A plot of the number of gaps of length $\geqslant l$ as a function of l.



Fig. 4. Photograph of part of the tracks of an electron shower in muscovite mica.

lying detection of particles in both emulsions and mica is believed to be similar, in that it is mediated by delta rays, it is reasonable to expect a similar behaviour. The results are shown in Fig. 3 and clearly support this hypothesis.

In Fig. 4 a photograph of part of a shower is shown. It is evident that the coarseness of the decoration prevents measurements of the energy of an electron from the multiple scattering of its track, but this might be estimated from the longitudinal or lateral extent of the observed shower³.

Because mica crystals are comparable in size with the lateral extent of showers the dominant factor in the detection efficiency is the angular spread of tracks within a shower as opposed to the high angular selectivity imposed for individual tracks. This enhanced efficiency of detection for showers could be useful in permitting a relative study of the high energy region of the muon spectrum, as the probability of an electromagnetic interaction of a muon as a function of energy is known⁴. A preliminary study indicates that the average probability for shower production is less than I per cent per muon, indicating a relatively low mean energy for the muons ($\ll 100 \text{ GeV}$).

(≪100 GeV).

With regard to the origin of the observed electron showers, it is relevant that the tracks of muons occurring in the same mica showed no definite evidence of a cos⁰0-type dependence on relative orientation, suggesting a predominantly neutrino induced muon origin. The implied

low mean energy of the muons is also consistent with a neutrino-muon origin for the showers.

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Order-Disorder Transformation in Potassium Graphite

During an X-ray diffraction investigation of the potassium graphites¹, we concluded that the reduction in potassium concentration within the intercalate layer, which occurs on passing from stage 1 (C_8K) to stage n ($C_{12n}K$), is accompanied by a loss of long range order within the layer. A study of the stage 2 compound has now shown that this disordered intercalate structure (form I) transforms reversibly to an ordered state (form II) at a temperature of about -175° C.

The specimen was prepared from annealed, hot-pressed pyrolytic graphite² by the two bulb method of Herold³. The second bulb, which contained the graphite, was in the form of a thin walled capillary tube; after the compound had been formed, this capillary tube was sealed off and transferred to the X-ray goniometer. The change in diffraction pattern on transformation from form I to II is shown diagrammatically in Fig. 1. Scattering from the intercalated potassium gives rise to a single streak (S) in form I, but in form II there are khl superlattice reflexions on at least six row lines (S1 to S6). Thus the development of order within the intercalate at the transformation temperature is not restricted to individual layers but extends to three dimensions.

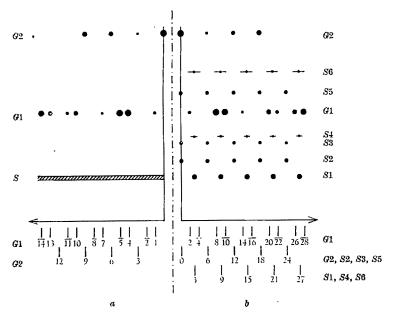


Fig. 1. Schematic representation of the change in the X-ray diffraction pattern during the order-disorder transformation in potassium graphite (stage 2). a, Form I, room temperature; b, form II, liquid nitrogen temperature. Each diagram is equivalent to a rotation photograph about the horizontal c-axis and numerical values of l to be associated with each layer line are indicated. Row lines of constant h and k are denoted by G if carbon atoms contribute to the reflexion and by S if the diffraction effect is due solely to potassium atoms.

A significant finding is that no detectable change in relative intensity or resolution of reflexions which index in form I as $10\overline{1}l$ and $11\overline{2}l$ (G1 and G2) occurs at the transformation temperature. From this it follows that two conclusions regarding the structure of form I are also valid for form II. These are¹: (a) the potassium atoms are located over the centres of hexagons of carbon atoms; (b) the graphite layer stacking sequence is AB|BC|CA|A. The first condition implies that displacements of the potassium atoms within any intercalate layer during the transformation to the ordered state must be expressible in terms of unit displacements which are of the type

 $\frac{a}{2}$ < 11 $\overline{2}$ 0 > (numerically equal to the translational repeat

distance, a, of the two-dimensional graphite lattice). Taken together, these two conditions impose a restriction on the intercalate layer stacking vector, t. The two-dimensional intercalate lattice must follow the graphite layer stacking sequence if potassium atoms are to remain over the centres of carbon hexagons. The vector t must there-

fore include a vector of the type $g = \frac{a}{3} < 10\text{T0}>$ which

changes the graphite layer orientation in the sequence $A \rightarrow B \rightarrow C \rightarrow A$.

In the simplest case, the periodicity of the stacking sequence of the graphite and intercalate sub-lattices would be identical and the intercalate stacking sequence could then be represented by $\alpha \beta \gamma \alpha \dots$ This would not account, however, for the position of reflexions on row lines S1, S4 and S6 which imply that the c-axis repeat distance doubles during the transformation from form I to II. The intercalate stacking sequence must therefore be of the type $\alpha \beta \gamma \delta \epsilon \eta \alpha \dots A$ simple relation between $\alpha \beta \gamma$ and δεη is suggested by the fact that there are extensive systematic absences within the superlattice row lines; hkl reflexions are present only when l = 6n in S2, S3 and S5 and when l = 6n + 3 in S1, S4 and S6. The sequence $\delta \varepsilon \eta$ is probably a displacement of the sequence $\alpha \beta \gamma$, parallel to the graphite planes and of a magnitude sufficient to introduce a phase shift of about 0 or π according to the numerical values of h and k to be associ-

ated with a particular row line. It is therefore proposed that the combined intercalate-layer and graphite-layer stacking sequence can be schematically represented by A α^1 A B β_1 B C γ_1 C A α_2 A B β_2 B C γ_2 C A α_1 A . . . where $\alpha,\ \beta$ and γ differ at least by successive displacements of g and where the change of subscript denotes a translation which approximately centres the intercalate lattice. This stacking sequence repeats after about 52 Å.

The displacement which changes at into α₂ can only arise through a direct interaction between these two layers, because the carbon atom environments of α_1 and α_2 are identical. Evidence for intercalate layer interaction over similar distances was also found in the order-disorder transformation of the graphite nitrates4. In these compounds also, the stacking repeat distance doubles during the transformation I->II, but the detailed interpretation of the diffraction patterns given by stages 2, 3 and 4 was complicated by the presence of some unmodulated row lines which were assumed to be caused by extensive intercalate stacking disorder. The simpler diffraction pattern for potassium graphite presumably reflects the difference in structural complexity between intercalate layers which contain atomic and molecular species, respectively. theless, the definition of reflexions in the row

lines which distinguish α_1 from α_2 falls off in the sequence S1, S4 and S6. A likely explanation is that even here there is some random element of structure, either within a single intercalate layer or in the intercalate layer stacking sequence.

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THE SOLID STATE

Cleavage of Zinc Single Crystals induced by Stress Waves

WE report some observations of the dynamic cleavage of specially oriented single crystals of zinc by the reflected stress wave technique. Kolsky¹ has used this technique for studying the brittle fracture of glass and polymeric solids.

Zinc crystals² with a diameter of about 8 mm, a length of about 60 mm and with their potential cleavage plane (0001) perpendicular to the rod axis, were loaded with the stress wave produced by hitting a transmitter bar with a flat-faced projectile, as shown in Fig. 1. Fractures were produced by the tensile wave reflected from the interface between the zinc and styrofoam. This procedure eliminates the problems involved in gripping the specimen and reduces the problem of specimen alignment. For this crystal orientation, the shear stress on the primary slip plane (0001) < 1120> is nominally zero. Earlier static tests3-6 on zinc crystals have indicated that the stress

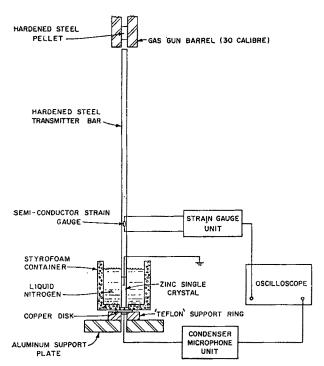


Fig. 1. Schematic diagram of testing arrangement,

required for twinning or for slip on secondary slip systems is comparable with the cleavage fracture stress.

The peak value of the stress wave transmitted to the zinc single crystal can be computed as the difference between the peak values of the incident and reflected waves in the transmitter bar. Cleavage was consistently observed for transmitted stress pulses with an amplitude greater than 16×10^8 dynes/cm² and a duration of $10~\mu sec$. Determination of the actual stress at fracture is difficult because the pulse is attenuated as it propagates in the zinc crystal as a result of plastic deformation and because it is not clear whether fracture has occurred at the peak value of the pulse or at some lower value. An attempt to allow for these factors was made by using a condenser microphone, to obtain a record of the displacement of the zinc-styrofoam interface. Further work will be necessary before reliable values of the fracture stress can be given.

The cleavage surfaces were examined optically and with various X-ray diffraction techniques. The dynamically fractured surfaces seemed to be optically more specular than any which could be produced by cleaving crystals with a razor blade at liquid nitrogen temperature. The general indication from Laue back-reflexion photographs, Schultz pictures and Berg-Barrett X-ray diffraction contrast micrographs is that the dynamically fractured crystals exhibit cleavage surfaces which are appreciably less deformed than the surfaces of crystals cleaved with An unfractured crystal which was repeatedly loaded became so deformed, however, that the Bragg condition was no longer obeyed over local areas greater than about 100µ in diameter.

The conclusion is that the technique is useful for studying cleavage fracture, at least in zinc crystals, in conditions for which the pre-fracture plastic flow is minimized.

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MOLECULAR STRUCTURE

Role of Van der Waals Interaction on Hindered Rotation about Single **Bonds in Simple Molecules**

The hindrance of rotation about single bonds has recently attracted considerable interest $^{1-3}$ because it greatly influences the physical and chemical properties of many organic molecules. The importance of rotations about single bonds in synthetic and biological macromolecules has also been emphasized and successful attempts have been made to derive the most stable conformations of linear macromolecules considering this effect⁴⁻¹⁰.

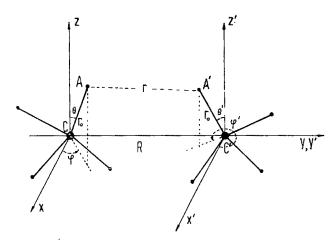


Fig. 1. Geometrical representation of two methane molecules in interaction $(r_0; CH=1.09 \text{ Å}, CF=1.32 \text{ Å}, CCl=1.77 \text{ Å}).$

In attempting to explain the presence of potential barriers which hinder rotations about single bonds, in molecules like ethane and ethane derivatives, there have been two principal approaches. According to several authors, the barriers arise from the lack of cylindrical symmetry in the molecular orbitals describing the obonds¹¹. Another approach emphasizes the importance of steric hindrance resulting from Van der Waals interactions between the non-bonded atoms which depend on rotation about the bonds¹².

Scott and Scheraga³ have tried to reconcile both views. While investigating the conformational analysis of macromolecules we have independently adopted a similar approach and have carried out systematic calculations on a number of molecules which we report here.

In order to evaluate the relative importance of Van der Waals interactions and torsional potential, we considered it useful to submit the various functions so far proposed to describe pairwise interactions between non-bonded atoms to an indirect experimental test. In particular, the carbon-carbon, hydrogen-hydrogen, fluorine fluorine, chlorine-chlorine and the mixed interactions were considered.

A reasonable criterion for such a test seemed to be the calculation of the intermolecular potential as a function of the distance between a pair of methane, carbon tetrafluoride or carbon tetrachloride molecules which has been derived from experimental data with a certain degree of accuracy¹³.

When the interactions between two methane molecules are calculated, it is necessary to consider the effect of the distance between the carbon atoms as well as the reciprocal orientation of the two molecules. Because the intermolecular potential was derived assuming spherical symmetry the following equation was used

$$V(R) = \frac{1}{4\pi^2} \int d\omega \mid d\omega' v(r)$$
 (1)

where $d\omega = \sin\theta \; d\theta \; d\phi; \; d\omega' = \sin\theta' \; d\theta' \; d\phi' \; {\rm and}$

$$v(r) = \Sigma_{j} \left(\frac{a_{j} \exp \left(-b_{j} r_{j}\right)}{r_{j} d_{j}} - \frac{c_{j}}{r_{j}^{6}} \right)$$

The summation on j is extended to all pairs of atoms in the two molecules.

The same equation was used to calculate the intermolecular potential between two carbon tetrafluoride and carbon tetrachloride molecules. The variables θ , φ , θ' , φ' , r and R are defined in Fig. 1. The parameters a_1 , b_1 , c_2 and d_3 of the various functions tested are listed in Tables 1, 2 and 3. It must be pointed out that when $d_1 = 0$, the function is of the Buckingham type, and if $b_1 = 0$ and $d_1 = 12$, it is of the Lennard-Jones type. Some authors used one type to describe the carbon-carbon interaction and the other for the hydrogen-hydrogen interaction. This means that the mixed carbon-hydrogen potential

Table 1. POTENTIAL FUNCTIONS BETWEEN CARBON AND HYDROGEN ATOMS

No. of	f Carbon-carbon			-carbon		Hydrogen-hydrogen				('arbonhydrogen			
set	Ref.	$a \times 10^{-3}$	b	c	d	$a \times 10^{-3}$	b	c	d	$a \times 10^{-3}$	b	c	đ
1 (a)	14	237.0	4.32	297.8	0.0	34.8	5.66	18-1	0.0	90.8	4.99	73.2	0.0
2	15	16.6	3.63	325.0	0.0	10.0	4.60	49.2	0.0	12.9	4.12	125.0	0-()
3	16	37-0	3.60	330.0	0.0	1.20	2.85	160.0	0.0	6.8	3.20	230-0	()-()
4	17	30.0	$3 \cdot 42$	420.0	0.0	30.0	5.00	43.0	0.0	30.0	4.12	140-0	0.0
5	3	528.0	4.58	370.0	0.0	9.17	4.54	45.2	0.0	81.5	4.56	128.0	0.0
6	9	564.0	4.60	364.0	0.0	8.35	4.60	47.0	0.0	60.1	4.60	128.0	0.0
7 (β)		237.0	4.32	298.0	0.0	6.60	4.08	49.2	0.0	31.4	4.20	121.0	0.11
8	18	301.0	0.0	327.0	12.0	6-60	4.08	49.2	0.0	44.8	2.04	125.0	8.0
9	5	301.0	0.0	327.0	12.0	3.72	3.07	89.5	0.0	33.5	1.53	589.0	6.0
10	10	286.0	0.0	370.0	12.0	4.46	0.0	46.0	12.0	38.0	0.0	128.0	12.0
11	19	396.0	0.0	364.0	12.0	7.26	0.0	47.0	12.0	56-8	0.0	128-0	12.0
12	20	408.0	0-0	373.0	12.0	13.5	0.0	51.5	12.0	58.9	0.0	133.0	12.0

Parameters a and c are given, respectively, in kcal AdMole⁻¹ and kcal AdMole⁻¹ unit.

(a) As this author did not propose a function for the carbon—carbon interaction, we have used the one proposed by Bartell¹⁸ fitted by a Buckingham type function. The same one has been adopted for the carbon—carbon interaction of set 7.

(b) This set of parameters has been derived from the Bartell's function in a way fully described in the text.

Table 2. POTENTIAL FUNCTIONS BETWEEN CARBON AND FLUORINE ATOMS

No. of		Carbon-carbon					Fluorine-fluorine				Carbon-fluorine			
set	Ref.	$a \times 10^{-3}$	b	c	d	$a \times 10^{-3}$	b	\boldsymbol{c}	d	$a \times 10^{-3}$	b	c	d	
1	3	528-0	4.58	370.0	0.0	60-2	4.60	118.0	0.0	152.0	4.59	200.0	0.0	
2(a)	5	237.0	4.32	298-0	0.0	7.2	0.0	0.0	9.99	159-0	1-46	193-0	0.0	
(b)						106-0	4.61	125.0	0.0					

The mixed interaction carbon-fluorine of set 2 was calculated with the same method as that described in the text for the carbon-hydrogen one. It is therefore slightly different from the corresponding one in ref. 6. Set 2a is valid for r < 2.9 Å and set 2b for r > 2.9 Å.

Table 3. POTENTIAL FUNCTIONS BETWEEN CARBON AND CHLORINE ATOMS

No. of		Carbon-carbon			Chlorine-chlorine				Carbon-chlorine				
set	Ref.	$a \times 10^{-8}$	b	c	d	$a \times 10^{-3}$	b	c	d	$a \times 10^{-3}$	b	c	ď
1 2 3 (a) (b)	14 3 5	237·0 528·0 237·0	4·32 4·58 4·32	298-0 370-0 298-0	0·0 0·0	260·0 314·0 13·0 221·0	3·67 3·75 0·0 3·62	1,811.0 2,520.0 0.0 1,430.0	0·0 0·0 7·87 0·0	248·0 425·0 239·0	4·17 3·97	732·0 975·0 653·0	0.0 0.0

The same remark as that given in Table 2 for the carbon–fluorine interaction is also valid for the earbon–chlorine interaction of set 3. Set 3a is valid for r < 3.0 Å and set 3b for r > 3.0 Å.

shows an r dependence characterized by a value of $b_i \neq 0$ and $d_i = 6$. To allow a direct comparison of the coefficients used by different authors, those corresponding to the same types of function are clustered in Table 1.

The intermolecular potential was calculated according to equation (1), with the aid of an IBM 7040 computer, and angular increments $\Delta \varphi$, $\Delta \theta$, $\Delta \varphi'$ and $\Delta \theta'$ of 30° were considered. It was observed that smaller increments would not increase the accuracy by more than a few per

The results for methane are shown in Fig. 2a and b. The intermolecular potentials calculated with most of the functions recently proposed are shown in Fig. 2b. From Fig. 2a and b, curves 7 and 8 are seen to give the best agreement with experimental results. Curve 7 differs from curve 8 in the choice of a Buckingham type function to describe the carbon-carbon interaction instead of a Lennard-Jones one and in the means used to derive the mixed interaction.

The parameters of the mixed interaction of curve 7 were, in fact, obtained by finding the geometrical mean of the attractive term and of the part of the repulsive term which depends on r. The remaining parameter a was obtained by imposing the condition that the function must be minimum at the sum of the Van der Waals distance given in Table 4. In curve 8, proposed by Bartell, the geometrical mean was calculated from the repulsive term, and the parameter c was then calculated from the same minimum requirement.

le 4. Potential functions used to calculate van der waals con-tribution to the rotation barrier of ethane-like molecules

V(r) =		<u>c</u> .s a	ь	c	d	<i>r</i> min
H-H		6.60	4.08	49.2	0.0	2.96
F-F	r < 2·3 r > 2·3	7·2 106·0	0·0 4·61	0·0 125·0	0·0 9·99	3.14
·ClCl	r < 3.0 r > 3.0	13·0 221·0	0·0 3·62	0·0 1.430·0	7·87 0·0	3.86
H-F		21.4	4.34	78-5	0.0	2.97
H-Cl		33.8	3.85	265.0	0.0	3.33
FCI		172.0	4.11	423.0	0.0	3.50

To calculate the coefficients of the mixed interactions hydrogen-fluorine and hydrogen-chlorine, a Van der Waals radius of 1.4 Å was taken for the hydrogen atom instead of 1.48 Å in the hydrogen-hydrogen interaction.

Table 5. COMPARISON BETWEEN OBSERVED AND CALCULATED POTENTIAL BARRIERS

	DAIMANAG											
Molecules	Van der Waals energy barrier	Total energy barrier	Experimental values									
1 CH ₃ -CH ₂ 2 CF ₃ -CF ₃ 3 COl ₃ -CCl ₃ 4 CH ₃ -CCl ₃ 5 CH ₃ -CCl ₃ 6 CF ₃ -CCl ₃ 7 CH ₃ -CH ₄ F 8 CH ₃ -CH ₄ F 9 CH ₃ -CH ₅ Cl 10 CH ₃ -CH ₅ Cl	0·42 1·95 9·34 0·69 1·71 5·69 0·51 0·60 0·84 1·27	3·12 4·65 12·04 3·39 4·41 8·89 3·21 3·30 3·54 3·97	3·0 4·0 12·0 3·7 3·0 13·0 8·3 3·4 4·0									
10 CH ₃ -CHCl ₂	1.21	5.81	9.0									

All angles are assumed to be tetrahedral. The bond distances used are

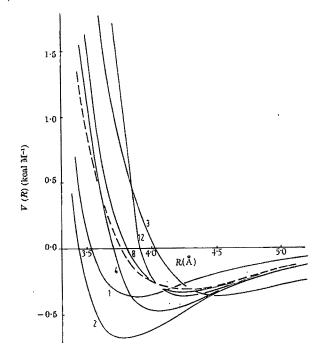
Carbon-carbon = 1.54 Å
Carbon-hydrogen = 1-09 Å
Carbon-fluorine = 1.33 Å
Carbon-fluorine = 1.76 Å

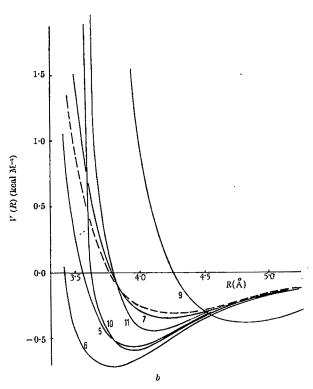
The energy values are given in kcal/Mole. The total energy barrier is the sum of the Van der Waals contribution and a unique torsional potential taken equal to 2.7 kcal/Mole.

Tables 2 and 3 give the data of an identical calculation carried out for the carbon tetrafluoride and carbon tetrachloride molecules.

From experimental curves shown in Figs. 3 and 4, it appears that curves 2 and 3, respectively, derived from Bartell's carbon-carbon interaction and the rare gas approximation introduced by Mason and Krevoy12, are the best. It should be pointed out that these functions were used in our laboratory a few years ago5 to derive quite successfully the most stable conformation of a lot of synthetic polymers.

Fig. 3 also shows the results of an electrostatic test. This test was carried out on the assumption that small charges approximately equal to one third of those producing the experimental dipole of a carbon-fluorine bond





. a and b, Calculated potential energy between two methane molecules. The dotted line represents experimental data.

are localized on the fluorine and carbon atoms. At first sight it seems that this procedure gives the best agreement within the whole range of R values. Nevertheless, it must be remembered that, in such a case, polarization contributions also occur. These terms are attractive and so could very well balance the repulsive electrostatic ones at intermediate distances.

In order to calculate the barriers to internal rotation in ethane and halogene substituted ethanes, the following equation has been used to obtain the potential energy as

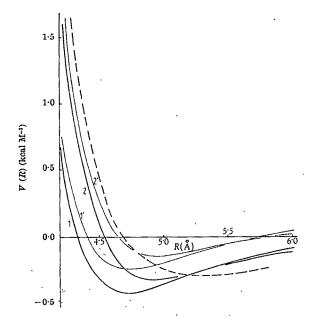


Fig. 3. Calculated potential energy between two carbon tetrafluoride molecules. Carves 1' and 2' were calculated with the same Van der Waals function as curves 1 and 2, but also taking into account an electrostatic contribution arising from a charge equal to $-0.07 \times e$ localized on each fluorine atom and $0.28 \times e$ on the carbon atoms.

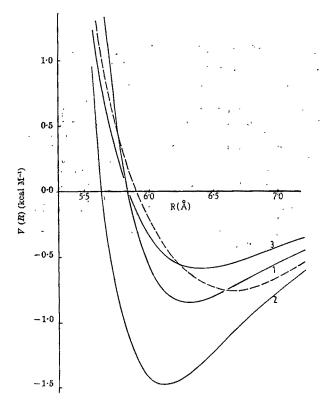


Fig. 4. Calculated potential energy between two carbon tetrachloride molecules.

a function of the angle of rotation about the carbon-carbon

$$E(\psi) = \Sigma_j a_j \exp(-b_j r) - c_j 2^{-8} + \frac{V_0}{2} (1 + \cos 3\psi)$$
 (2)

When the parameters of curve 7 described in Table 2 were used to calculate the Van der Waals contribution corresponding to the first term of equation (2) and a value of $V_0 = 2.7$ kcal was used for the torsional potential

barrier, the results listed in Table 4 were obtained. The agreement between experimental^{2,3} and calculated potential barriers seems rather good in view of the simplicity of the approach used.

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CHEMISTRY

Reactivity of Trapped Free Radicals in Biological Systems

IRRADIATED poly-crystalline amino-acids lend themselves to the kinetic investigation of radical stabilities, because radicals can be induced readily in solid amino-acid by irradiation and because radical concentrations at different temperatures can be followed conveniently by electron spin resonance spectroscopy. In this study, radicals were formed in a number of amino-acids containing sulphur by irradiation at 2537 Å with a low-pressure mercury-vapour germicidal lamp¹. Electron spin resonance spectra were determined on a JEOL-3BX electron spin resonance spectrometer. Samples were usually irradiated continuously at room temperature until the concentration of radicals remained constant and decay rates were then studied at higher temperatures; it was assumed that the radical concentration present was proportional to the signal strength, which was estimated directly from the recorded spectral trace using a planimeter.

L-Homocysteine, L-cysteine hydrochloride and L-penicillamine, but not L-cystine, all gave decay curves which, in the absence of air, fitted a single second order function for the majority of the decay times. A typical curve is shown in Fig. 1. Because irradiation with 2537 Å light is known to form radicals preferentially near the crystal surface*.

* This was also confirmed by irradiating a single crystal of L-cysteine hydrochloride, when appreciable radical concentrations could only be detected in the irradiated portions. We are indebted to Professor J. R. Bolton for suggesting this experiment.

one would anticipate the observed decay curve to be the composite of a series of second order curves, one for each of the RS radical pairs present depending on their distance from each other. That is, radicals nearer the surface, being closer to each other, would be anticipated to combine more readily than more isolated radicals formed further away from the crystal surface. Because most of the observed points lie on a single second-order decay curve, it is assumed that an initial, faster, randomizing, reaction occurs. A probable, more specific, explanation is that a reaction of type (1) occurs first, which is followed by a second, slower, rate-determining step (2).

RS· (formed during irradiation) + RSH
$$\rightarrow$$
 RSH + RS·* (1

$$RS^{*} + RS^{*} \rightarrow Products$$
 (2)

In these equations, RS.* represents the eventually trapped species, the recombination rate of which is measured in the experiments. RS. may itself represent the formation of a secondary radical (compare with ref. 2) and radicals such as H· and R· are presumably also formed. The electron spin resonance spectra at 77° K and room temperature, however, indicate predominantly the presence of RS. species only. That is, signal shape and g values show the electron to be located predominantly on the sulphur atom and no significant change in signal shape was detected during the build-up or decay of the radical concentrations. No appreciable line-broadening was detected between 77° K and room temperature.

The simplest way to explain the presence of randomly distributed radicals is to assume that, given favourable steric conditions, hydrogen transfer occurs between neighbouring SH groups (step 1) until the unpaired electron is located on a sulphur atom (RS·*), which is no longer near to another hydrogen atom and no further reactions of type (1) then occur. The actual mechanism of step (1) is uncertain, but may involve an intermediate of type RSHSR (compare with ref. 3, where a similar species has been postulated to account for the low energy required for the transfer of hydrogen atoms in solution). The occurrence of step (1), incidentally, can account for the relatively small saturation concentration of radicals, formed on irradiation⁴, and for the previously noted phenomenon of radical site migration in irradiated solids⁵.

Of considerable interest are the observed energies of activation for step (2), which were found to lie between about 9-25 kcal/mole. Because step (1) must possess a smaller energy of activation, the data suggest that effective free radical site migration can occur in these solids with a relatively low energy of activation (compare also with ref. 6 where, for migration processes within proteins, activation energies of 70 cal/mole and 2·2 cal/mole have

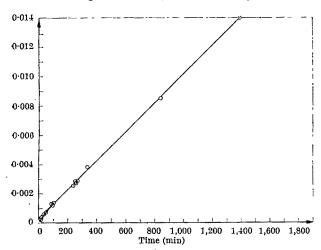


Fig. 1. Decay diagram for 2537 Å-irradiated polycrystalline L-penicillamine in vacuum at 120° C, plotting time versus $(\{R^-\}_b - \{R^-\}_o)/(\{R^-\}_b - \{R^-\}_\omega)$, where $\{R^-\}_b$, $\{R^-\}_t$ and $\{R^+\}_\omega$ are the relative radical concentrations at time 0, t and ω , respectively.

been reported). This finding, it substantiated by further experiments, indicates a mechanism of how free radicals, once formed—for example, through inhaling of tobacco smoke or soot—may migrate through biological materials to reach sensitive sites.

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Origin of Linewidth Alternation in Semiquinone-Alkali Metal Systems

The electron spin resonance spectra of semiquinone-alkali metal systems in 1,2-dimethoxyethane (DME) have recently been interpreted in terms of (a) two types of ionpair, A or solvated type and B or contact type, which appear separately or together depending on the temperature and (b) an equilibrium between these two types of ion-pair which is thought to be the cause of marked linewidth alternation. We have also studied these systems over a wide temperature range, but have drawn quite different conclusions.

We will consider only those spectra which show hyperfine coupling from diamagnetic gegenions because if this is detected there can be no doubt of the presence of ion-pairs². In particular the sodium salt of durosemiquinone in

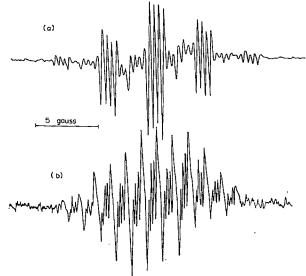


Fig. 1. Electron spin resonance spectra of the sodium salt of duro-semiquinone in DME at $(a) + 20^{\circ}$, $(b) - 90^{\circ}$.

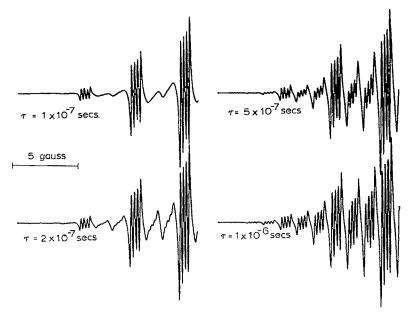


Fig. 2. Computer simulations, for various τ (ref. 7), of the electron spin resonance spectrum of the sodium salt of durosemiquinone assuming an intramolecular migration between equivalent sites.

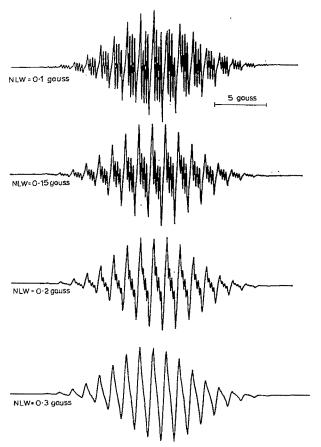


Fig. 3. Computer simulations, for different natural linewidths (NLW), of the electron spin resonance spectrum of the sodium salt of duro-semiquinone assuming no intramolecular migration, that is, large τ (ref. 7).

t-pentanol, DME and tetrahydrofuran (THF) will illustrate our case.

If an equilibrium exists between two types of ion-pair in which the same sodium ion is involved so that its nuclear spin state is retained, either one or two sets of

hyperfine lines will be detected depending on the rate of equilibration. If this is rapid, the outer pair of lines will usually be broader than the inner pair2 because the hyperfine lines arising from the spin states, $m_s = \pm 3/2$, span a larger range of field than the $m_s = \pm 1/2$ lines. This should be particularly apparent from the quartet at the centre of the durosemiquinone spectrum, where, in the absence of a large g-shift between the different ion-pairs. the central anion hyperfine component is unaffected by the exchange. No variation in the linewidths of the central quartet has been observed for the sodium salt of durosemiquinone in DME, as shown in Fig. 1 (see also Fig. 2 in ref. 1) or in t-pentanol3, although in the overall spectra alternating linewidth effects, indicative of an exchange, are dominant. This shows that an equilibrium between two structurally different ion-pairs, which have very different sodium hyperfine coupling constants, is not responsible for the observed We conclude that the more spectra. common concept2 of intramolecular migration of the cation between two equivalent sites on the anion is the only one so far suggested which will agree with all the

observations. In the absence of hyperfine coupling from diamagnetic gegenions the conclusions are ambiguous, but there seems to be no reason to assign a different mechanism.

Although different types of ion-pair have been detected by electron spin resonance6, the only clear cut example of this with semiquinones has been with 2,6-dimethyl-pbenzosemiquinone^{4,5}, the difference being one of site rather than solvation. The symmetrically substituted semiquinones, for example, durosemiquinone, are unable to behave in this way and so we have examined our spectra for any evidence that two types of ion-pair1 do occur in these systems. Our spectra (Fig. 1) seem to be very similar to those previously published1, over a range of temperature. We have, however, been able to analyse each spectrum in terms of one radical with hyperfine coupling constants: $a_1^{\text{Me}} = 1.25$; $a_2^{\text{Me}} = 2.55$; $a^{\text{Na}} = 0.23$ gauss at -90° C or $a_1^{\text{Me}} + a_2^{\text{Me}} = 3.8$; $a^{\text{Na}} = 0.37$ gauss at 20° ('. Using the coupling constants at -90° we have calculated theoretical spectra with a natural linewidth of 0-1 gauss and for various rates of intramolecular migration expressed as a function of the lifetimes, τ , in Fig. 2. The effect of different natural linewidths on the spectrum for slow rates of migration, that is, at low temperatures, is quite significant and is shown in Fig. 3. Comparison of these calculated spectra with the experimental (see also Fig. 2 of ref. 1) shows that the experimental spectra are well interpreted with one species with an intramolecular migration of the cation between equivalent sites. It seems that the spectrum assigned to two different species1 is purely an unfortunate artefact of overlapping lines.

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PHYSIOLOGY

Antidiuretic Activity in the Pituitary Gland of the Koala Bear

Arginine or lysine vasopressin is found in the pituitaries of most placental mammals and arginine vasopressin has been tentatively identified in a monotreme¹ and in four species of marsupials1,2. We have investigated antidiuretic activity in the pituitary of a young koala bear (Phascolarctos cinereus), which is of interest because this animal is rarely, if ever, known to drink, its water intake being derived solely from eucalyptus leaves.

The koala studied was about 6 months old and thus quite immature, and had died by falling out of its mother's pouch during the night. The pituitary gland was extracted according to standard methods³ and assayed against 'Pitressin' (Parke, Davis and Co.) in the hydrated rat⁴ with an initial and maintained water load equivalent to 6 per cent of its body weight. We have found this slightly lower level of hydration to produce a satisfactory flow of urine and to improve the sensitivity to injected vasopressin. Sections of hypothalamus and medulla were similarly extracted and assayed for antidiuretic activity.

The antidiuretic activity of the whole pituitary was equivalent to 235 mu or 7.95 mu/mg dry tissue which, considering the age of the koala, seems comparable with that reported for other marsupials. It is tentatively ascribed to vasopressin because the log dose-response curves of the standard and extract were parallel, the standard and extract produced similar effects on urine volume and conductivity both in onset and duration and the activity was destroyed by incubation with 0:01 molar sodium thioglycollate for 30 min (ref. 4). There was no detectable antidiuretic activity in the extracts of hypothalamus and medulla, the lower limit of sensitivity in the assay animal being $1.25 \mu U$.

The koala was collected by Dr P. A. Robertson of Lone Pine Sanctuary, Brisbane. We thank the Australian Wool Board for financial assistance.

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Siphuncular Tube of Nautilus

SINCE Robert Hooke¹ suggested that the siphuncle (which he took to be the gut) of Nautilus produced gas to force liquid from the shell chambers and so buoy up the animal, there have been many speculations on its function. These speculations required that the siphuncular tube was watertight², or air-tight³⁻⁵, or permeable to gas⁶⁻⁸, or permeable to liquid^{9,10} or permeable to both gas and liquid¹¹. The only experimental information, however, on the transmission properties of the siphuncular tube is that it is permeable to water¹⁰. The experiments described here show that the wall of the siphuncular tube is permeable to sea water (with a linear relationship between flow rate and pressure), impermeable to air and able to withstand pressures equivalent to well above 450 m of water.

A fresh shell of Nautilus macromphalus was sawn open along the dorsoventral plane. Stainless steel tubes were bonded with 'Araldite' to the siphuncular tube openings

so that sea water could enter into the first chamber adjacent to the living chamber, and could leave from the fifth chamber. The inflow tube was connected to the pressure apparatus (Fig. 1) and the outflow tube to a bleed valve. Initially, sea water was forced in under a head of 41 m of water, and immediately small beads of liquid appeared on the surface of the siphuncular tube. In each segment, the beads coalesced into a single drop of liquid at intervals of about 5 min. When the pressure was increased, the flow rate of liquid was found to be directly proportional to the pressure (Fig. 2). At a 205 m head, the five chambers were almost half full of transmitted liquid after an hour.

In a further experiment, sea water under 205 m head was forced into the siphuncular tube segments in the earliest twenty shell chambers. Liquid appeared on the siphuncular tube segments in all chambers except the earliest one. With this exception, all the siphuncular tube segments seem to be functional, even the earliest seven segments which supposedly formed in the egg. Thus at 200 m depth a Nautilus shell would fill in about 2 h if the siphuncle did not prevent it.

The salinity of the effluent at various pressure heads up to 200 m was analysed and found to be unaltered from that of the inflowing sea water, so that the siphuncular tube seems to act as a permeable, not a semi-permeable. membrane. A reduction in salinity would have explained

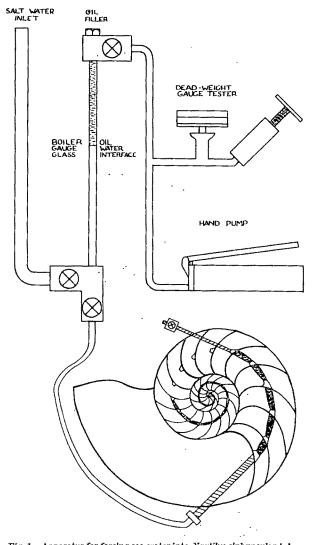


Fig. 1. Apparatus for forcing sea water into *Nautilus* siphuncular tube. Permeable parts of siphuncular tube in black.

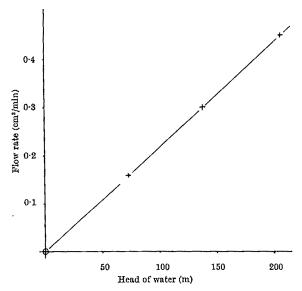


Fig. 2. Flow through the walls of the siphuncular tube.

the low salinity values that Denton and Gilpin-Brown found in the chamber liquid of some Nautilus specimens¹⁰.

A long column of air was trapped in a high pressure nylon tube and forced into the siphuncular tube segments under 103 m head of water. No bubbles of air appeared on the surface of the siphuncular tube, and the water-air interface in the nylon tubing did not move during a period of 10 min. Thus, when the siphuncular tube is moist as it is in life, no gas will pass through. This finding, along with the invariably low gas pressure encountered in *Nautilus* shell chambers^{9,10}, seems to discount the hypothesis that the siphuncle generates gas into the shell chambers in order to balance the hydrostatic pressure. Thus it would seem that the only way gas can enter the chambers is by simple diffusion10.

With no assistance from gas pressure, the shell, including the siphuncular tube, must withstand the considerable hydrostatic pressures encountered by Nautilus at depth (up to 900 p.s.i. at 600 m). Denton and Gilpin-Brown proved that the shell is strong enough, and also found that two pieces of siphuncular tube could resist a tensile stress of 500 p.s.i. (ref. 10). This latter test was inconclusive, however, because the pieces of siphuncular tube had been in formalin for 3 yr, and the direction of stress was different from that experienced in a live Nautilus. To determine the strength more realistically, a hydraulic jack was used to force sea water into the five segments previously tested, until the siphuncular tube ruptured. Failure, under a head of 480 m, was at the artificial interface between 'Araldite' and the siphuncular tube, the remainder of the siphuncular tube being even stronger. Thus the siphuncular tube seems able to withstand the full hydrostatic pressures encountered by Nautilus.

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Effect of Exercise on the Thyroid Gland

WHILE testing the hypothesis that exercise increases the rate of renewal of thyroidal iodine in the rat we found that the total amount of iodine in the thyroid gland depends on activity (exercise). Usually a month or more is necessary1 to replace completely the iodine in the thyroid gland so that the total can be estimated by the technique of isotopic equilibrium2,3, and we assumed that if rats were exercising the increased expenditure of calories would be associated with an increased utilization of dietary iodine for the production of thyroid hormone, which would in turn lead to an increased turnover of iodine in the thyroid and a decreased isotopic equilibration period. The experimental results did not confirm this, but they did reveal that the exercising rats stored only about half as much iodine in the thyroid as did the nonexercising controls.

In the initial test, sixteen male Wistar rats weighing 125-150 g were placed in small individual wire cages, eight of which were equipped with revolving activity drums and cyclometers. The rats were fed a low iodine diet and iodine-129 was added to the water supply so that each rat received about 10 µg of iodine/day. After 28 weeks, iodine-125 was added to the water supply, and 2 weeks later the rats were killed. The thyroids were removed and their iodine-129 and iodine-125 radio-activities compared with that of a standard sample of the radioiodinated water supply. Total thyroidal iodine and the percentage of iodine renewed in the last 2 weeks were calculated. Live body weights, iodine consumption and number of revolutions of the activity drums were recorded weekly during the period of 30 weeks.

In the second test, sixteen male Wistar rats weighing about 100 g each were placed in the small cages. Again eight of the cages were equipped with activity drums. A low iodine diet was given and iodinated water labelled with iodine-125 was supplied so that each rat received about 5 µg of iodine/day. After 5 weeks the rats were killed and the total amount of iodine in their thyroids was determined by comparing the iodine-125 radioactivity of their thyroids with that of standard samples of the radio-iodinated water supply. Numbers of turns of the activity drums had been recorded weekly for those rats in the exercise cages.

In the first experiment, non-exercising rats had an average of 9.0 ± 0.9 µg of iodine in their thyroids; the exercising rats had an average of $5.8 \pm 0.5 \mu g$ of thyroidal iodine. The percentage of the thyroidal iodine renewed in 2 weeks was 62.5 ± 1.6 for the non-exercising rats and 59.8 ± 2.7 for the exercising animals (Table 1).

In the second test, the non-exercising rats had an average of 20.0 ± 1.9 µg of iodine in their thyroids compared with $9.9\pm1.2~\mu g$ in the exercising group. The results are summarized in Table 2.

In both experiments, the non-exercising rats had, on average, approximately twice as much total thyroidal iodine as the exercising rats. On the other hand, a significant difference in the rate of renewal of thyroidal

iodine was not found. When both sets of data are combined and tested for a correlation between the amounts of exercise and the amounts of iodine in the thyroid, a negative correlation (0.025 < P < 0.05, statistical test based on Spearman's Rand correlation coefficient) is found. It is concluded that the more rats exercise the less the storage of iodine in the thyroid gland.

Previous studies have not led to a general concept of the role of the thyroid gland in exercise. Ricter⁴ showed that thyroidectomy produced a decrease in spontaneous activity in rats which was restored to pre-thyroidectomy levels by the administration of thyroid hormone. The feeding of thyroid hormone to rats, however, does not seem to increase spontaneous activity (ref. 5 and unpublished results of C. E. Easley and myself). Muscular exercise has been reported not to increase the rate of degradation of thyroid hormone^{6,7}, but Escobar del Rey and Morreal de Escobars did find that radiolabelled thyroxine disappeared more quickly in rats when longer periods of exercise were employed. Carriere and Isler⁹ found that forced muscular exercise as well as the psychological stimulation resulting from frequent changes of housing prevented the stimulation of the thyroid gland of mice caused by a diet deficient in iodine.

Previous studies have been handicapped by the superimposed effects of stress. Bogorock and Timiras¹⁰ and others11 have suggested that exercise experiments have antagonistic effects on the thyroid. The increased metabolism could cause a stimulation of the thyroid while the stress associated with the forcing of the exercise would tend to depress thyroid function. Brown-Grant et al.12 found that depressed thyroid function is a general response to stress in animals, but Korn¹³, on the other hand, found that the response to stress, as indicated by condition activity depression, is not affected by the thyroid gland.

Table 1. EFFECT OF EXERCISE ON THE RATE OF RENEWAL OF IQDINE IN

T	HE THYROID GLAND OF R	RATS
Live body weight (g)	Activity (thousands of turns/week*)	Percentage of isotopic equilibrium
559	0	56.1
494	0	57-8
536	0	61-1
528	0	61.8
585	0	62.2
527	0	64.8
510	0	66.5
518	0	69.7
532 ± 3 †		62·5 ± 1·6 †
521	0.76	51.2
490	1.05	56.3
586	1.19	58.8
599	1.26	76.0
508	2.62	54.4
580	5.09	64-2
480	10.54	55.3
460	11.28	62-0
528 ± 19†		59·8 ± 2·7†

^{*} Averaged over the last 12 weeks of the experimental period. \dagger Standard error of the mean.

Table 2. EFFECT OF EXERCISE ON THE TOTAL AMOUNT OF IODINE IN THE THYROID GLAND OF RATS

Expe	riment 1	Expe	riment 2
Activity (thousands of turns/week)	Iodine in the thyroid gland (µg)	Activity (thousands of turns/week)	Iodine in the thyroid gland (µg)
0 0 0 0 0 0	13·38 11·86 10·36 9·40 7·06 6·84 6·73 6·56	0 0 0 0 0 0	28·50 24·63 22·20 21·33 20·84 16·54 13·30 12·77
	9.02 ± 0.93*		20·01 ± 1·94*
0·76 1·05 1·19 1·26 2·62 5·09 10·54 11·28	8·84 5·79 5·99 3·95 6·47 5·15 5·05	N.D. 5-33 5-82 7-68 13-54 25-54 37-51 44-90	13·15 11·53 11·11 6·91 14·88 6·96 9·78 5·17
4.22	5.81 ± 0.51 *	20.05	$9.94 \pm 1.19*$

^{*} Standard error of the mean.

In this experiment, stress is not involved because the exercise is spontaneous and the treatment of the exercise group is no different from that of the non-exercise control group. The data can be interpreted in two ways. Either thyroid activity is depressed as a result of the exercise6,9, or the utilization of thyroid hormone is increased so that more dietary iodine is converted to circulating hormonal iodine and less to stored iodine. I prefer the second explanation. It has long been known that decreased or absence of thyroxine is associated with a decreased utilization of oxygen and decreased energy exchange in respiring tissues14. Perhaps the converse is also truethat is, with the increased expenditure of energy and associated increased respiration there is an increased utilization of the thyroid hormones. This is consistent with the suggestion that activity of the thyroid hormones is associated with their metabolism. Thus, at a given steady state of iodine metabolism, exercising animals will not be able to store as much hormonal iodine as nonexercising animals.

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Obligate Cation Exchanges in Red Cells

Coupling between the movements of sodium out of, and potassium into, human erythrocytes was discussed some time ago1. Although the concept of coupling between the active movements of sodium and potassium ions has been widely accepted^{2,3} it has been less usual to regard the down gradient "leak" movements as coupled. The stoichiometry of the active process met the difficulty that more sodium ions are extruded than potassium ions gained. To maintain electro-neutrality some other ion must move in association with potassium and sodium ions; this process has been somewhat neglected.

Results recently obtained by exposing erythrocytes to specific inducers of cation permeability such as valinomycin, gramicidin or nigericin provide evidence that the outward movements of cations down gradients are linked to contrary inward movements of other cations or protons. Participation of the latter largely explains the non-equivalence between movements of sodium and potassium ions and can account for the differing ratios of sodium to potassium ions obtained in different conditions.

Chappell and Crofts4 applied gramicidin to red cells in a choline and sodium salt medium and obtained internal concentrations of sodium ions greater than those outside at the expense of the depletion of the content of potassium ions. They also observed a shift of protons from the medium into the cells.

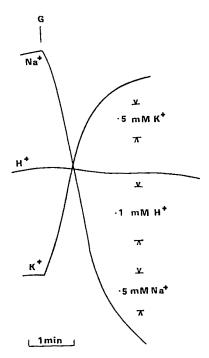


Fig. 1. Exchange between internal sodium ions and external potassium ions caused by grumicidin B (G) at 0.2 $\mu g/ml$, applied to a suspension of dog erythrocytes in 300 mmolar sucrose, 30 mmolar potassium chloride and 20 mmolar tris-chloride. The changes in the concentrations of sodium, potassium and hydrogen ions were monitored by ion-specific electrodes; movement from cell interior to solution is shown by a downward deflexion. Concentration of red cells was 0.042 ml. cells/ml.

There is a parallel between these cation exchanges in which the discharge of one gradient (that of potassium ions) sets up another (of sodium ions) and Rosenberg and Wilbrandt's induction of uphill transport of one sugar at the expense of counterflow of another with which the cells had been previously loaded.

The rapid ion exchanges induced by gramicidin can be illustrated with recordings obtained with ion specific glass electrodes (Fig. 1). With low concentrations of gramicidin (0·2 µg/ml.) there is little shift of pH (Fig. 1), but after addition of 1 µg/ml. of gramicidin the analyses of samples taken after 2–3 min showed that part of the emerging cation was replaced by protons. The figures in the first line of Table 1 show discharge of potassium ions from human erythrocytes setting up a gradient of sodium ions and those in the second line show discharge of sodium ions from dog erythrocyte setting up a gradient of potassium ions.

The results with the higher concentration of gramicidin show that proton movements cannot be neglected and the use of nigericin to increase permeability produces a more extensive cation for hydrogen ion exchange. Figure 2 illustrates the time course of this process, which is accompanied by some cation exchange as shown by the analyses in the third and fourth lines of Table 1. The cation exchange is sufficient to produce an outward gradient of the original extracellular cation, and a factor contributing to this is the shrinkage which accompanies the cation for proton exchange.

The requirement for another positively charged ion to replace the cell's potassium ions if it is to be discharged is also exposed by tests with the two agents valinomycin and trifluoromethoxy carbonylcyanide phenylhydrazone (FCCP). Valinomycin specifically increases permeability

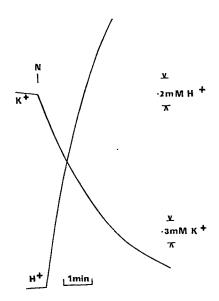


Fig. 2. Exchange chiefly between internal potassium ions and external protons caused by nigericin (N) at 0.5 μ g/ml. applied to a suspension of human erythrocytes in 300 mmolar sucrose, 10 mmolar sodium chloride and 20 mmolar *tris*-chloride. Cell concentration was 0.042 ml. cells/ml. of suspension.

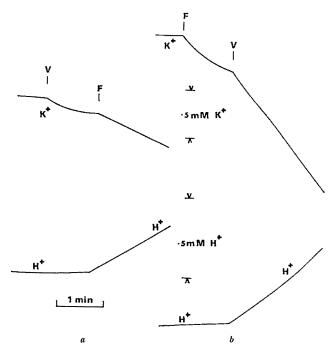


Fig. 3. Exchange between internal potassium ions and external protons caused by simultaneous presence of valinomycin (V) at 0-4 μ g/ml, and FCCP (F) (0-5 μ molar and 2-5 μ molar in a and b, respectively) applied to a suspension of human erythrocytes in 300 mmolar sucrose, 10 mmolar sodium chloride and 20 mmolar t16-chloride. Cell concentration was 0-045 ml, cells/ml.

Table 1. Accumulation of a cation in the erythrocyte at the expense of discharge of a gradient of another cation by a permeability-inducing

Permeability-inducing	Type of	Final exp. alkali chloride	Cell I		Cell (m)	II)	Proton move- ment into cell	Nature and conc. ratio (int./ext.)		
ngent	red cell	(mM)	Before	After	Before	After	water m.equiv./l.	of entering cation		
Gramicidin A 1 µg/ml.	Human	NaCl 14.0	142	52	23	110	14	Na 110/14		
	Carine	KCl 21.5	25	90	140	28	18	K 90/21.5		
Nigericin 1 µg/ml.	Human	NaCl 20.0	167	75	20	59	57	Na 59/20		
" "	Canine	KCl 23.0	25	75	140	65	5	K = 75/23		
Tonicity was maintained	l with 300 mmo	lar sucrose, 20 mmol	ar <i>tris-</i> chlori	de, pH 7.4,	and alkali chle	oride (third	column).			

of phospholipid bilayers to potassium ions and similar ions6 while FCCP produces a specific permeability to hydrogen ions7. Either agent when applied to a suspension of human erythrocytes in sucrose and sodium chloride causes only a limited loss of potassium ions, but when the second of the two agents is added a sustained exchange of potassium ions for hydrogen ions proceeds (Fig. 3). This resembles a result obtained with lipid micelles by Chappell and Haarhoffs in similar conditions.

Evidently in the evaluation of the stoichiometry of ion movements, the participation of protons must be taken into account. When metabolic acid is being produced, some of the protons can be used to replace sodium ions which are extruded and this in turn would account for the observed shrinkage. The obligate nature of the cation exchange, whether during energy-linked movements of sodium and potassium ions against their gradients, or when at least one cation is discharged down a gradient, shows that any chloride ion in the system does not act as a co-ion to accompany a lone cation. In other words, the anions do not shint the forces operating to accumulate one cation or proton as another cation emerges from what behaves as an internal cation exchanger. An agent which reduces the energy requirement for cations to enter the membrane material can accelerate movements in both directions, whether active or passive, as valinomycin does when applied to mitochondria. agent which also provides proton permeability in addition to that for alkali cations tends to discharge cations, as nigericin does when applied to mitochondria

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Possible Mechanism for the Endocrine Control of Breeding in a Cirripede

THE breeding of the barnacle Balanus balanoides (L.) is controlled by light and temperature; a temperature below a critical value of 10° C and a light periodicity of less than 12 h/day are necessary before the barnacle will breed 4-8 weeks later. As part of the cyclic activity of the oviducal glands during the natural breeding phase of B. balanoides 2-3 weeks before fertilization an elastic sac is formed within each gland2 which envelops the eggs as they pass down the oviduct, forming a membrane binding the egg mass together.

A series of experiments was carried out in this laboratory to determine the influence of light and temperature on the state of the nervous system and glands associated with breeding. This communication describes the effect of these exogenous factors on the oviducal gland.

A population of B. balanoides was brought into the laboratory at the end of July 1965; from their size they were at least second-season specimens, with well developed ovaries and testes. They were kept in flowing sea water at temperatures between 15° and 20° C and were exposed to artificial light between 0800 h and 1730 h daily. On September 29 two parallel groups of barnacles were transferred to a low-temperature room (4° C), where one group was exposed to constant illumination and the other to constant darkness. The populations were sampled at regular intervals and a histological study was later made of these specimens. The barnacles kept in cold, dark conditions had bred by December 8; those in cold, light conditions had not. Breeding had not occurred in the second group by January 17, 1966, when they were transferred to dark conditions in the low-temperature room. Breeding occurred on February 17.

The results of the histological study of the oviducal glands of the two groups are given in Table 1, which shows the presence or absence of the elastic sac usually secreted by the oviducal gland. Constant light conditions had obviously inhibited the formation of the elastic sac produced by the barnacle under natural breeding conditions.

Table 1. ELASTIC SAG FORMATION IN OVIDUCAL GLANDS OF BARNACLES KEPT UNDER LIGHT AND DARK CONDITIONS IN A LOW-TEMPERATURE ROOM

Date	Conditions	Elastic sac in oviducal gland
September 29	Aquarium room	Absent
October 26	${ m Light \atop Dark}$	${ { m Absent} \atop { m Present} }$
November 12	${ m Light \atop Dark}$	$\begin{cases} Absent \\ Present \end{cases}$
December 8	${ m Light \atop Dark}$	Absent Egg mass

Table 2. ELASTIC SAC FORMATION IN OVIDUCAL GLANDS OF BARNACLES

TRANSPERRED	TU	COTDIDVER	CONDITIONS ON	JANUARY	0	
Date			State of	gland		
January 6 January 12 January 19 January 26 February 3			No sac Secretion Sac being form Sac Egg mass in m		ty	,.

Another series of barnacles from the original population was kept in a warm laboratory in an aerated static-sea water tank until January 6. The temperature of the sea water varied between 16° and 20° C and the barnacles were illuminated daily from 0800 h to 1730 h. Breeding had not occurred by January 6 and the elastic sac had not been produced in the oviducal glands. The barnacles were then placed under dark conditions in the low-temperature room and sampled at intervals. Breeding had occurred by February 3. Table 2 shows the conditions of the oviducal glands in these barnacles.

These results show that the effect of light on the breeding of B. balanoides is, in part, to inhibit cyclic activities of the oviducal gland. This is unlikely to be a direct photo-effect on the gland, so it is probable that there is inhibition from the photo-receptors and nervous system. A possible mechanism is indicated by the observation that when barnacles are illuminated the photo-receptors generate a sustained depolarizing potential which inhibits second-order neurones in the supra-oesophageal ganglia; this inhibition is removed when the illumination is stopped3. There may be a neurosecretory role in this control, for neurosecretory fibres have been reported in the median photo-receptor nerve of Balanus cariosus⁴ and I have found neurosecretory cells in the sub- and supra-oesophageal ganglia of B. balanoides.

The role of temperature in the inhibition of breeding of B. balanoides is uncertain. The absence of elastic sac formation in the barnacles kept until January 6 under a less than critical light exposure, yet in warm conditions, indicates that temperature has an inhibiting effect on

The control mechanism for oviducal gland secretions. this could be analogous to that in the Cecropia silkworm Hyalophora where there is a low-temperature requirement (6°-15° C) before neurosecretory cells will initiate the termination of pupal diapause5.

At present little is known about endogenous control systems in Cirripedia. My observations indicate a possible control mechanism for breeding in B. balanoides which would bring cirripedes into line with the general pattern of endocrine control in Arthropods and provide a basis for further experiments.

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Monosynaptic Stochastic Stimulation of Spinal Motoneurones in the Cat

THE artificial nature of a synchronous afferent volley was pointed out many years ago1 and, although neurophysiological knowledge has been considerably advanced by use of the synchronous volley technique, it has definite limitations in providing a full understanding of reflex mechanisms in the intact animal. Each annulo-spinal ending in a muscle under tension discharges in a quasiperiodic manner, and the differing stretch thresholds in these receptors² cause the average discharge frequency of each ending to differ. The timing of the initiation of impulses in one ending will be statistically independent of

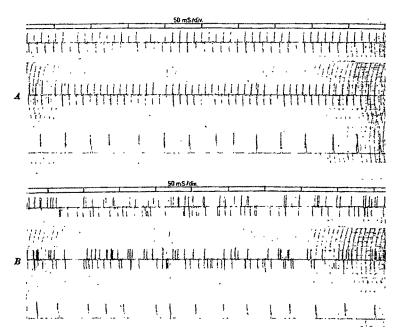


Fig. 1. A, Pen recording showing four asynchronous trains of periodic stimuli, each at a nominal frequency of 100/sec, and the simultaneous discharge in a ventral root filament. Individual stimulus strengths were confined to less than max. GpI, and adjusted such that simultaneous stimuli processes on at least three separate pathways were necessary for the cell to discharge. Stimulus impulse deletions indicate where two combined stimulus impulse trains drift into phase. B, Four independent Poisson or random stimulus impulse trains, with mean impulse rates approximately 100/sec, applied in place of the four periodic processes in A. All other stimulus and recording conditions are identical to those in A.

the times of occurrence of impulses in other endings. The convergence of these asynchronous impulse patterns onto a particular motoneurone represents a situation very different from that of synchronous stimulation and introduces the effects of temporal summation. If the object of the experiment is to establish relationships between afferent and reflex impulse patterns, natural stimulation by stretching muscle has severe limitations, because the temporal impulse patterns arriving at a particular motoneurone cannot be obtained. In the experiments described here, an attempt has been made to overcome this problem while retaining a stimulus which is physiologically realistic.

All experiments were performed on cats which had been made spinal under ether anaesthesia. Many branches of posterior biceps-semitendinosus or gastroenemius-soleus muscle groups were prepared for stimulation by fine dissection down into the muscle. A motoneurone responding monosynaptically to synchronous stimuli on either of these nerve groups was isolated by way of a ventral root filament. Only afferent branches which caused a detect. able depolarization of the motoneurone were used in the experiment. These branches were found by the presence of a reflex spike when the depolarization caused by stimulation of the branch under study was added to a slightly subthreshold depolarization produced by synchronous stimulation of other effective branches. Each useful afferent branch was then separately stimulated by either a periodic or random pulse process such that the stimulus processes for each nerve branch were asynchronous. Stimulus strengths were usually much less than maximum GpI and were adjusted to ensure that at least two separate afferent fibre groups required simultaneous stimulation to produce sufficient summation to evoke a reflex discharge. Stimulus frequencies were varied between 10/sec and 200/sec. The individual stimulus frequencies were adjusted to be within ± 10 per cent of a nominal stimulus frequency.

The nature of asynchronous or stochastic stimulation

is illustrated in the pen recordings in Fig. 1. These were obtained by replaying tape recorded stimulus processes and ventral root discharge at a reduced speed and combining two stimulus trains onto a sing'e pen, using polarity to distinguish between them. Fig. 1A shows the effect of applying four trains of periodic stimuli, of slightly different frequency, and randomly phased to four afferent nerve groups of the cell the discharge of which is being recorded. In the experiment recorded in Fig. 1B, the same cell was subjected to stimulus processes of random or Poisson type with the same average stimulus rate and which were delivered at the same stimulus intensity as in the previous case. In both cases, temporal and spatial summation combine to discharge the cell, and the discharge is noticeably regular in its interspike interval duration. A slowly discharging cell does not have the same regularity.

The long term discharge response of motoneurones stimulated with a suddenly applied and maintained stochastic stimulus shows considerable variation. Recordings from the same motoneurone pool with constant stimulus conditions provide discharges which vary from extremely phasic to extremely tonic in nature, with all intermediate combinations of these discharge characteristics. These results suggested a continuous distribution between tonic and phasic properties within a functional motoneurone pool, as other investigators^{3,4} have recently suggested, rather than a distinct division into tonic and phasic motoneurones.

Motoneurones which discharge tonically were stimulated with a range of frequencies and the stimulus strengths were kept constant on all stimulated pathways. At least 60 sec of continuous recording was made at each stimulus frequency and the mean discharge frequency was calculated from the entire record. The relationship is shown in Fig. 2, where a curve has been drawn for periodic asynchronous stimulation and points are shown for the Poisson pulse rates which were available. This and other similar results indicate an approximately exponential relationship between the mean afferent and efferent frequencies in the afferent range of 30-120/sec (an important physiological range). Frequency limiting usually occurs at an efferent frequency of 10-15 impulses/sec and the afferent frequency at which this discharge rate occurs depends on the individual stimulus intensities. threshold is exceeded by three, but not two, afferent groups which have been simultaneously stimulated, the stimulus frequency is usually in the range of 100-120 pulses/sec. This frequency limiting has been a consistent finding with various electrical stimulation methods involving GpI fibres only^{8,7}. Frequency limiting has previously been ascribed to the effects of recurrent inhibition8. In this experimental arrangement, the motoneurones of the excited pool will excite Renshaw cells asynchronously and recurrent inhibition will be operative. experiments measuring frequency transfer characteristics before and after the infusion of dihydro-beta-erythroidine (hydrobromide) indicate that recurrent inhibition does not change the frequency at which limiting occurs. We believe that repolarizing current during "after hyperpolarization" and the depolarization limitations of the Ia afferent pathways are the principal factors in limiting the discharge frequency.

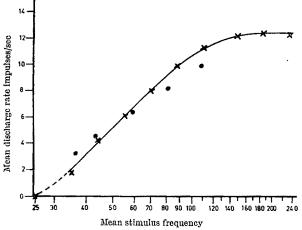


Fig. 2. Results taken from a unit responding tonically to stimulation of gastroenemius-soleus nerves. All branches of medial and lateral gastroenemius and soleus muscle groups were arranged on four separate stimulators. The stimulus strength on each was adjusted such that, at a nominal frequency of 100/sec for each stimulator, all but two combinations of pairs were effective in discharging the cell when asynchronous stimulation was applied from two stimulators at a time. Measurements were taken in order of increasing frequency, with a 3 min period without a stimulus between each record. No discharge was obtained at a mean stimulus frequency of 25/sec, which was the next available frequency below 35/sec. The discharge could have ceased at an intermediate frequency. There was no alteration in stimulus strength when Poisson stimuli were used. ×, Asynchronous periodic stimulation; •, Poisson stimulation.

The frequency transfer curve specifies the stationary transfer properties of a single monosynaptic reflex pathway with a fixed number of stimulated afferent fibres. A more complete description of the reflex pathway would be a family of frequency transfer curves with "recruitment" as a parameter.

The technique of stochastic stimulation offers a method of establishing reflex discharges from neurones excited by means of converging peripheral pathways in a controlled and approximately physiological manner. Modulation in the excitability of the neurone may then be quantitatively measured by changes in the discharge frequency of that cell. A precise knowledge of the afferent temporal patterns is also helpful in investigating input-output relationships.

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Strychnine Block of Neural and Drug-induced Inhibition in the Cerebral Cortex

THERE have been conflicting reports about the actions of strychnine on cortical inhibitions. Inhibition of cortical neurones by direct cortical stimulation is resistant to strychnine^{1,2}. The "recurrent" inhibition of cortical neurones during stimulation of the pyramidal tract has been said to be both resistant to strychnine^{1,3} and antagonized by strychnine⁴⁻⁷.

This investigation of the actions of strychnine on neural and drug-induced inhibitions in the cerebral cortex was started when we realized that strychnine antagonized the depressant actions of noradrenaline and 5-hydroxytryptamine (5-HT) on neurones in the left precruciate cortex.

All experiments were performed on cats anaesthetized with nitrous oxide and methoxyflurane ('Penthrane', Abbott). Drugs were applied iontophoretically from five barrelled micropipettes, the central barrel of which was used for recording the extra cellular spike potentials of cortical neurones. A Hewlett-Packard electronic counter coupled to an ink-recorder was used to analyse the discharges of cortical neurones which were either spontaneous or evoked by L-glutamate. L-Glutamate pulses of 5-10 sec duration were repeated at regular intervals. Changes in cell excitability were assessed by comparing the spontaneous firing frequencies or those induced by L-glutamate, during and after the application of depressant compounds or during stimulation. The duration of inhibition evoked by direct stimulation was measured as the period of suppression of spontaneous or induced firing. Ipsilateral stimulating electrodes were placed in the lateral hypothalamus (All. 5, L4, D-3), mesencephalic reticular formation (A3, L3, D-1) and on the exposed pyramidal tract. A fine bipolar co-axial electrode was inserted directly into the precruciate cerebral cortex to a depth of 1 mm for cortical stimulation.

Strychnine was applied iontophoretically from a barrel containing 10 mmolar strychnine in a solution of 200 mmolar sodium chloride. When applied by currents of 20-50 n.amp, strychnine had a depressant action on neuronal activity. Firing induced by L-glutamate was

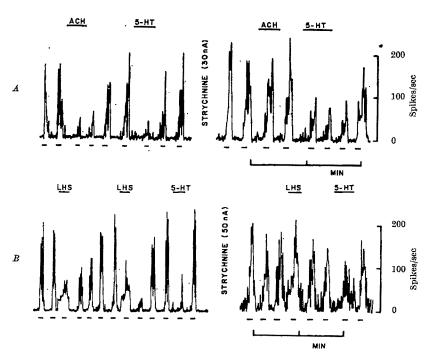


Fig. 1. A, Effect of strychnine (30 n.amp for 2 min) on the depression of L-glutamate (40 n.amp) firing by 5-HT (30 n.amp) and ACh (30 n.amp). B. Depression of L-glutamate (40 n.amp) firing by repetitive (10/sec, 6 V) stimulation of lateral hypothalamus and 5-HT (40 n.amp) was antagonized by strychnine (50 n.amp for 2 min). Applications of L-glutamate or 5-HT and ACh are indicated by horizontal lines below and above the records, respectively. Recording was interrupted during and for a period of 3 min immediately after application of strychnine

depressed for 2-3 min after strychnine application had Cell excitability was frequently enhanced after ceased. this.

The depressant effects of 5-HT, noradrenaline and acetylcholine (ACh) on cortical neurones have already been described⁸⁻¹⁰. On animals anaesthetized with nitrous oxide and 'Penthrane', all three compounds have potent depressant actions. 5-HT depressed 89 per cent of the 131 neurones tested, noradrenaline depressed 49 per cent of 43 neurones tested and ACh 32 per cent of 162 neurones tested. On many of these cells the actions of the depressant drugs could be reduced or abolished by strychnine. Examples of a reduction of depression by 5-HT and almost complete block of depression by ACh are shown in Fig. 1A.

Long lasting inhibitory effects of repetitive stimulation of pyramidal tract or reticular formation on cortical neurones which were also depressed by ACh have been described elsewhere¹⁰. Application of atropine or hyoscine to such neurones usually abolished the response to ACh and reduced or abolished the neurally evoked inhibitions. Strychnine has now been observed to have a similar effect on these inhibitions.

Destruction of tissue in the lateral hypothalamic nucleus is associated with a marked decrease in the concentrations of noradrenaline and 5-HT in the neocortex of cats11,12. It seems that cats have a monoaminergic pathway from the brain stem to the cortex through the medial forebrain bundle, which has been described in rats13.

Stimulation in the lateral hypothalamus evokes short latency negative focal potentials in the feline precruciate neocortex which seem to have a maximum amplitude between depths of 800-1,300μ. A short latency inhibition lasting 100-150 msec was also frequently observed. Less usual (in sixteen of the seventy-one cells tested) was a long lasting inhibition of firing, both spontaneous and induced by L-glutamate, which had a duration of up to 1 min after stimulation had ceased (Fig. 1B).

The short latency inhibition associated with stimulation of the lateral hypothalamus was, like the comparable inhibition evoked by direct stimulation. resistant to strychnine (see also refs. I and 2). On all occasions on which it was tested, however, strychnine abolished the long lasting inhibition evoked by stimulation of the lateral hypothalamus. An example of this is shown in Fig. 1B. Stimulation of the lateral hypothalamus (10/sec for 15 sec) greatly reduced the response of this cell to L-glutamate pulses, and 5-HT almost abolished the Strychnine (50 n.amp) was applied for 2 min and, when recording continued 3 min later, stimulation of the lateral hypothalamus had an excitant action and the depressant action of 5-HT was greatly reduced.

It is apparent from these results that strychnine depresses some synaptic inhibitions in the cerebral cortex and not others. In particular, the long lasting inhibitory effects of stimulation of pyramidal tract-reticular formation and lateral hypothalamus seem to be depressed while the relatively short inhibitory effects of stimulation of the surface and the lateral hypothalamus are unaffected. In a similar study of the effects of strychnine on the depression of Renshaw cells in the spinal cord. no antagonism between strychnine and noradrenaline was observed although synaptic inhibitions were reduced 14. In these experiments, the amounts

of strychnine used were not great enough to produce extracellular concentrations which alone reduced the sensitivity of Renshaw cells to excitants and this may be an explanation of the different results obtained. The full significance of these actions of strychnine on neural and drug-induced inhibitions has yet to be evaluated. The fact that strychnine will antagonize depressions produced by such disparate compounds as the monoamines and ACh, however, suggests that it may effect the membrane permeability changes induced by these compounds.

These results also support the idea that 5-HT and noradrenaline containing pathways project from the lateral hypothalamus to the cerebral cortex.

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RADIOBIOLOGY

Suppression of Experimental Allergic Encephalomyelitis in Mice by Irradiation of the Target Organ

THE pathogenesis of experimental allergic encephalomyelitis (EAE) is not definitely known and yet it is frequently used as an experimental model for the study of autoimmune disease of the central nervous system. Waksman contends that the principal event giving rise to EAE after injection of the antigen is the passage of immunologically competent cells or histiocytes through small venules into the nervous system¹. These cells originate in the blood stream and are held responsible for the destruction of elements containing antigen in the central nervous system by an undefined mechanism. It has been found that EAE can be suppressed, either by generalized physical or chemical cytotoxic agents, or by destruction of the regional lymph nodes draining the inoculation site²⁻⁹. This communication reports the suppressive effect of localized irradiation of the brain in mice during the induction of EAE, and compares the effect with that of total body irradiation.

Random bred adult $BSV\tilde{S}$ mice from the mouse colony of Rockefeller University were used. The mice were sensitized by intraperitoneal injection of Haemophilus pertussis vaccine and 4 days later they were given an intradermal injection of mouse brain with adjuvant, which was repeated 10 days after the first sensitization. The mice were irradiated at intervals after the first sensitization, with varying doses administered to the total animal, to the head only or to the body only. A typical schedule of sensitization and irradiation is shown in Table 1. The techniques of sensitization and irradiation have been described in detail in previous publications^{10,11}. The irradiated animals were divided into two groups; one received a high dose (400 r. × 5) and the other a low dose (100 r. × 5). Among the controls used, some were sensitized and not irradiated and others were irradiated but not sensitized. All animals were killed 30 days after the first sensitization and their brains were removed for histological examination. The severity of the pathological change in the brain was graded from 0 (negative) to 5+ (marked positive). As a control for the effect of irradiation on immune reactions in mice, reciprocal skin homografts were performed in BSVS mice which were then given irradiation to the head only in the same schedule as that employed for the EAE animals. The lesions in the sensitized BSVS mice were those characteristic of EAE-infiltration of the meninges, ependyma and perivascular spaces with lymphoid cells¹². These were designated mild positive or 1+, positive or 3+ and marked positive or 5+. All the sensitized non-irradiated animals had such lesions, and these were predominantly 3 to 5+ in severity. Irradiation of the head only did not prolong the rejection times of reciprocal skin grafts in the $BS\,VS$ mice.

The results of total body irradiation, head irradiation only or body irradiation only at high irradiation dose (2,000 r.) are shown in Table 2 and the results of low irradiation dose are shown in Table 3. The lesions in the irradiated mice are usually predominantly mild (1 to 2+) as shown in Tables 4 and 5. The lethal effect of total body irradiation is easily seen. In the sensitized animals receiving 2,000 r. to body and head, six out of seventeen died before day 15, possibly too early for pathology of EAE to be evident. The deaths were probably caused by the effect of total body radiation.

Table 1.	SCHEDULE	OF SENSITIZATION-IRRADIATION EXPERIMENT
Day		Treatment
-4 0 3 7	•	H. pertussis vaccine intraperitoneally Mouse brain and adjuvant intracutaneously Irradiation treatment 1 Irradiation treatment 2
10		Mouse brain and adjuvant intracutaneously
11 15		Irradiation treatment 3 Irradiation treatment 4
20 30		Irradiation treatment 5 Mice killed for histological examination

Table 2. EFFECTS OF HIGH IRRADIATION DOSE (2,000 r.) ON EAF IN MICE

Group	No. of mice	pathology per cent positive
Sensitized No irradiation	32	100
Sensitized Head irradiation only	38	63
Sensitized Body irradiation only	18	56
Total irradiation only	16	31
Not sensitized Head irradiation only	12	0
Not sensitized Body irradiation only	· 13	. 0

Table 3. EFFECTS OF LOW IRRADIATION DOSE (500 r.) ON EAE IN MICE

Group	No. of mice	pathology per cent positive
Sensitized No irradiation	32	100
Sensitized Head irradiation only	19	74
Sensitized Body irradiation only	20	65
Not sensitized Head irradiation only	8	0
Not sensitized Body irradiation only	8	0

Previous workers² have shown that excision of regional lymph nodes at any time in the first 2 days after sensitization prevented the development of EAE and there was a significant reduction even in animals whose nodes were not removed until 12 days after sensitization. Physical cytotoxic methods such as irradiation of the regional lymph nodes 4 days before sensitization also reduced the incidence of EAE while irradiation of other nodes or irradiation after sensitization had no effects. Whole body irradiation administered to rats before sensitization was effective in preventing EAE while irradiation after sensitization was not as effective. Mice

Table 4. Incidence and severity of lesions in sensitized and unsensitized mice receiving high x-ray (2,000 r.) to head, body, or head and body Effect of high X-ray on EAE in BSVS mice

		1311000	Or HIGH ME ING ON ME				
	0	±	+	++	+++	++++	++++
Sensitized Head	00000	0	00000	0	00000	• 0	000•
Body	00000	• •	••	•	••••		
Head and body	0 • • • • • * * * *		•••*		•		
	*						00000
No X-ray		00	0000	00000	0000	000	00000
Not sensitized Head	00000				•		
Body	00000 000** **	0					

X-irradiated on days 3, 7, 10, 14, 20 (2,000 r. total)

Killed (paralysed or end of experiment).

Death-day 15 to day 30.

Death before day 15.

Table 5. Incidence and severity of lesions in sensitized and unsensitized mice receiving low x-ray (500 r.) to head or body

		тапесь	OI IOW A-149 OH 12.	ME IN DO A PUMP	,		
	0	±	+	++	+++	++++	++++-
Sensitized Head	00000			•	000	0	0000
Body	00000 00	0	0000	•	00		00000
No X-ray		00	00	00	000	0	20000
Not sensitized Head Body	00000 00000 0000						•

X-irradiated on days 3, 7, 10, 14, 20 (500 r. total) O Killed (paralysed or end of experiment).

• Death-day 15 to day 30.

are sensitive to irradiation, and soon die when large doses are administered to the whole body. When irradiating the head alone, we took care not to produce necrosis of the pharynx and subsequent inability to eat and swallow. Two doses of radiation were selected in order to find a level which would be effective in suppressing EAE and yet would enable the mice to survive. At 500 r., more mice survived but there was a slightly higher incidence of positive lesions in the brains than when only the head was irradiated. By increasing the dose to 2,000 r., there were fewer brain lesions but more animals died from radiation sickness.

Chemical cytotoxic agents such as methotrexate, 6-mercaptopurine, corticosteroids and nitrogen mustard administered systemically have also been successful in various degrees in suppressing or preventing EAE4,5,7-9.

All these methods seem to be acting through a general suppression of the reticuloendothelial system and immune phenomena. There are obvious disadvantages in this and it was considered feasible to prevent EAE by action of immunosuppressive or cytotoxic agents aimed at the target organ or the brain itself. Neural tissue is very resistant to effects of irradiation while lymphoid elements are very sensitive. In previous experiments, the attempted immunosuppressive effect has been directed at the cellular or humoral sites which produce antibody. this study, the immunosuppression has been directed at the point of attack of the antibody or the target organ and a significant degree of suppression of EAE has been achieved. It would not be improper to conclude from these results that Waksman's theory is valid. The entrance of sensitized lymphocytes into the nervous system is impaired by irradiating the head which destroys these cells, and hence the disease is suppressed.

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BIOCHEMISTRY

Quantitative Evaluation of Tritium in Autoradiography and Biochemistry

TRITIUM emits 6-particles of such low energies that absorption even in very thin layers of organic matter has to be taken into account. Calculations of the absorption of β-particles within a layer in which tritium is incorporated and in a superimposed layer of organic matter are presented in this communication. For a biological specimen the absorption can be taken as proportional to density.

The emission spectrum of tritium was computed using a nuclear coulomb factor, F(Z, W), evaluated from the National Bureau of Standards tables1.

 $N(W) dW \propto F(Z, W) \cdot (W^2 - 1)^{\frac{1}{2}} \cdot (W_0 - W)^2 \cdot W \cdot dW$ where N(W) dW =the number in interval dW, $W = m/m_0$ for the electron, and W_0 corresponds to the maximum electron energy assumed to be 18 keV.

The average kinetic energy as obtained from this calculation was 5.7 keV, in agreement with the evaluation of Jenks et al.2, the spectrum having a peak at 2.3 keV (Fig. 1). This peak is close to the experimental and theoretical values of Curran et al.3. For electrons of these energies the ratio of energy loss by radiation to that by ionization is less than 2×10^{-4} . The percentage of the particles traversing a given thickness of material, and their emergent energies, can therefore be estimated using equations (2) and (3),

Range = $R(mg/cm^2)$ = 412 · $E^{(1\cdot265-0\cdot095\cdot\ln E)}$ (2)where E is the kinetic energy in MeV.

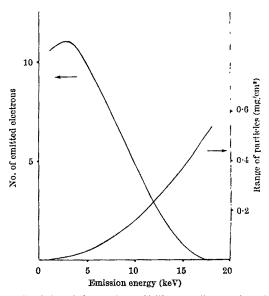


Fig. 1. The beta emission spectrum of tritium, and the range in mg/cm² of the emitted particles. The range shown is an average for particles of that energy.

Energy loss =
$$\frac{\mathrm{d}t}{\mathrm{d}s} = \frac{6.728}{W} \times \ln(W \times 17.33) \,\mathrm{keV}/\mu$$
 (3)

A series of calculations was made on an Elliott 803 computer for an evenly loaded layer of thickness L, covered by an unlabelled layer of thickness t (Fig. 2). For emission from tritium the percentage (P) of emitted particles reaching the surface and their energies were found (Fig. 3). For L < t/2 and $t < 3\mu$ a fit (within 2 per cent) to the curve is given by

$$P = \frac{1}{2}(48e^{-2\cdot3937t} + 51e^{-6\cdot115\sqrt{t}} + 0\cdot0058t^2 - 0\cdot0832)$$
 (4) t is in μ for material of density $1\cdot5$ g/cm³. If $t < 1\mu$, only the first two terms are significant. For thick, evenly loaded tissue (Fig. 4) the percentage reaching the surface is

$$\int_{t_1}^{t_2} P.dt / \int_{t_1}^{t_2} dt$$
 (5)

If the thickness is $T \mu$, this equals

$$P = \frac{1}{2T} \cdot \left| -20.04 e^{-2.3937t} - 16.68t^{\frac{1}{6}} \cdot e^{-6.115\sqrt{t}} - \right|$$

$$2.729e^{-6.115\sqrt{t}} + 0.0019t^3 - 0.0832t \begin{vmatrix} t_2 \\ t_1 \end{vmatrix}$$
 (6)

For $t_1 = 0$ and $T > 4\mu$ this reduces to $12 \cdot 15/T$.

These computed results for the percentage of β-particles emerging agree very well with the experimental results of Fitzgerald et al.⁴ for an aluminium absorber; the discrepancy is less than 3 per cent if "zero" absorber thickness is taken as corresponding to 0·004 mg/cm² of absorber. Maurer et al.⁵ plotted grain density against absorber thickness, and obtained a curve similar to the one in Fig. 4. Discrepancies between Maurer's curve and the one in Fig. 4 can be explained by the variation of grain yield with the energy of the emergent β-particle. Ada et al.⁶ measured the grain yield per emergent electron, using protein films of various thicknesses and of density 1·3 g/cm³.

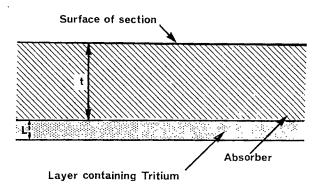


Fig. 2. Position of layer containing tritium within the section.

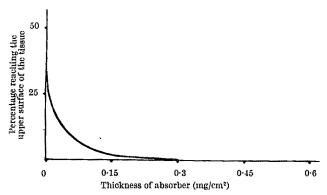


Fig. 3. Absorption, within the tissue, of the beta particles from a layer containing tritium. The thickness of this layer is 0.0075 mg/cm².

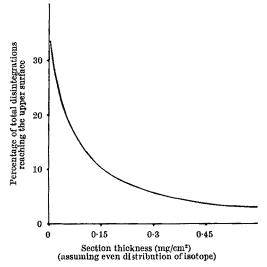


Fig. 4. Absorption, within the tissue, when the tritium is evenly distributed throughout the tissue.

They obtained values ranging from 0.28 grains/emergent electron for 0.5μ to approximately 0.65 grains/emergent electron at 5.0μ . Oja et al. measured the efficiency of K5 emulsion with sections of liver labelled with tritium. If a density of 0.75 g/cm² is assumed for their tissue, then their results are compatible with the values given for P in Fig. 4.

Maurer et al. found "infinite thickness" to be 0.05 g/cm^2 , that is less than 3μ of nucleoplasm, 2μ of cytoplasm and 0.6μ of nucleoli. In any section over which grain counts are made, therefore, small variations in thickness and the differing densities of the organelles, intercellular spaces, and so on will make quantitative evaluation of the tritium present impossible unless a direct tritium assay is undertaken.

Usually it clearly cannot be assumed that a tracer is evenly distributed in a specimen of even thickness of material of the same density. To estimate the influence of different distributions of tritium within a structure, such as a cell, it was assumed that the area was subdivided into ten equal areas and that the tracer was spread differently over these areas (Table 1). For example, if all the tritium was in a homogeneous layer 4μ thick the relative number of β-particles emerging towards an emulsion is 0.25, while if all the tracer is redistributed in a layer 0.25μ thick the relative yield is 1.52, that is six times greater. This apparently extreme and somewhat artificial model is encountered when interphase nuclei are labelled with tritiated thymidine and compared with the resultant labelled mitoses in squashes.

				Ta	ble 1				
Thickness (µ)	4	3	2	1	0.5	0.25			β/day
Thickness $(\mu g/cm^2)$	400	300	200	100	50	25	Total β/day	Rel. vol.	unit loading
β /unit area	1	0.97	0.93	0.81	0.57	0.38	(1)	(2)	(1)/(2)
No. of areas	10 5 3 1 5 6 2	5 1 2 1	$\begin{smallmatrix}1\\2\\2\\2\end{smallmatrix}$	1 22 5 5	1 1 4	1	10 9·38 8·65 8·97 7·73 9·01 7·54 8·92	40 35 26·75 24·5 15 25 25 22	0·25 0·28 0·32 0·37 0·52 0·36 0·30 0·40
	1	1	1	1	1	1	1 0·97 0·93 0·81 0·57 0·38	4 3 2 1 0·5 0·25	0·25 0·32 0·46 0·81 1·14 1·52

Density of material assumed to be $1\cdot 0$ g/cm³. Concentration of tritium was assumed to be constant per unit density. An even layer of 4μ was assumed to have ten emerging β per day. If a thin layer containing tritium is covered by unlabelled tissue, the figures in the final column, (1)/(2), will be further reduced.

The grain yield per electron for energies from 0 to 18 keV is not known and a further correction may have to be applied before the results of our calculations can be converted to number of grains per disintegration.

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to which the paramagnetic label is connected. When an iminoxyl free radical with a covalent bond is fixed in relation to the rigid tertiary structure of the protein by additional hydrophobic forces, the electron spin resonance spectrum is almost the same as the electron spin resonance signal of iminoxyl free radicals in rigid glasses. In other cases where there is a much greater mobility of a free radical attached to a protein, the electron spin resonance spectra can also be recorded.

In this work, we used a radical which attaches to SH protein groups with a high degree of specificity.

Electron Spin Resonance Investigation of Conformation Changes in Serum Albumin with the Help of Iminoxyl Paramagnetic Label

In iminoxyl free radicals¹ there is an odd electron localized usually at the nitrogen-oxygen bond. Because of the tensor anisotropy of dipole-dipole interactions and g-tensor anisotropy, the electron spin resonance spectrum of these radicals is quite sensitive to changes in the mobility of free radicals. The correlation time, τ , which can be found by width analysis of certain components of the electron spin resonance spectrum, is a characteristic of the tumbling motion rate of the free radicals².³. Using iminoxyl free radicals, with various substituents, reacting specifically with certain functional groups of proteins, it is possible to obtain free radicals labelled with macromolecules⁴.⁵

Because the correlation time of the free radicals is short in comparison with the rotation time of the protein macromolecules in solution, electron spin resonance spectra are essentially influenced only by the mobility of the free radical in relation to the protein molecule and the rate of mobility of the macromolecule chain fragment

One of its merits, extremely high stability, should be mentioned. It hardly changes its properties even after being kept in the open air for 2 yr.

The method of attaching a paramagnetic label to the molecules of bovine serum albumin (Koch Light Laboratories, Ltd.) was similar to that described by Griffith and McConnel⁵.

The electron spin resonance spectrum of the iminoxyl free radicals attached to molecules of bovine serum albumin (Fig. 1b) corresponds to a situation where a free radical is completely immobilized relative to the protein molecule. In protein globules the polypeptide chains are twisted in a very complex manner, and therefore the points of application of the additional bonds, immobilizing the paramagnetic label, are not necessarily in the same fragment of the polypeptide chain to which the free radical is attached. This is why it is quite possible that even small changes in tertiary structure of proteins may cause a relative rise in the mobility of the free radicals. It is known that to a certain extent the tertiary structure of proteins can be changed independently of the secondary structure. This can be seen in the action of polar solvents of the dioxane type compared with water. In proteins with many intermolecular sulphur-sulphur bonds, these polar solvents modify the tertiary structure, but do not

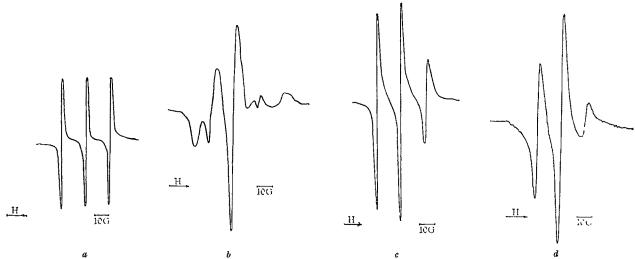


Fig. 1. a, Electron spin resonance spectrum of iminoxyl free radical in a water solution. b, Electron spin resonance spectrum of iminoxyl free radical attached to molecules of bovine serum albumin, 0.1 molar phosphate buffer, pH 6.8. c, Electron spin resonance spectrum of iminoxyl free radical attached to molecules of bovine serum albumin in a 10 molar solution of a urea. d, 50 per cent dioxane.

substantially affect the melting temperature of the α spiral. In 65 per cent dioxane the axis relation of serum albumin, a/b, increases three-fold compared with water solution of the protein. The percentage of spiral fragments increases by 1.5 at the same time.

Adding sufficient quantities of urea or dioxane to the solution of bovine serum albumin, labelled with free radicals, eliminates the wide signal of the immobilized paramagnetic label in both cases, and gives a narrower signal of a radical possessing relatively great mobility (Fig. 1c, d). Urea, however, destroys the secondary as well as the tertiary structure of bovine serum albumin and gives a greater growth of mobility of a free radical than the dioxane which chiefly modifies the tertiary structure of protein. τ , calculated for these two cases, was $1\cdot09\times10^{-8}$ sec for bovine serum albumin in 10 molar urea, and 2.04×10^{-4} sec for bovine serum albumin in a 50 per cent water-dioxane mixture.

 $ilde{\mathbf{A}}\mathbf{s}$ the relationship of narrow components in the process of denaturation did not change substantially and only the share of relatively free and completely immobilized free radicals did, the areas of the narrow and wide signals, S_2 and S_1 , respectively, can be easily determined, knowing their relationship to one of the corresponding components in the case of molecules of native and fully denatured bovine serum albumin.

The curve of urea denaturation of bovine serum albumin (Fig. 2) represents a typical melting curve of the protein

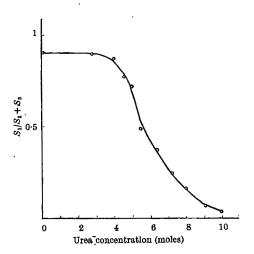


Fig. 2. Dependence of $\frac{S_1}{S_1 + S_2}$ on urea concentration.

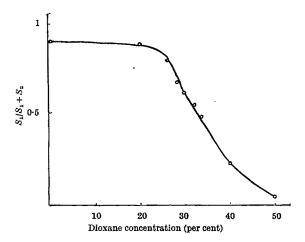


Fig. 3. Dependence of $\frac{S_1}{S_1 + S_2}$ on dioxane concentration.

secondary structure, and coincides well with the relationship obtained from the optical activity measuring method8,9.

The changes in the bovine serum albumin tertiary structure, affected by dioxane, take place in a rather limited range of concentrations of dioxane solution (Fig. 3).

The possibility of studying, with the help of a paramagnetic labelling, the changes in the protein tertiary structure when not accompanied by destruction of the secondary structure may be extremely useful, because the optical activity measurement method is not always applicable. The differences in correlation time for the bovine serum albumin, treated with urea and dioxane, should not be over-estimated, because it is not clear whether the lesser mobility of the iminoxyl free radicals in a water-dioxane mixture is caused by the withdrawing of the label from the point of application of the hydrophobic forces or by a changing flexibility in the fragment of the polypeptide chain to which the free radical is attached. When bovine serum albumin is denatured with urea both these effects must take place, but it is difficult here to estimate the role of each of them.

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Relative Contribution of Carbon Dioxide Fixation and Acetyl-CoA Pathways in Two Nervous Tissues

CARBON dioxide fixation has been demonstrated in nervous tissue1-4, but its importance has not been assessed. By comparing the relative amounts of radioactivity incorporated from pyruvate-1-14C and -2-14C into aspartate and citrate, the relative contribution of acetyl-CoA and carbon dioxide fixation pathways in the conversion of pyruvate to citrate could be assessed.

Based on the two known pathways from pyruvate to citrate, and assuming an immediate randomization of the dicarboxylic acids (Fig. 1), C-1 of pyruvate would enter into citrate as C-1 and C-6 by way of carbon dioxide fixation and oxaloacetate. Both these carbons of citrate would be decarboxylated before reaching succinate in the tricarboxylic acid cycle (TCAC). C-2 of pyruvate would appear as C-2 and C-3 via this same pathway, and as C-5 via acetyl-CoA. All these carbons would be retained in citrate after the TCAC turned over once. Thus the specific activity of citrate from pyruvate-1-14C would represent only the carbon dioxide fixation or oxaloacetate pathway, and the difference between the specific activity of citrate from pyruvate-2-14C and pyruvate-1-14C would represent the acetyl-CoA pathway plus the recycling of

labelled citrate. In aspartate, a similar situation would be true, but the cycling aspect would seem to be emphasized because the acetyl-CoA from pyruvate-2-14C would not enter into aspartate directly. The possibility of the recarboxylation of decarboxylated C-1 of pyruvate should not be great, because carbon dioxide fixation would supply the same carbons of citrate and aspartate as of C-1 of pyruvate. Furthermore, the specific activity of this carbon dioxide would be greatly diluted by the internal non-radioactive carbon dioxide.

In the lobster nerves (Table 1), the specific activity of aspartate (2,430) from pyruvate-1-14C was much greater than the difference of the specific activities of aspartate (750) from pyruvate-2-14C and pyruvate-1-14C. The ratio was 0.3. This implies a low contribution of radioactivity by recycling of the TCAC, and pyruvate appears to be the primary precursor for aspartate and oxaloacetate. Similar results for citrate were 52,000 and 74,000, respectively, and the ratio was 1.4. This implies an almost equal contribution of radioactivity from pyruvate to citrate by means of the carbon dioxide fixation pathway and the acetyl-CoA pathway.

Table 1. Specific activities of aspartate and citrate derived from pyruvate-1- and $2^{-14}\mathrm{O}$

Tissue		Sub- strate m molar	μc./ μmole	Aspartate c.p.m./ µmole	Citrate c.p.m./ µmole
Lobster nerve	(A) Pyruvate-2-14C (B) Pyruvate-1-14C A-B	2 2	2·5 2·5	3,180 2,430 0·3	126,000 52,000 1·4
•	B			0.9	1.4
Rat striatum slices	(A) Pyruvate-2-14C (B) Pyruvate-1-14C	1·5 1·4	3·4 3·5	555,000 42,000	1,230,000 : 114,000
	$\frac{A-B}{B}$			12	10

Lobster nerve or rat brain striatum slices were incubated for 3 h at 15° C or 1 h at 37° C in their Ringer solutions, respectively^{3,2}. After incubation, the tissue was homogenized in trichloroacetic acid, and the protein precipitate was centrifuged off. Trichloroacetic acid was then removed from the supernatant with ether, and the aqueous solution, after removal of the ether, was charged on a 'Dowex-1'-acetate column's. Aspartate and citrate were cluted and their specific activities determined as described previously^{3,10}. Each value represents the average from three experiments.

In the slices of rat striatum, both ratios for aspartate and citrate remained high (Table 1). This suggested a relatively small contribution of the carbon dioxide fixation pathway as well as a relatively high rate of turnover of the TCAC. A greatly simplified estimation placed the ratio of radioactivity incorporation via the carbon dioxide fixation pathway to the acetyl-CoA pathway at approximately 1:10. This finding is in good agreement with those observations in vivo^{5,6} and

(1)
$${}_{,}^{CH_3}$$
 ${}_{,}^{C^{\bullet}OOH}$ $\longrightarrow {}_{,}^{CH_3}$ $-{}_{,}^{C^{\bullet}OO}$ $\longrightarrow {}_{,}^{CH_2}$ $-{}_{,}^{C^{\bullet}OOH}$ $\longrightarrow {}_{,}^{CH_2}$ $-{}_{,}^{C^{\bullet}OOH}$ $\longrightarrow {}_{,}^{COH_2}$ $-{}_{,}^{COOH}$ $\longrightarrow {}_{,}^{CH_2}$ $-{}_{,}^{COOH}$

Fig. 1. Schematic diagram of the pyruvate to citrate pathways showing only the paths of C-1 (×) and C-2 (•) of pyruvate. (1) Acetyl-CoA pathway with acetyl-CoA as the intermediate and the decarboxylation of C-1 of pyruvate. (2) Carbon dioxide fixation pathway with oxaloacetate as the intermediate and the randomization of the oxaloacetate carbon via the dicarboxylic acid shuttle.

indicates that the principal route of pyruvate metabolism in brain is via acetyl-CoA.

Oxaloacetate (or malate) has been generally considered as a key intermediate in the TCAC. The loss of carbons from the TCAC must ultimately be replenished by the carbon dioxide fixation reaction to yield oxaloacetate (or malate). In the lobster nerve, oxaloacetate was considered as the primary product of carbon dioxide fixation?. The magnitude of this reaction, as measured by the amount of radioactivity incorporated from carbon dioxide2 or pyruvate, might be related to a significant loss of TCAC intermediates and related compounds (unpublished). In contrast, in rat brain striatum slices, such loss appeared to be relatively small (unpublished) which agreed well with the low contribution of the carbon dioxide fixation pathway.

Although these two nervous tissues showed a quantitative difference in carbon dioxide fixation and the rates of turnover of the TCAC, the necessity of having carbon dioxide fixation to supply oxaloacetate for replenishing the loss of carbon skeletons during the operation of the TCAC remained the same. The low rate of carbon dioxide fixation in brain slices could account for the difficulty in demonstrating such a vital mechanism.

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Conditions of the Colour Change of Prodigiosin

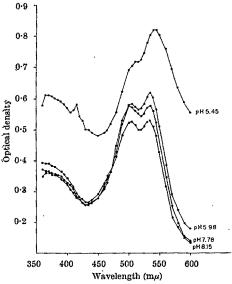
THERE have been several reports that prodigiosin, the pigment produced by Serratia marcescens, is red in acid solution with an absorption maximum at 535-540 mu but is yellow-orange in alkaline solution with an absorption maximum at 470 mm (refs. 1 and 2). This colour charge in strong acid or alkaline solution is recognized by anyone working with the pigment. The observation that the colour of a colony on an agar plate changes from orange to red when the pH, changing with growth, becomes more alkaline rather than more acid, and the report³ that eluates of single pink chromatographic bands show, on redevelopment, separation into both pink and orange bands, suggest that pH is not the only factor involved in the colour change of prodigiosin from red to orar ge.

To investigate this problem, prodigiosin was removed by a single extraction with an organic solvent from

.48-72 h cultures of a red pigmented strain of Serratia marcescens grown aerobically in trypticase soy broth or synthetic glycerol medium. Prodigiosin (30 ml.) was extracted from slightly alkaline cultures with 10 ml. of chloroform. No attempt was made to extract all of the pigment, but rather to remove as little as possible of other cell components; neither was any attempt made to purify the extract and it may have contained more than one pigment. The clear pink extract was distributed into tubes in 10 ml. quantities, and the solvent was removed by evaporation under a vacuum. Dried pigment was stored at room temperature; because of its instability and photosensitivity, it was not put into solution until minutes before use. Dried pigment was dissolved in 0.2 ml. of acetone and then water, buffer or acetone was added as desired. Usually prodigiosin was diluted 1:25 to give an optical density of between 0.5 and 1.0, using the Beckman \hat{DU} spectrophotometer; this is referred to later as a low concentration of pigment. A high concentration of pigment (1:10 dilution) gave an optical density reading greater than 1.0.

Aqueous solutions of high or low concentrations of prodigiosin in M/30 or M/150 phosphate buffer at different pHs are all red to pink in colour and have a peak at 535-540 mu with a shoulder, or distinct peak, at 500-505 mμ throughout a rather broad pH range. These peaks

can be seen in Fig. 1.



Aqueous solutions of a low concentration of prodigiosin in the presence of M/30 phosphate buffer at different pHs.

Large concentrations of prodigiosin in acetone in the presence of M/30 phosphate show red with a glass electrode reading of less than seven and yellow when the reading is greater than seven; acetone solutions containing high concentrations of pigment in the presence of M/150 phosphate are all yellow regardless of the pH of the buffer used. The colour of acetone solutions containing a low concentration of pigment with buffer of either strength remains in the yellow region. A scan of low concentration of pigment in acetone can be seen in Fig. 2. Although the glass electrode readings given are those taken before scanning, a continuous reaction seems to be taking place in the organic solvent and all readings tend to increase with time. At the end of the scanning procedure all values were 7-7.5, regardless of whether the initial adjustment was made with sodium hydroxide or with phosphate buffer. The change seemed to be related somewhat to the amount of prodigiosin used, suggesting that the pigment itself is contributing to this picture. The glass electrode reading of the acetone alone in these conditions remained constant,

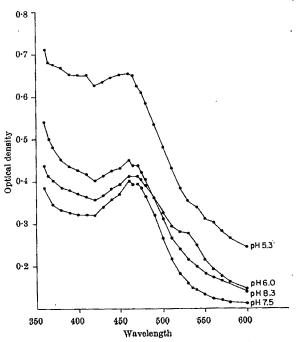


Fig. 2. Acetone solutions of a low concentration of prodigiosin in the presence of M/30 phosphate buffer.

These observations suggest that colour change in prodigiosin may be more closely related to its state of exidation or reduction than merely to pH. Certainly, there are several sites in this tripyrrole structure at which electron transfer could occur. To test this idea, aqueous and acetone solutions of prodigiosin in the presence of phosphate buffer (pH 7-7.5) were mixed with several known oxidizing or reducing substances. Results are shown in Table 1.

In aqueous solution, the only colour change came when known oxidizing agents were added to the pigment. There seems to be a complex interaction with permanganate which changes with time. A scan of the prodigiosinferricyanide mixture shows a flattening and broadening of the 535-510 mu peaks with only a very small peak appearing in the 470 mu region.

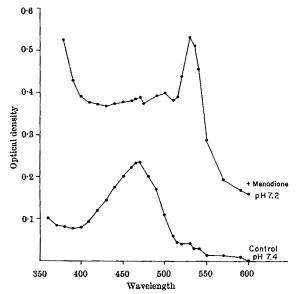
Table 1, COLOUR CHANGE OF PRODIGIOSIN AFTER ADDITION OF OXIDIZING OR REDUCING SUBSTANCES

	Colour of so addition of subs	electrode reading of acetone	
	Aqueous	Acetone	solution ·
Potassium permanganate (0.001 molar)	Orange	Yellow	7.5
Potassium ferricyanide (0-01 molar)	Yellow- orange	Yellow	7.8
Sodium dithionite (0.1 molar)	Pink	Pink	6.3
Ferrous chloride (0.01 molar)	Pink	Pink	6.5
Ferrous sulphate (0.01 molar)	Pink	Pink	6.5
Cysteine (0.1 molar)	Pink	Pink	6-8
NADH ₂ (5 mg/ml.)	Pink	Yellow	7.3
Menadione, bisulphite (0·1 molar)	Pink	Pink	7-2

Prodigiosin was dissolved in acetone, then diluted 1:20 in M/30 phosphate buffered water or acetone. The colour of the pigment in aqueous solution is pink and in acetone is yellow. To 1 ml. of diluted pigment one drop of the various solutions was added as indicated.

In acetone solution, the addition of sodium dithionite, ferrous compounds, cysteine or menadione resulted in a change of colour from yellow to pink. These reducing agents are acid in solution, and so they were brought to neutral pH before they were added to prodigiosin. In some cases, there was a decrease in the glass electrode reading after the colour had changed. These readings, however, were well within the range which may exhibit

the yellow 470 mµ maximum in other conditions (Fig. 2). The scan of the change after the addition of menadione given in Fig. 3 shows the shift of most of the major peak at 470-530 mµ with a smaller peak in this case at 500 mµ. The results are very much the same when iron salts are used. The menadione used was the bisulphite water soluble compound, and further experiments with unsubstituted oil soluble menadione are necessary to determine whether colour change in prodigiosin is also associated with the unsubstituted quinone.



Acetone solution of prodigiosin before and after the addition of menadione in the presence of M/150 phosphate buffer.

One interpretation of these results is that prodigiosin may exist in an oxidized (yellow) state or a reduced (red) state. The aqueous reduced form is not readily oxidized by the compounds used and there may be some breakdown of the pigment, for the absorbance curve is decreased and spread out. In acetone solution there seems to be direct electron transfer between acetone and prodigiosin. When conditions are carefully controlled so that acetone is in excess of pigment and electrons, pigment remains yellow or oxidized; on the other hand, when pigment or pigment plus electrons are in excess of acetone, there is no longer sufficient electron acceptor and the pigment remains in the red reduced state. Such an interaction between acetone and prodigiosin would seem to be a possible biological phenomenon. An enzyme catalysed acetone-NAD-isopropanol reaction was used for most accurate calculations of free energy change in reduction of NAD⁵.

Thus prodigiosin may readily accept and give up electrons in its environment, its colour being related to the electron intensity of the environment. This would the electron intensity of the environment. be compatible with a change in colour resulting from a change in hydrogen ion concentration; it would explain the orange to red colour change of colonies at a time when pH is rising, for reducing products of metabolism are also increasing. Such a mechanism could also account for the continued finding of some orange pigment from red chromatographic bands. In addition, this property would account for the repeated observation that this strain of organisms, in undisturbed deep liquid culture of synthetic medium in which the only carbon source is citrate, produces bright orange pigment at the surface but deep red-purple pigment at the bottom of the culture. The pH throughout the culture is considerably greater than seven at all times, but it is well known that there is more of a reducing environment at the bottom of a deep culture than at the surface.

If prodigiosin can act as an auto-oxidizable electron acceptor, then perhaps it plays a part in the respiration of the organism; this remains to be examined carefully. EMMA G. ALLEN

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Stable Agar by Gamma Irradiation

DRY agar slowly deteriorates during storage, probably because of the degradation partly inherent in it and because of impurities which have not yet been identified, but processing stages designed to reduce sulphate2 and nitrogen seem to increase its stability. Until recently it has been the practice to extract agar from seaweeds immediately after collection. Recent experiments carried out in this institute to determine the effect of gamma radiation on dry seaweeds have led to some interesting observations which are reported here.

Samples (5 g) of various dried (100° C) red seaweeds (22 mesh) collected from the Saurashtra coast were sealed in 'Pyrex' tubes (15×100 mm) and exposed to a 1,000 c. cobalt-60 γ-source for low doses of 0.9-6.4 × 104 r./g. The agar was extracted from these samples in the way we have described before3, and the gel strengths were determined immediately and after various periods of storage. The results obtained, which are shown in Table 1, have led us to the following conclusions. (a) The quality of gel produced increases with dose in the low dose range up to a critical dose and this dose differs from species to species. (b) The instability of agar observed with the various species when stored for up to 2 months is not seen in any of the species which receive gamma irradiations. Furthermore, the effect of gamma radiations—in addition to stabilizing the agar—is to enhance the gelling power which remains steady at that value during the whole period studied. The results can be explained on the basis that the sulphate content in the agar molecule is reduced on irradiation which may result in an increased stability of agar on storage3.

Table 1. STABILITY AND GRLLING POWER OF AGAR BEFORE AND AFTER GAMMA IRRADIATION OF DRY SEAWEEDS

Dose	Gel stre	ngth (g/cmi) versus st	orage period	(days)
$(r./g \times 10^{-4})$	0	15	30	45	60
(21)81.20	ŭ				
			lidiella acero		
0	86 (0.29)*	79 (0.26)	74 (0.24)	70 (0.22)	68 (0.21)
1.43	125 (0.28)	127 (0.30)	124 (0.29)	121 (0·29)	124 (0.30)
2.86	151 (0.30)	154 (0.29)	147 (0.30)	147 (0.30)	149 (0.29)
4.80	145 (0.29)	147 (0.29)	143 (0.29)	147 (0.30)	147 (0.29)
		Gelid	lium micropt	erum	
0	78 (0.43)	72 (0.38)	68 (0.34)	66 (0.31)	64 (0.30)
ĭ·43	106 (0.46)	108 (0.42)	109 (0.44)	110 (0.44)	109 (0.45)
2-86	98 (0.44)	98 (0.43)	97 (0.46)	96 (0.45)	97 (0.47)
4.80	77 (0.45)	76 (0.42)	77 (0-45)	76 (0.43)	79 (0.13)
	Gracilaria milardetii				
0	65 (0.91)	62 (0.88)	60 (0.81)	58 (0.76)	54 (0.72)
1.43	75 (0.89)	73 (0.89)	79 (0-89)	81 (0.90)	76 (0.90)
2.86	89 (0.89)	90 (0.88)	92 (0.88)	88 (0.87)	87 (0.88)
4.80	96 (0.90)	95 (0.90)	97 (0.91)	98 (0.89)	97 (0.88)
6.40	78 (0.91)	79 (0.91)	81 (0.88)	80 (0.88)	77 (0.89)
0.40	19 (0.81)		•		17 (0 00)
		Hyp	nea muscifor	mis	
0	72 (0.88)	66 (0.82)	61 (0.77)	58 (0.74)	54 (0.72)
1.43	82 (0.82)	84 (0.84)	86 (0.86)	82 (0.84)	80 (0.84)
2.86	91 (0.84)	90 (0-83)	88 (0.82)	88 (0.84)	80 (0.85)
4.80	98 (0.81)	97 (0.87)	99 (0.86)	100 (0.82)	102 (0.84)
6.40	101 (0.81)	100 (0.88)	104 (0.89)	100 (0.90)	101 (0.82)
# (The melwards harely to denote the execute of some found (a)					

* The values in brackets denote the amount of agar found (g).

Mean of three values taken of an individual collection, the variation encountered being ±1.0 per cent.

These findings suggest that stable agar can be stored over longer periods by exposing the seaweeds after collection to high energy radiations of very low doses $(0.9-6.41\times10^4 \text{ r./g.})$ in a γ -cell of a Van de Graaff

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Autoradiographic Detection of Reactive Protein-SH and its application to Anuran Haemoglobin Chains

THERE is a need for a simple method of detecting reactive sulphydryl groups in proteins and polypeptides after separation by zone electrophoresis. Most of the methods for detection and measurement of reactive sulphydryl groups are ultimately based on spectrophotometric measurements and only suitable for the sulphydryl groups of proteins in solution. An alternative method for the detection and comparison of the relative sulphydryl content of electrophoretically separable components of a mixture of polypeptides and/or proteins has been devised. This method which eliminates the need for previous isolation and purification of the protein mixture is based on autoradiography of mercury-203 bound to sulphydryl groups. We report here its application to several proteins with a known sulphydryl content and its use to show the absence of these groups in all tadpole haemoglobin chains and their presence in both chains of several frog haemoglobins.

The conditions used for radioactive labelling of proteins or polypeptide sulphydryl groups by reaction with chlormerodrin containing mercury-203 can be varied to suit most electrophoretic systems. Thus low pH and high concentration of urea were employed in these experiments to produce haemoglobin (Hb) chain separation and expose the maximum number of sulphydryl groups. After the addition of chlormerodrin*, the buffered protein solution was allowed to stand for several hours to permit the following reaction

R—²⁰³HgCl+HS—Protein — R—²⁰³Hg-S—Protein where

$$R = NH_2 - C - NH - CH_2 - CH - CH_2 - CH_3 - CH_3$$

After electrophoresis on starch gels, nigrosine or amido black was used to stain for protein. Protein bands which bound mercury-203 were detected by autoradiography. Fig. 1A shows the results obtained using this technique on human HbA, ribonuclease, twice crystallized ovalbumin and insulin. Fig. 1B indicates that ribonuclease and insulin, both of which have disulphide bonds but no free sulphydryl groups, did not bind mercury-203 in these

*We have found evidence for the instability of chlormerodrin in the conditions of starch gel electrophoresis (pH about 2.3). This instability did not interfere with this method, for the protein patterns obtained from electrophoresis of frog or human haemoglobin with or without the addition of chlormerodrin were the same. When chlormerodrin is broken down at low pH, the final product would be protein-8-Ha-Cl. The formation of dimers of the type protein-8-Hg-S-protein was apparently not significant in the presence of the 0.32 normal formic acid with 0.03 normal hydrochloric acid buffer used.

Table 1. BINDING OF MERCURY-203 BY PROTEINS TREATED WITH LABBILED CHLORMHRODRIN

Protein (purity)	Binding of mercury-203	Maximum No. of sulphydryl groups* (ref.)
Frog Hb (see Fig. 2)	++	8-10 (14)
Human Hb (whole haemolysate)	+ +	6 (14)
Ovalbumin (2x crystalline)	++	5 (6)
Aldolase [(NH ₄) ₂ SO ₄ suspension]	++	28 (14)
Cytochrome c (horse heart)	+	0 (1)
Ceruloplasmin (crystalline, human)	+	1 (15)
Mercaptalbumin (crystalline, human)	Trace	0-1 (2)
Bovine albumin (crystalline)	Trace	0-1 (2)
Tadpole Hbs (see Fig. 2)	Trace †	0-1 (14)
Ribonuclease (crystalline)	Q	0
Insulin (amorphous)	0	0
Sperm whale metmyoglobin	0	0

Abbott Laboratories Neohydrin-203 was used; this is chlormerodrin or 1-[3-(chloromercuri)-2-methoxypropyl]-urea containing mercury 203. Sperm whale metmyoglobin was given by Dr F. R. Gurd.

O, No exposure; trace, slight exposure; +, definite exposure; ++, very dense exposure of Kodak royal blue X-ray film in 24 h by the mercury-203 bound to protein. Approximately 5-20 µg of each protein at pH 2-3 in 6 molar urea was treated with excess chlormerodrin containing mercury-203 for 8-10 h before electrophoresis on starch gels.

* Maximum number of sulphydryl groups assumes complete dissociation into chains or sub-units in 6 molar urea, pH 2·3, used in the starch gel electro-

† Due to non-haem proteins.

conditions. HbA and ovalbumin contain free sulphydryl groups and showed positive binding of mercury-203. Table 1 shows the additional qualitative results obtained with several proteins and indicates good agreement between the ability to bind mercury-203 and the sulphydryl content reported in the literature. The data in Table 1 were obtained from several acid-urea-starch gels in conditions which should result in the exposure of most, if not all, reactive sulphydryl groups. Human haemoglobin has only two reactive sulphydryl groups at neutral

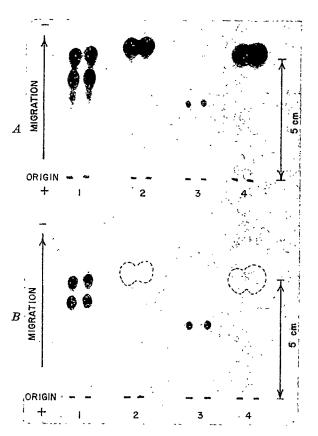


Fig. 1. A, Starch gel electrophoresis, pH 2·3, with formate: hydrochloric acid buffer, 6 molar urea, amido schwarz stain: (1) human HbA; (2) ribonuclease; (3) twice crystallized ovalbumin; (4) insulin. Samples 1, 2 and 4 were from a single gel and sample 3 was from a different gel run in the same conditions. B, Autoradiographic detection (X-ray film) of mercury-203 bound to sample shown in A. Broken lines indicate areas containing protein but no radioactivity. Note lack of binding by ribonuclease and insulin which contain disulphide but no sulphydryl groups.

pH, but when the chains unfold, four more groups are exposed². Disulphide bonds apparently do not bind mercury-203 in these conditions as shown by the negative results for insulin and ribonuclease in Fig. 1. An apparent exception is cytochrome c, which is reported³ to have both sulphydryl groups bound to vinyl side chains of the haem group. This binding is evident during starch gel electrophoresis of horse heart cytochrome c where the red colour remains associated with the protein. (Electrophoresis of haemoglobin in these conditions results in the removal of haem which then stays near the origin.) The bonds to the haem group of cytochrome c may have been partially broken to allow some binding of mercury-203.

Extensive differences in the molecular properties of tadpole and frog haemoglobins have raised the question of the presence of a chain in common between these two forms and analogous to the common α chain of adult (HbA) and foetal (HbF) human haemoglobins⁴⁻⁶. Baglioni and Sparks⁷ compared the peptide chains of tadpoles and frog globins using starch gel electrophoresis at low pH in 8 molar urea and found only one of similar mobility in both forms. DeWitt and Ingram⁶ have reported the occurrence of two N-terminal acetylated chains in the major haemoglobin of both tadpoles and frogs but have not determined the identity of the N-terminal amino-acid.

They report that the non-acetylated N-terminal amino-acids were glycine in the frog and valine in the tadpole. Herner and Riggs, were unable to detect a hybrid of haemoglobin after mixing tadpole and frog haemoglobins; if they had a chain in common, no hybrid would have been formed. If the two haemoglobins, however, had a different chain with similar mobility (in the experimental conditions), then any hybrid formed would have been undetectable. Thus neither of these reports firmly establishes the presence or absence of a common chain. Elzinga¹⁵ devised starch gel electrophoresis conditions which clearly showed that there were no similarities in mobility of haemoglobin chains of R. catesbeiana tadpole and frog. In contrast to the work of Elzinga¹⁰ and other recent reports (ref. 11 and personal communication from A. Riggs and S. Aggarwal), Hamada and Shukuya¹² have published a study of R. catesbeiana indicating an a chain, with no half cystine, common to both tadpole and frog haemoglobins.

We have confirmed the report⁴ that whole R. catesbeiana haemolysates contain between eight and ten sulphydryl groups in a haemoglobin molecule, whereas tadpole haemoglobin has none. (In an extension of earlier work in this laboratory on amphibian haemoglobins, we have re-examined the half cystine content of R. catesbeiana frog haemoglobins. Oxidation of protein by performic acid to convert cysteine and cystine to cysteic acid and extrapolation of values from amino-acid analysis after sealed tube acid hydrolysis of globin from frog haemolysates have consistently indicated between eight and ten half cystine residues in a mole of haemoglobin.) Similar studies on the four major haemoglobins purified from frog haemolysates by preparative scale disk electrophoresis13 indicate no significant differences in half cystine content. Thus Hamada and Shukuya's12 study of amino-acid composition indicating four half cystines in a mole of frog haemoglobin are not consistent with these data. The relative amount of mercury-203 bound to tadpole, frog and human haemoglobins has been estimated by counting the protein precipitated by trichloroacetic acid from whole haemolysates treated with chlormerodrin-203. Similar quantitative studies were made after separation of the haemoglobins into chains on acid-urea-starch gels. These two methods gave results which were consistent with each other and in good agreement with the half cystine content of these haemoglobins established by amino-acid analyses.

Fig. 2 shows the results obtained by applying the method of sulphydryl group detection to the four haemo-globins of frog and five haemoglobins of tadpole (R. catesbeiana) after separation and partial purification by acrylamide disk electrophoresis. The acrylamide gel containing the tadpole haemoglobin with highest mobility (No. 1 in Fig. 2) was shown by starch gel electrophoresis at low pH to contain also a minor slow moving component which did bind some mercury-203. Because the amount of this protein relative to other proteins is so small and the fact that purified tadpole haemoglobins never contain more than a trace amount of half-cystine, we believe that it is unlikely that this minor component containing reactive sulphydryl is a haemoglobin chain. Fig. 2Bshows that all major frog haemoglobin chains bound mercury-203, as did the two slow moving protein bands seen in frog haemoglobin No. 4. These bands are thought to be partially dimerized frog haemoglobin chains (see ref. 15 for discussion of frog haemoglobin dimerization), although the possibility that they are non-haemoglobin proteins similar to those of the tadpole has not been excluded. It is apparent from Fig. 2B that all frog haemoglobin chains did not bind equal amounts of mercury-203

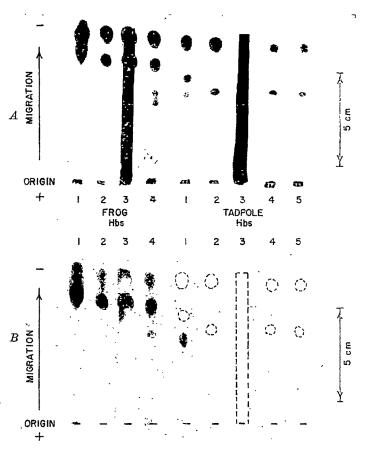


Fig. 2. A. Starch gel electrophoresis. pH 2·3, with formate: hydrochloric acid buffer, 6 molar urea, amido schwarz stain, of tadpole and frog haemoglobins (Hbs) which had been separated and partially purified from their respective haemolysates by disk electrophoresis on polyacrylamide. Samples are numbered in order of decreasing mobility on acrylamide. Tadpole Hb 1 contained a contaminant with lower mobility than either of the major chains from this Hb. To reduce the possibility that a chain with no sulphydryl groups was "buried" under a chain containing sulphydryl, strips of gel containing Hb No. 3 from both tadpole and frog were removed and re-electrophoresed in a second gel to increase the resolution and check for heterogeneity. Slightly better separation of the chains was obtained, but no additional chains were found. B. Autoradiographic detection (X-ray film) of mercury-203 bound to samples shown in A. Broken lines indicate areas containing protein but no radioactivity. Binding by slow moving non-haemoglobin band in tadpole sample 1 is evident, but no major Hb chain of tadpole bound mercury-203. All major chains of frog showed binding, although the faster chains seem to have bound less than the slower chains.

and thus did not have equal numbers of reactive sulphydryl groups*. Although free glutathione cannot account for these observations, the possibility of bound glutathione has not been rigorously excluded.

These results indicate that reactive sulphydryl groups of frog haemoglobin are found on all chains detectable in these conditions of electrophoresis and therefore that none of these chains is the same as any of those found in the tadpole. In agreement with Elzinga¹⁰ and others^{11,12}, these data indicate that a complete switch in the peptide chain content of tadpole and frog haemoglobins occurs at the time of metamorphosis.

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*The relative distribution of sulphydryl between frog chains can be estimated by comparison of the relative intensities of protein stain and autoradiographic exposure. In an additional experiment, protein bands were cut from the gel and counted in a well-type scintillation counter. The ratio of counts indicated a relative reactive sulphydryl ratio of 1:3 for the faster compared with the slower chain.

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Autoradiographic Study of the Effects of Cortisol on DNA Synthesis in Lymphatic Tissue

THE inter-relationships between adrenal corticosteroids and lymphatic tissue have often been described. involution of lymphatic tissue produced by adrenal corticosteroids can be related to different aspects of the growth of lymphatic tissue and to the metabolism of lymphocytes. The effects on growth of lymphatic tissue are a combination of lymphocytokaryorrhexis and the inhibition of mitosis¹. Metabolic effects include inhibition of DNA synthesis^{2,3}, RNA synthesis^{3,4}, protein synthesis3,5-7 and glucose utilization7-8, as well as producing changes in certain enzymes10.

Studies performed in our laboratories2 have demonstrated that cortisol significantly decreased DNA synthesis measured as incorporation of ¹⁴C-2-thymidine in mouse thymi and lymph nodes within 2.5 h after hormone

The results of these experiments were treatment. expressed as d.p.m./mg of DNA. There were no decreases in the total amounts of DNA and RNA in these organs when measured by colorimetric methods2. This communication reports the results of an experiment designed to determine if this decrease in DNA synthesis was a result of a direct inhibition of the biosynthetic pathway for DNA or the result of a purely destructive or inhibitory effect of cortisol on certain lymphocytes.

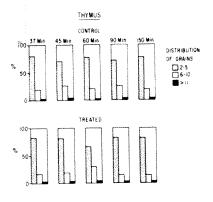
Autoradiography was undertaken in an attempt to investigate this problem. The results of this autoradiographic study should make it possible to distinguish between a purely destructive or general inhibitory effect and an actual biochemical inhibition of DNA synthesis following cortisol treatment. If cortisol acted by destroying or inhibiting cells, then the total number of cells labelled after treatment with cortisol would decrease and there would be no change in the number of grains in the labelled cells. If cortisol interferes with a particular aspect of cellular metabolism and thus produces an inhibition of DNA synthesis, the converse situation would be expected to occur, that is, no decrease in the number of labelled cells but a decrease in the number of grains in each labelled cell.

Thirty normal male mice of the CBA strain, ranging from 10 to 14 weeks of age, were used as experimental animals. The body weights varied from 17 to 25 g. All animals were adrenalectomized 24 h before injection with cortisol acetate or steroid carrier solution. mice were randomized, and divided into ten groups of three. The mice were examined 37, 45, 60, 90 and 150 min after injection of cortisol, a control and a treated group in each case.

A stock solution containing 1 mg of cortisol acetate, given by Dr Elmer Alpert, in 0.25 ml. of a suspension of 0.08 per cent carboxymethylcellulose and saline was prepared. A similar carrier solution was injected into control animals. All injections were made intraperitoneally.

At 7, 15, 30, 60 and 120 min after injection with cortisol acetate or carrier solution the animals received an injection of 1 µc. of 3H-1,2-thymidine (specific activity 14.5 c./mmole)/g of body weight in sterile saline. The animals were killed after 30 min. Thymi, spleens and lymph nodes (two axillary and two inguinal) were removed and fixed in acetic acid-alcohol (1:3) for 1 h and then in formol-saline for 24 h. Tissues were embedded in paraffin and cut 2µ thick. Autoradiographs were prepared by dipping the slides in Kodak NTB-2 liquid emulsion, and were exposed for 30 days. The slides were developed and stained with haematoxylin and eosin. The autoradiographs were examined microscopically and the results obtained were based on a count of 10,000 cells for each time period, 5,000 cells in each of the control and hormone The areas which were counted were treated groups. selected at random, and included both cortical and medullary portions of the organs. The counts were made on at least three different sections for each time period, control, or treated. Cells with two or more grains were considered to be labelled. The data were analysed statistically, using an analysis of variance.

The data in Table 1 show the percentage of labelled cells (labelling index) in the spleens, thymi and lymph nodes, in control and treated groups, at the five times after treatment with cortisol. No statistically significant differences in labelled cells between the control and treated spleens can be seen at any of the time periods studied. The 37 min values obtained from spleens of both control and treated animals are significantly larger than the rest of the measurements made on spleens at the other time intervals. There is a significant difference in labelled cells between lymph nodes of the control and treated animals 150 min after treatment. Treatment with cortisol significantly decreased the total percentage of labelled cells in the thymi 45, 90 and 150 min after treatment.



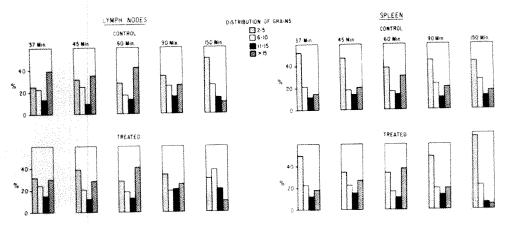


Fig. 1. Distribution of grains in the labelled cells.

The distribution of grains in the labelled cells of all three tissues is shown in Fig. 1. Distribution is broken down into groups of cells containing two to five, six to ten, eleven to fifteen, and more than fifteen grains in each labelled cell. In the case of the thymi, the last two groups are included together, because there were so few cells containing more than ten grains. No differences in distribution of grains were seen in the three organs studied at any of the times. It is specially important that in the thymi and lymph nodes, no significant differences in grain distribution were found between control and treated animals despite significant differences in the labelling The labelled cells of the control and treated indices. animals were morphologically similar, and were the larger, more immature lymphocytes.

The lack of effect of cortisol on spleens in this work may be a consequence of the large amount of myelopoietic tissue in the spleens of mice which has been reported previously¹¹. There were no differences in the distribution of grains between control and treated thymi and lymph nodes at any of the time periods which we studied. The lack of significant differences in the distribution of grain counts, when there were differences in labelling index, indicated that cortisol inhibits DNA synthesis in lympho-

Table 1. PERCENTAGE OF TOTAL CELLS LABELLED

	ens			T	h madaa
Control % ±		Control	rmi Treated	Control	Treated
9-75 ±	$8.93 \pm$	11·27 ±	9-21 ± 0-66	2·55 ± 0·27	3·58 ± 0· 6 5
7.00 ±	4·95 ± 0·77	12·52 ± 1·18	7·55 ± * 0·66	$\frac{2.88 \pm}{0.36}$	2-96 ± 0-37
7·00 ± 0·54	3·57 ± 0·61	12·24 ± 1·18	0.59	0.38	3·40 ± 0·23
7·57 ± 1·30	5·63 ± 0·90	0.59	0.70	0.37	3·86 ± 0·38 4·68 ± *
6-26 ± 0-80	4·28 ± 0·86	0.51	0.39	1-29 I 1-21	0.86
	Sple Control % ± 9·75 ± 1·17 7·00 ± 0·54 0·54 ± 1·30 6·26 ±	$\begin{array}{c c} \textbf{Spleens} \\ \textbf{Control} & \textbf{Treated} \\ \% \pm S.E. \\ \textbf{9.75} \pm & 8.93 \pm \\ 1.17 & 0.69 \\ 7.00 \pm & 4.95 \pm \\ 0.88 & 0.77 \\ 7.00 \pm & 3.57 \pm \\ 0.54 & 0.61 \\ 1.30 & 0.90 \\ 6.26 \pm & 4.28 \pm \\ \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$

cytes, in an all or none manner. Lymphocytes not affected by the hormone incorporated tritiated thymidine at the same rate as those of the control animals.

In biochemical studies², the cells which were inhibited or destroyed by cortisol were still present in the tissue when chemical extractions were performed. The number of cells able to incorporate thymidine into DNA in animals treated with cortisol would therefore be less than in controls and thus DNA synthesis seems to have been decreased. If the specific activity (d.p.m./mg of DNA) of the DNA could be measured in a cell not affected by cortisol it should be the same as that in control cells. The d.p.m./mg of DNA, however, would be altered by the presence of DNA from the dead or inhibited cells when measured chemically. The results of the autoradiographic study support this conclusion.

As we have noted, a significant difference first appeared between control and treated thymi at 45 min after treatment with cortisol. This initial decrease in labelled cells in treated animals does not appear until 150 min after treatment in lymph nodes. Rieke, Caffrey and Everett¹² believe that the lymph nodes are composed primarily of a population of long lived lymphocytes which may circulate for as long as 60 days, whereas thymi are composed primarily of a population of short lived lymphocytes which circulate for about 5 to 6 days. If this is so, an equal number of thymocytes can be expected to turn over ten times for each generation of lymphocytes in lymph nodes, and therefore the effects of cortisol would be detectable earlier in the thymus than in the lymph nodes.

The maximal concentration of cortisol in all three lymphatic tissues has been shown to occur at 7-15 min after intravenous injection¹³. It seems then that cortisol must, when it reaches its target cell, initiate a series of events which are responsible for the ultimate death of the cell. The results of this study indicate that the inhibition of

DNA synthesis after treatment with cortisol is not the result of a direct interference with a biosynthetic pathway, but is simply a result of the reduction in the number of cells able to incorporate thymidine. Other effects of cortisol on lymphatic tissue, such as decreased glucose utilization and decreased protein synthesis, also may be the result of this phenomenon.

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BIOLOGY

Nucleoside Synthesis under Potentially Prebiotic Conditions

Ir is widely believed that the "prebiotic" formation of polynucleotides was a key step in the evolution of life. One of the least understood steps in this synthesis is the formation of nucleosides. Sugars, purines and pyrimidines can be made in relatively high yields from simple materials, and nucleosides can be converted to nucleotides under reasonably plausible "prebiotic" conditions, but the yield of nucleosides reported in most prebiotic syntheses is poor. We have therefore investigated certain aspects of prebiotic nucleoside synthesis.

We found that well defined adducts of deoxyribose or ribose with adenine, cytosine or guanine are formed when the dry bases and sugar are heated together at temperatures in the range 130°-170° C for a few minutes. Three compounds were obtained from adenine and deoxyribose, one of which, obtained in good yield, had an ultraviolet spectrum almost identical with that of deoxyadenosine and behaved identically on chromatography in aqueous ammonia (pH 10) and other solvent systems. It is not deoxyadenosine, however, because it hydrolyses much too rapidly in alkaline solution. Whereas deoxyadenosine withstands heating to 100° C at pH 13 for an hour with very little hydrolysis, the thermal adducts are all hydrolysed almost completely within 15 min. Carbon² has reported two diastereoisomeric compounds formed from deoxyribose and adenine in hot aqueous solution and tentatively identified them as 2,3-dideoxy-(9-purinyl)-pentoses. Two of our products are very similar to these in spectra and stability and may be the same.

In view of these findings, our two laboratories have separately investigated the photochemical reaction between adenine and deoxyribose in the presence of cyanide. We confirm the original finding that material with the same R_F as deoxyadenosine is formed³. This important product is not deoxyadenosine, however, because it is

much less stable to 0.1 molar alkali than is the natural

Thus adenosine cannot be present among the photoproducts in greater than 0.4 per cent yield and further investigation will show whether the indications that deoxyadenosine is indeed formed in small amounts are correct.

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Gastrulation in Papilio polytes L.

Gastrulation has often been observed and described in the Lepidoptera, but it remains a controversial topic. Differences of opinion concern chiefly two points: (a) how does gastrulation take place; and (b) does the process take place in the same way along the whole embrye or differently in different parts of the embryo?

Schwartze¹ stated that gastrulation in Lepidoptera proceeds forwards from the back. This is to a large extent the opposite to what I have observed in our studies of Papilio polytes (Papilionidae, Lepidoptera) and is the exact opposite of the observations of Eastham² and Drummond³. Furthermore, Schwartze maintained that in Lepidoptera gastrulation is brought about by: (a) invagination to form a tube; (b) cell proliferation; and (c) overgrowth; and that these different processes can occur in different regions of the same embryo. Toyoma4 and Schwangart5 confirmed this suggestion.

According to Eastham² gastrulation takes place in the same way throughout the length of the embryo, and the germ band undergoes the following sequence of changes. (a) Proliferation of cells along the middle line followed by differentiation of this region as the middle plate; (b) an invagination of the middle plate; and (c) overgrowth of the middle plate by the lateral plates.

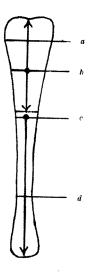


Fig. 1. Diagrammatic representation of the scheme of gastrulation. The process commences independently at level b anteriorly and at level c posteriorly and then continues in the directions indicated by arrows.

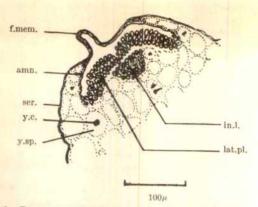


Fig. 2. Transverse section of the embryo at the completion of gastrulation through level d indicated in Fig. 1. amn, Amnion; f.mem, fold of the embryonic membranes; in.l, inner layer; lat.pl, lateral plate; ser, serosa; y.c, yolk cell; y.sp, yolk spherule.

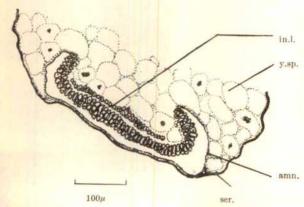


Fig. 3. Transverse section of the embryo at the completion of gastrula-tion through level a indicated in Fig. 1. Abbreviations as in Fig. 2.

Johannsen⁶ held a similar view (but he did not observe cell proliferation). Drummond's observations3 were similar to those of Eastham², who also said that Schwartze, Toyoma and Schwangart were wrong in their conclusions because "what they saw in separate regions are in fact separate processes which the germ band undergoes in sequence"7.

In view of these controversies, my observations of Papilio polytes are of interest. I found that in this insect gastrulation is initiated independently in the anterior and the posterior regions of the embryo (Fig. 1). In the anterior region, the process starts in the first part of the thorax and then extends forward to the head and backward to the posterior thoracic segments. In the abdominal region, the process starts at the anterior end and then extends posteriorly. In the head and thorax gastrulation involves cell proliferation while in the abdomen there is a medial sliding of the lateral plates to enclose the invaginating middle plate. The state of the embryonic membranes in these two regions has afforded further proof of this. The amnion and the serosa in the abdominal region show a pronounced fold at the end of gastrulation which would be expected after a considerable decrease in the width of the embryo as a result of the medial sliding of the lateral plates (Fig. 2). There is no such fold in the anterior region of the embryo for there is no movement of the lateral plates towards each other and therefore no decrease in the width of the embryo (Fig. 3).

It is also interesting to note that gastrulation by medial sliding of the lateral plates to enclose the middle plate, as distinct from the growth of the lateral plates over the middle plate, has not yet been reported in any other members of the Lepidoptera. It may further be concluded

that gastrulation shows different characteristics in various sub-groups of Lepidoptera.

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Induction of Gamete Compatibility and Seed Formation in Axenic Cultures of a Diploid Self-incompatible Species of Petunia

In reviews of the control of fertilization in flowering plants, experimental methods of overcoming self-incompatibility have been discussed1,2. For example, induction of autotetraploidy by colchicine produced compatibility in a diploid self-incompatible species of Petunia3, but self-pollination of the excised whole pistils cultured in vitro did not, as one of us has shown⁴. We report here the elimination of self-incompatibility in the diploid (n=7) Petunia axillaris (Lam.) B.S.P. by fertilization in vitro, a technique devised in this laboratory5 and improved by us.

Self-incompatibility was confirmed by selfing 108 potentially fertile flowers in the field. Although germination of pollen grains was as good as that after cropollination, in 24 h the pollen tubes grew only half the distance from stigma to ovary. Also, unlike the cross-pollinated flowers, in selfed flowers the pollen tubes had not reached the ovary 48 h after pollination. Consequently, none of the selfed flowers set any fruit or seed.

To prevent chance natural pollination in our material, flower buds were emasculated and bagged 1 day before anthesis, which was easily judged by the extent of unfolding of the petals. One day after anthesis unpollinated flowers were brought into the laboratory and their sepals and petals were removed. The pistils were surface sterilized in fresh chlorine water for 10 min, washed twice in sterile distilled water and wiped with sterile filter paper. The stigma and style were removed and the ovary wall was carefully peeled with sterilized forceps, leaving behind the mass of bare ovules supported on the annular disk (present at the base of ovary) and a short length of pedicel. Thus 110 ovule masses were prepared. Of these, twenty were not pollinated, forty-two were cross-pollinated and the remaining forty-eight were heavily sprinkled with selfpollen grains collected axenically from flower buds which were ready to open (Fig. 1a). All 110 ovule masses were implanted individually in aseptic conditions in test tubes each containing 10 ml. of autoclaved nutrient medium (pH 5.8) containing in 1 1. 500 mg of Ca(NO₃)2.4H2O, 125 mg of KNO₃, 125 mg of KH₂PO₄, 125 mg of MgSO₄. 7H₂O, 0.025 mg of CuSO₄.5H₂O, 0.025 mg of Na₂MoO₄, 0.5 mg of ZnSO4.7H2O, 3 mg of MnSO4.4H2O, 0.5 mg of $\mathrm{H_3BO_3}$, 10 mg of $\mathrm{FeC_6O_5H_7.5H_2O}$, 7.5 mg of glycine, 0.25 mg of calcium pantothenate, 0.25 mg of pyridoxine hydrochloride, 1.25 mg of niacin, 0.25 mg of thiamine hydrochloride, 500 mg of casein hydrolysate, 40 g of sucrose, and 8 g of Difco 'Bacto-Agar'. The cultures were grown under diffuse daylight (10–12 ft.-candles) at $25^{\circ}\pm2^{\circ}$ C.

All twenty unpollinated cultures wilted during the first week of culture. In the forty-eight self-pollinated cultures, the pollen grains germinated in 3 h and the pollen tubes grew luxuriantly among the bare ovules and on the placentae (Fig. 1b). One day after culture, pollen tubes were also seen growing in the micropyle of ovules. In 3 days eleven out of forty-eight self-pollinated cultures turned brown and stopped growing. In each of the remaining selfed cultures between ninety and a hundred ovules enlarged sufficiently to suggest a growth stimulus caused either by fertilization or by parthenogenesis.

Embryological examination of ovules from ten self-pollinated cultures removed periodically up to 24 days after selfing demonstrated fertilization but not parthenogenesis (Fig. 1c). Five days after culture the fertilized ovules, that is, young developing seeds, were inescapably evident in the ovule mass in which the unfertilized ovules had already shrivelled and could not be seen by the unaided eye (Fig. 1d). In another 2 days the young seeds turned mealy white and resembled seeds of the same age developing after cross-pollination both in vivo and in vitro. Microtome sections of seeds formed by selfing in

vitro showed filamentous to globular proembryos and cellular endosperm (Fig. 1c). Twelve days after selfing the proembryo grew into heart shaped embryo and the characteristic testa was obvious. Mature seeds bearing dicotyledonous adult embryos were formed 24 days after selfing (Fig. 1e).

Because ten out of thirty-seven selfed cultures were killed for embryological studies, only twenty-seven were grown to seed maturity. From these a total of 1,404 mature seeds was obtained, with an average of fifty-two seeds per culture. The two highest yielding cultures gave

186 and 203 seeds, respectively.

To test germinability of seeds reared in self-pollinated cultures, seeds were sown immediately after collection—sixty on water-soaked filter paper and ten on fresh nutrient agar medium of the same composition as that on which the seeds had developed. In 15 days twenty-three seeds germinated on filter paper and seven on agar medium. Fig. 1f shows one of the seedlings formed on

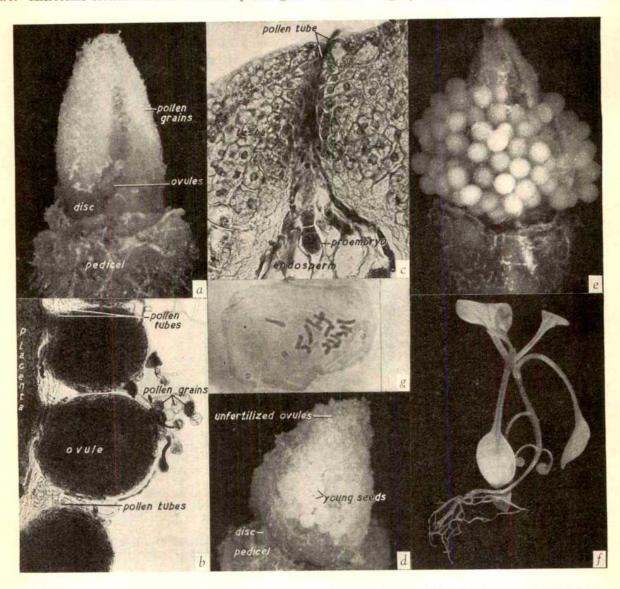


Fig. 1. Elimination of incompatibility in selfed axenic cultures of *Petunia axillaris*. For composition of medium see text. a, Whole mass of bare ovules sprinkled heavily with self-pollen grains (seen as greyish white deposit), and ready for culturing. Note outlines of a few ovules near the base and slightly upwards along middle region of the mass, the hypogynous disk and a short length of hairy pedicel (×13). b, Transverse section showing a fragment of the ovule mass 1 day after selfing. Germinated pollen grains are seen on the surface of ovules and there are meshes of pollen tubes among the ovules as well as on the placental margin (×121). c, Longitudinal section of a 7 day old seed; only the micropylar part is shown. There are pollen tube remains in the micropyle, a four celled filamentous proembryo and a few endosperm cells. The extraovular part of the pollen tube is also distinct (×306). d, Five day old culture showing enlargement of several self-fertilized ovules. Unfertilized ovules are poorly visible. Placentae and pedicel also show general enlargement, but not the disk. Compare with a (×11). c, Twenty-four day old culture showing many mature seeds, almost all with a clear testa. Barely anything is traceable in the seedless region c, Twenty-four day old culture showing many mature seeds, almost all with a clear testa. Barely anything is traceable in the seedless region the top which previously bore several unfertilized ovules (×9·5). f, Fourteen day old diploid seedling raised in culture of mature seed at the top which previously bore several unfertilized ovules (×9·5).

agar medium. Chromosomes of root tip squashes of sixteen seedlings in Feulgen reagent revealed fourteen bivalents

Of the forty-two cross-pollinated cultures, nine succumbed, fifteen were used for embryological studies and the rest were grown to seed maturity. Their embryology was similar to that of self-pollinated cultures. The total number of seeds was 991 and the average was 55.05, which is only a little more than 52, the number obtained for selfed cultures.

Gamete compatibility was effectively induced and formation of diploid seedlings accomplished in aseptic cultures of bare masses of ovules of the self-incompatible species, Petunia axillaris. This is an improvement on the work of Stout and Chandler, who could not obtain a diploid progeny of a selfed diploid parent3. Our work, now in progress, has also revealed that the technique can be successfully applied to bring about self-fertilization in part of an ovule mass, while the remainder of the ovule mass is left intact. The intact part is then available for other experiments of genetical, physiological and embryological interest.

We are indebted to the late Professor P. Maheshwari for encouragement, and thank Professor B. M. Johri for facilities. The Council of Scientific and Industrial Research, New Delhi, gave a research fellowship to one of

us (K. R. S.).

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Cytokinesis in the Green Alga Fritschiella

CYTOKINESIS in the algae, including the green algae, is generally believed to be characterized by centripetal development of the new cell wall. In the green land plants, on the other hand, a cell plate forms in the phragmoplast between two telophase nuclei and expands centrifugally until it separates the daughter cells. It is generally accepted that the land plants evolved from specialized green algae which invaded the land, and it is reasonable to assume that various cellular processes, including cytokinesis, were modified early in the evolution of the terrestrial plant cell from its ancestral green algal cell, and that these modifications were passed onto the land plants as well as to some modern green algae.

This hypothesis led to an investigation of cytokinesis in several ulotrichalean algae, including the subaerial parenchymatous Fritschiella tuberosa lyengar. Soil containing Fritschiella was sent by Dr A. K. Mitra from Allahabad, India. The alga was isolated and grown on a mineral agar supplied with micronutrients and material was prepared for study under the electron microscope using glutaraldehyde-osmium fixation and 'Epon' embedding. Fig. 1 is a section of a cell in which two telophase nuclei can be seen separated by a cell plate quite characteristic of higher land plants. Dictyosome activity can be seen in the region, and vesicles apparently similar to those of dictyosome origin seem to be fusing with the cell

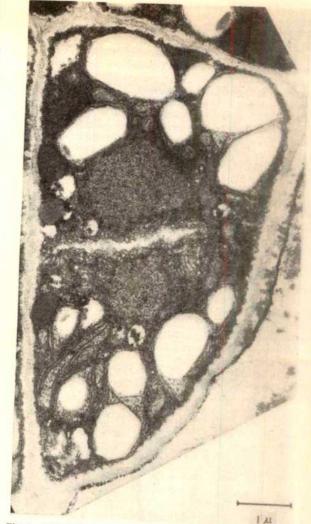


Fig. 1. Fritschiella tuberosa. Electron micrograph of dividing cell in late telophase showing developing cell plate separating two daughter nuclei. Glutaraldehyde-osmium fixation, 'Epon' embedding.

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Disulphide Bridges of Immunoglobin GI Heavy Chains

The immunoglobulins G1 represent the major component of human immunoglobulins, with a basic four chain structure consisting of two heavy and two light chains joined by disulphide bridges1. (The nomenclature used is based on that recommended by the World Health Organization (1966).) The light chains are made up of two sections an invariable C-terminal section characteristic of each type, and a variable N-terminal section specific to each clone²⁻⁴. Peptide maps of Fd fragments^{3,6} suggest that a similar situation holds in heavy chains. In light chains the two sections are folded, each by a single disulphide bridge, to give two loops of almost equal size—the invariable C-terminal loop and the variable N-terminal loop?

The Fe region of human G immunoglobulins includes two intrachain disulphide bridges which impose on it a ring structure, again made up of an N-terminal and a C-terminal loop, resembling the ring structure of light chains. In this communication, we present evidence which suggests further similarities between disulphide bridges of light and heavy chains. We also present results concerning a peptide containing both the heavy-light and heavy-heavy interchain disulphide bridges.

The experimental approach used in these studies has been described previously⁷⁻⁹. Disulphide bridge diagonals were prepared to localize cystine peptides after digestion by pepsin, or pepsin and trypsin. Partial reduction and carboxymethylation were used in the preparation of

labelled carboxymethyl-cysteine peptides.

Disulphide bridge diagonals of a pathological (Dee) Fab fragment (made up of Fd and light chains 10) indicated the presence of four intrachain disulphide bridges in addition to the inter-heavy-light chain bridge. The two bridges usually derived from light chains could be easily identified using isolated light chains (type K, Inv b+). The remaining two intrachain bridges belonged to the Fd fragment. One of them was common to a second pathological Fab fragment (Car). The spots derived from this intrachain common bridge were also found in very good yields in normal human Fab fragments, in other isolated heavy chains of the same type and in the whole molecule. These results suggested that the fragment (Fd) of heavy chains contains, like light chains, a common and a variable disulphide bridge

The peptides from the common bridge were isolated and their partial sequence is shown in Table 1. The two cysteic acid peptides from the other bridge of Dec (not present in protein Car) could be isolated and their sequences studied. The results showed the presence of the

following disulphide bridge

It is interesting that neither of these two peptides is present in the N-terminal sequence, up to residue 84, of another myeloma heavy chain of the same type¹¹. Cleavage of the Fab fragment from protein Dee by cyanogen bromide has shown that the fragment can be split into two components. One is unretarded in 'Sephadex G-100' (6 molar urea, 0.2 molar formic acid) and contains the heavy-light bridge peptides, all the light chain intrachain disulphide bridges and the common intrachain bridge of Fd. The other fragment contained the variable disulphide bridge.

Disulphide bridge diagonals of pepsin digestion (conditions in which the danger of disulphide interchange is minimized) of intact protein Car showed the composite pattern of disulphide bridged peptides of its Fab and Fc fragments. A new set of peptides, however, could be readily identified. In particular a new peptide could be

Table 1. COMMON INTRACHAIN DISULPHIDE BRIDGE OF Fd IN yG1 AND THE HOMOLOGOUS BRIDGE OF yG4 COMPARED WITH THE COMMON BRIDGE OF LIGHT CHAINS AND WITH THE "C" AND "N-TERMINAL LOOP" BRIDGES OF FC Heavy chains

1	
Leu-Thr-Cys-Leu	Phe-Ser-Cys-Ser-Val-Met-His-Glu
Val-Thr-Cys-Val	Tyr-Lys-Cys-Lys-Val-Ser-Asn-Lys
Leu-Gly-Cys-Leu	Tyr-He-Cys-Asn-Val-Asp-His-Lys
Leu-Gly-Cys-Leu	Tyr-Thr-Cys-Asn-Val-Asp-His-Lys
(Val Leu)Val-Cys-Leu	$ \frac{1}{\operatorname{Tyr} \left(\frac{\operatorname{Ala}}{\operatorname{Ser}} \right) \operatorname{Cys} \left(\frac{\operatorname{Glu}}{\operatorname{Gln}} \right) \operatorname{Val-Thr-His} \left(\frac{\operatorname{Gln}}{\operatorname{Glu}} \right) } $
	Val-Thr-Cys-Val Leu-Gly-Cys-Leu Leu-Gly-Cys-Leu

Table 2. TRYPTIC PEPTIDES DERIVED FROM AN OXIDIZED PEPTIDE PEPTIDE OF PROTEIN CAT DIAGONALS, CONTAINING THE THREE HALF CYSTINES INVOLVED IN INTERCHAIN BINDING

The original peptide contained Lys as N-terminal, but the order of peptides 1 and 2 could not be established. Peptides 3 and 4 are ordered as in Fig. 1.

- 1 Lys-Val(Glu,Pro)Lys
- 2 Lys(Asp,Val)Lys
- 3 Ser-Cys-Asp-Lys
- 4 Thr-His-Thr-Cys-Pro-Pro-Cys-Pro-Ala-Pro-Glu-Leu

isolated which appeared to be bridged only to the light chain derivatives. This peptide contained three cysteic acid residues and after tryptic digestion gave rise to the peptides shown in Table 2. One of the derivatives was identical to the one involved in binding the light chain, while another contained two cysteic acid residues which could only be bridged with one another either as an intrachain bridge or by forming a symmetrical bridge, thus constituting the inter-heavy chain link. Models of the peptides Cys-Pro-Pro-Cys showed that it is sterically impossible to make an intrachain bridge in such a sequence to make an intrachain bridge in such a sequence to see succeeding communication) and this, added to the similarity with protein Vin (see succeeding communication) convinced us that these two cysteines were involved in inter-heavy chain bonding. Whether this bonding is parallel or anti-parallel is not known.

Partial reduction and carboxymethylation⁹ (digesting for only 4 h with trypsin) of protein Car and its Fab fragment gave rise to four and two carboxymethylated peptides, respectively, containing the bulk of the label. The results obtained are shown in Fig. 1. The studies of the resulting peptides permitted their ordering as in Fig. 2, which shows a site of papain digestion giving rise to Fc and Fab fragments. Confirmation was provided by finding Thr as the N-terminal residue of the Fc fragment from protein Car. Leu was, however, also present, suggesting that soluble papain splits the heavy chain in more than one site. Normal Fc studied in parallel also contains

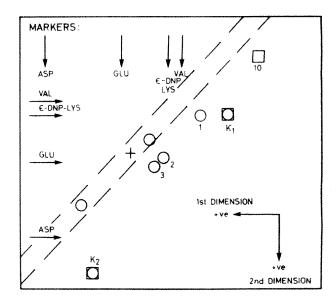


Fig. 1. Radioactive peptides obtained by partial reduction and carboxymethylation of protein Car () and of its Fab fragment (). The diagonal "map" was prepared from a tryptic-peptic digest as described previously. The digest was subjected to electrophoresis at pH 3-5, the paper was oxidized with performic acid vapour and run in a second dimension, again at pH 3-5, to complete the diagonal. The circles on the diagonal were radioactive spots containing carboxymethylated methlonine but not CMCys. The other peptides, isolated as carboxymethyl cysteine peptides, are

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Thr and Leu as N-termini, and in addition Asp and Glu, which could derive, at least in part, from light chain contamination.

The presence in a pathological YG1 protein of two inter-heavy chain disulphide bridges, both of which were reduced in mild conditions, confirms preliminary results, and is in full agreement with the results obtained independently by Steiner and Porter on another YG1 pathological protein where the full sequence around the four intra-chain half-cystines has been established12.

On the basis of studies of disulphide bridges and the general similarity of

peptides present in light and heavy chains (note, for example, that the sequence Tyr-Tyr-Cys of the variable section of all light chains is also present in heavy chains) a loop of about sixty-five residues seems to be a fundamental characteristic repeated several times in the IγG molecule. This is represented by the F'c fragment. obtained by sub-fragmentation of Fc with papain, carry ing only one intrachain disulphide bridge corresponding to the C-terminal half of the Fc fragment*. The similarity between the sequences around one of the disulphide bridges of Fd and the two bridges of Fc agrees with the hypothesis13,14 that the heavy chain originated from gene doubling of a light chain-type precursor (see Table 1).

Ser-Phe-Asn-Arg-Gly-Glu-Cys Ser-Cys-Asp-Lys-Thr-His-Thr-Cys-Pro-Pro-Cys-Pro-Ala-Pro-Glu-Leu Inter-heavy chain Papain

Fig. 2. The sequence around the interchain bridges and a site of papain cleavage of IgGl.

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Disulphide Bridges of a Human Immunoglobulin G Protein

HUMAN heavy chains can be classified as one of four types1,2 γ1, γ2, γ3 or γ4—which have been shown by fingerprint studies to be generally similar in their Fe regions^{3,4}. Similarities in the C-terminal regions and the disulphide bridges^{6,7} of the Fc regions of these types have also been observed. The preceding paper presents sequences around the disulphide bridges of the Fd portions of yl chains

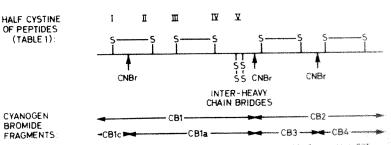


Fig. 1. Disulphide bridges and cysteine-containing cyanogen bromide fragments of the heavy chain of an IgG protein (Vin). The relative positions of cysteines I-IV are suggested by comparison with light chains (see Table 2, and Table 2 of preceding paper); the position of the inter-heavy-light chain bridge (cysteine VI) within fragment CB1a is unknown.

and gives evidence suggesting that, as in light chains*, one of the two intrachain bridges investigated is common to all IgG1 proteins, while the other is a "variable" bridge.

This communication describes the corresponding disulphide bridges of an IgG protein (Vin) of a different type-The presence of two interchain bonds between heavy chains is clearly demonstrated. Protein Vin was described as a γ2 protein in earlier papers^{7,9}. More recent results suggest. however, that it is more likely to be a 74 protein.

The heavy chain of the pathological IgG protein Vin (supplied by Dr G. P. Klein) contains, by amino acid analysis, eleven cysteine residues in a total of 450 residues. Peptides accounting for all the cysteines were isolated

from a tryptic digest of totally reduced and carboxymethylated heavy chains. tion was carried out with 2.8 mmolar dithiothreitol in 6-6 molar guanidine, 0-1 molar tris-hydrochloric acid at pH 8-2, and blocking by the addition of a three-fold excess of 14C-iodoacetic acid. Of the eleven cysteines, four comprise the disulphide bridges

of the Fe region, and one (in sequence VI of Table 1) is known to be bridged to light chains. The roles of the remaining six cysteines (peptides I-V of Table 1) were investigated by the diagonal technique19 applied to the whole unreduced molecule or its cyanogen bromide fragments. The results indicate that four are involved in intrachain bridges and two in inter-heavy chain bonding.

Cyanogen bromide cleavage of the whole molecule, followed by fractionation on 'Sephadex G-100' (in 6 molar urea, 0.2 molar formic acid11), gave rise to four fragments containing cysteine. Each of the two smallest fragments (CB3 and CB4 of Fig. 1) included two cysteine residues; those of CB3 formed the disulphide bridge nearest to the N-terminal of the Fe region, and those of CB4 formed the Fe "C-terminal loop". Sequences around these cysteine residues are presented in ref. 7. Incomplete cleavage at a methionine in a position between the two Fe bridges accounted for the presence of a third larger fragment. The fourth fragment, CB1, was the largest and accounted for all the remaining cysteine residues of the molecule.

In a chymotryptic diagonal of fragment CB1 three cystine peptides related to tryptic peptide V (Table 1) were obtained. Each cystine peptide was oxidized by per-

Table 1. CARBOXYMETHYL CYSTEINE PEPTIDES FROM A TRYPTIC DIGEST OF PROTEIN VIN HEAVY CHAINS Peptide

Leu-Ser-CMCys-Ala-Ala-Ser-Gly-Phe Ala-Glu-Asp-Thr-Ala-Val-Tyr-Tyr-CMCys-Ala-Arg Ser-Thr(Ser, Glu, Thr, Ser) Ala-Ala-Leu-Gly-CMCys-Leu-Val-Lys Thr-Tyr-Thr-CMCys-Asn-Val-Asp-His-Lys-Pro-Ser-Asn-Thr-Lys Tyr-Gly-Pro-CMCys-Pro-CMCys-Pro-Ala(Ser, Glu)Pheiii IV V

V2 Leu-Gly-Gly-Pro-Ser-Val-Phe-Leu-Phe-Pro-Pro-Lys-Pro-Lys

Gly-Pro-Ser-Val-Phe-Pro-Leu-Ala-Pro-CMCys-Ser-Arg

Products of the chymotryptic cleavage of cystine peptide V are shown as V1 and V2, but were isolated as the cysteic acid derivatives.

Table 2. A COMPARISON OF THE "VARIABLE" DISULPHIDE BRIDGE PEPTIDES FROM A * LIGHT CHAIN WITH PEPTIDES FROM y1 HEAVY CHAINS AND THE HEAVY AND LIGHT CHAINS OF PROTEIN VIN

Protein	Ref.	Chain type	1.00	
Daw Dee Vin Rad Vin	(11) (13) (8)	71 71 74 2	Leu-Thr-Cys-Thr-Phe-Ser-Gly-Phe Arg-He(Ser,Cys,Lys,Ala,Ser,Gly) Leu-Ser-Cys-Ala-Ala-Ser-Gly-Phe Leu-Ser-Cys-Arg-Ala-Ser-Gln-Val He-Thr-Cys-Gly-Gly-Asn	Tyr-Tyr-Cys-Thr-Gly Ala-Glu-Asp-Thr-Ala-Val-Tyr-Tyr-Cys-Ala-Arg Pro-Glu-Asp-Phe-Ala-Val-Tyr-Tyr-Cys-Gln-Gln

The peptide from Vin λ chain was isolated from a peptic diagonal of separated light chains.

formic acid and the resulting cysteic acid peptides were purified and analysed. The cysteic acid peptides obtained (V1 and V2) are shown in Table 1. Of the three original cystine peptides, one gave rise on oxidation to V1 alone, one to V2 alone and one to both V1 and V2: no other cysteic acid peptides were observed in association with any of these derivatives. The presence of a cystine peptide in which V1 is bridged to V2 (in addition to the presence of the symmetrically bridged peptides V1-V1 and V2-V2) is clear evidence of inter-heavy chain bonding in this sequence. The molecule contains no free sulphydryl groups, and so it was concluded that both cysteines are involved. Both cysteines react with iodoacetate after partial reduction, giving rise to a di-carboxymethyl cysteine peptide isolated after pepsin and trypsin digestion⁹. It is not known whether these disulphide bridges are formed in a parallel or an anti-parallel fashion. Model building suggests that both modes are possible, but that intrachain bonding between half cystines in the sequence Cys-Pro-Pro-Cys is sterically forbidden.

The remaining four cysteines (in sequences I-IV) are considered to form intrachain disulphide bridges, for they are resistant to partial reduction leading to chain separation. Two peptides related to tryptic peptides III and IV were isolated from a pepsin and trypsin diagonal of the whole molecule. They form the disulphide bridge shown in Table 1 of the preceding communication and are very similar to peptides derived from pathological IgG1 proteins and from pooled normal IgG. A disulphide bridge is most probably formed between the only remaining half-cystine peptides I and II, although this has not been directly demonstrable by the diagonal technique; however, a small fragment (CB1c of Fig. 1), including peptide I, is released on reduction and carboxymethylation of cyanogen bromide fragment CB1. Fragment CB1c probably derives from the N-terminal rather than the C-terminal section of the heavy chain portion of CB1 (see comparison of peptides in Table 2).

These results establish the presence of two intrachain and three interchain bridges in the cyanogen bromide "Fd-like" fragment of protein Vin. The similarity of peptides III and IV with the yl chain Fd "common" bridge (see Table 1 of preceding paper) may indicate that the intrachain bridge between peptides III and IV is present in all 74 proteins. A similar comparison of peptides I and II with a Yl "variable" bridge (Table 2) suggests that I and II may form a "variable" bridge; this is supported by their similarity to the "variable" cysteine peptides of light chains (Table 2). The homology in this region between Vin heavy chain and a selected z chain is greater than that between the heavy and light (\(\lambda\)-type) chains of protein Vin.

The interchain bridges are also similar to those of yl proteins in that two cystines bridge the heavy chains (ref. 12 and preceding communication) and one links the heavy and light chains,; however, it is remarkable that although the inter-heavy chain bridges appear closely similar in sequence to those of YI, the inter-heavylight chain bridges are obviously not in homologous sequences. It appears that their relative positions in the two molecules differ.

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GENETICS

Sex Chromosome Abnormalities

DURING the past decade several surveys have related the incidence of sex chromosome abnormalities among the general population with that among mentally deficient patients in hospital¹⁻³. In 1963 Wegmann and Smith⁴ carried out a buccal smear survey among a male population composed of juvenile delinquents and felons. They found the incidence of positive for sex chromatin lower (1:658) than in the neonatal population (1:377) and suggested that the XXY aberration does not contribute disproportionately to juvenile delinquency or felonious behaviour. Nevertheless, evidence is beginning to suggest that genetic factors influence criminal behaviour. Jacobs et al. have called attention to the criminal behaviour of males with the XYY sex chromosome complement.

Because there are conflicting opinions about the biological effect of the extra Y and because much remains to be known about this cytogenetic aberration, we undertook a chromosome survey at a maximum security penal institution with the primary purpose of detecting XYYmales.

The institution was the Ohio Penitentiary which has approximately 3,000 male inmates. The only criterion used for the selection of one hundred men was based on height. Men 73 in. or more tall were asked to volunteer blood for the "Tall Man Project". Of the one hundred volunteers (fifty-two Caucasians and forty-eight Negroes) four were found to have a chromosome abnormality. Two individuals, both Caucasian, had forty-seven chromosomes with an XYY sex complement, while the other two, one from each race, also had forty-seven chromosomes with an XXY sex chromosome constitution.

The two with Klinefelter's syndrome were 77 in. and 74 in. tall. In addition to exhibiting the classical clinical features of the syndrome, both men were homosexuals.

The two men with XYY were 76 in. and 75.5 in. tall. Physical examination of one did not reveal any abnormalities, but the other displayed stasis ulcerations of both lower extremities. Neither was married and both denied fathering any offspring. Both men had committed numerous offences and had been in correctional institutions many times. One was thought to have an aggressive personality, while the other had not. Both were of normal intelligence. Plasma testosterone concentrations were within normal limits in each case.

The 2 per cent incidence of Klinefelter's syndrome in our preliminary survey is in keeping with that of Forssman and Hambert, who in 1963 reported a survey of the nuclear sex of 760 male patients in three Swedish institutions for criminal and "hard to manage" males of

Grey, H. M., and Kunkel, H. G., J. Exp. Med., 120, 253 (1964).

subnormal intelligence. They also found 2 per cent to be positive for chromatin. Jacobs et al.5 cite a similar study in two comparable institutions in England, where 2.2 per cent of the males were positive for chromatin. Whether criminal institutions per se have a higher incidence of Klinefelter patients compared with institutions solely for the mentally deficient is not fully known. There is some evidence that XXY cases may be slightly more prevalent in the former. Both our XXY cases were homosexuals, however, and in view of the fact that homosexuality is extremely common in penal institutions, it is interesting to speculate about a possible relationship. There is at present no information of this nature drawn from a criminal population. If significant, such information might be helpful in understanding and perhaps managing this problem.

In the thirty or more cases of the XYY syndrome that have been recorded the most outstanding clinical feature seems to be height. For some unknown reason these patients are unusually tall. Jacobs et al.5 have stated that in their original group studied a man more than 72 in. in height had an approximately 50 per cent chance of being XYY. Selecting for height, aggressive behaviour and intelligence, Welch and his group? were unable to support a hypothesis suggesting an association between these features and the XYY individual. Welch? points out that one factor in his study may be that most of his cases were Negro, and as yet the alteration has not been reported in this race. Whether there is a racial predilection in the XYY syndrome remains to be answered. In a controlled study of seven XYY patients Hope et al. s concluded that these patients do not differ markedly from controls either in intelligence or tested hostility. Furthermore, discrepancies can be noted in their concentrations of plasma testosterone. Migeon has evaluated testosterone concentrations in fourteen XYY patients and has noted that eight (including our two) have normal levels while six have a definite elevation (unpublished results). Whether the XYY syndrome indicates a familial tendency to non-disjunction has also been questioned. Further investigations are obviously necessary to elucidate the role of the extra Y chromosome in this disorder.

We thank Dr R. Brooks, W. W. Gilbert and Warden E. Maxwell for making this study possible. This work was supported in part by a grant from the US National Institutes of Health.

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PATHOLOGY

Tumour Production in Glandular Stomach of Rat by N-Methyl-N'-Nitro-N-Nitrosoguanidine

WE reported1 that the subcutaneous injection of a potent mutagen², N-methyl-N'-nitro-N-nitrosoguanidine (NG), into rats induced many transplantable sarcomas at the injected loci. Schoental³ reported the occurrence of squamous cell carcinoma in the fore-stomach of rats to which NG was administered by stomach tube. Druckrey et al.4 also described the production of sarcoma in rats by the subcutaneous injection of NG. The carcinogenicity of this mutagen seemed to be well documented.

This communication deals with the production of tumorous lesions by NG in the glandular stomach of rats. with very high frequency when NG was continuously

administered dissolved in drinking water.

NG (K and K Laboratories, Inc., New York) was dissolved in water at a concentration of 33 mg or 83 Wistar strain male rats, initially 6 weeks old, were fed freely commercial CE-2 animal diet and water containing NG. Experimental rats grew at the same rate as controls. The animals were carefully autopsied when they died or they were killed after 12 months on continuous NG. In nine of thirteen rats so far autopsied, the tumorous lesions have been found in the glandular stomach, especially in the pylorus region. Four of the rats showed histologically definite serosa involvement with the rupture of muscle layer as shown in Figs. 1 and 2.



Fig. 1. Stomach of a rat killed after 12 months administration of NG solution (83 mg/l.) with a tumour at the pylorus.

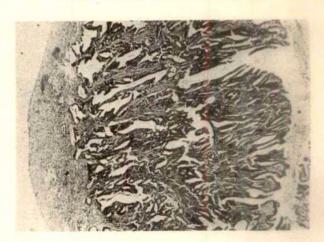


Fig. 2. Adenocarcinomatous growth with the serosa involved; same rat as in Fig. 1.

The remaining five rats have similar but early epithelial growth either at the pylorus or at the antrum. The surviving animals are being observed, in expectation of the eventual occurrence of the tumour with metastasis.

It is interesting that the fore-stomach of all the rats autopsied was quite normal. NG seems to be much more potent in producing tumour in the glandular stomach than 2,7-bis(acetamide) fluorene, 4-nitroquinoline 1-oxide, N-nitroso-N-methyl urethane or aflatoxin⁵⁻⁸.

Some of the animals showed adenocarcinoma of the duodenum, myosarcoma of the jejunum or sarcomatous lesions of mesenthelium origin. Organs other than the digestive tract were almost unaffected, except in one case which showed liver adenoma.

The mechanism by which NG causes this high incidence of tumorous lesions preferentially in glandular stomach is obscure, and is being investigated. This work was supported in part by a grant from the Ministry of Educa-

We thank Dr T. Baba for carrying out histological diagnoses.

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APPLIED SCIENCE

Tensile Strength of Granular Materials

RECENTLY Carr1 and Douglas2 have commented on the equation for the tensile strength of granular materials

$$\sigma \simeq \Gamma \frac{Bt}{\bar{D}^3} \tag{1}$$

as derived by V. Smalley and myself³, where σ is the tensile strength, Γ is a numerical constant, B is the bonding force of particle to particle, t is the thickness of the fracture zone (measured parallel to the stress direction) and D is the diameter of the spherical particles which make up the ideal granular material. As originally formulated³ the constant 1' had a value of 4.6.

Carr¹ suggested a possible modification which could be introduced into Γ, which required selecting the value 8.0, from the results of Bernal and Mason4, to be the coordination number of the spheres in the random aggregate rather than using the mean value of 8.5. Bernal and Mason distinguished two types of contact in their random sphere packing, close and near. In a liquid bonded system in the pendular state, bonds should exist at both of these types of contact, so the co-ordination number which should be applied is the one for mean total contacts in a close random pack of spheres, that is, 8.5. If the dry case is being considered it might be justifiable to consider just the close contacts, in which case the co-ordination number would be 6.4. In neither case is the 8.0 value strictly applicable.

B. B. Morgan, of the British Coal Utilisation Research Association, in a private communication, however, had previously pointed out two necessary modifications in the method which do change the value of \(\Gamma \). In particular, in considering the number of bonding forces in the system it is more convenient to consider that one bond unit

exists for every two contact points on individual spheres rather than consider that every contact point represents a bond unit. This revision alone introduces a factor of two into the original formulation. The other anomaly indicated by Morgan was the unjustified appearance of the $\sin \theta$ term in equation (7) (in ref. 3) and its resulting disturbance; also a B has been omitted from equation (10) (in ref. 3). If the Morgan modifications are applied, the tensile strength equation becomes

$$\sigma \simeq 2.8 \, \frac{Bt}{D^3} \tag{2}$$

The numerical constant changes but most of the equation does not; the dependence of the tensile strength on D^3 remains. In other words, the number of bonds in the fracture zone depends on a volumetric control. For this to be valid, the model requires that the fracture section should be considered as having a finite thickness t and thus a real volume. Fracture takes place in the section in a manner somewhat similar to that described by Orrs: when an agglomerate of spheres is pulled into two pieces. the spheres must go with one or other of the two parts. If those in the fracture zone choose to remain with the part in which they are most deeply buried, the effective fracture region about these spheres can be as much as 0.5 the area of a sphere and as little as zero. The fracture scheme described by Douglas2 in which the particles come apart without themselves contributing to the strength is not applicable.

As Carr pointed out, the original equation of V. Smalley and myself is dimensionally consistent, and is also a valid representation of a specific model fracturing system in which t cannot be directly related to D. If t can be expressed in terms of D, then a dimensionally consistent expression of the form evolved by Rumpf⁶ is possible

$$\sigma \simeq 2 \frac{B}{D^2} \tag{3}$$

But there seems to be a paradox here, because to fit in with the dimensional requirement t must apparently increase with D, but in the original Rumpf formulation twas not only constant, it was actually zero in all cases. The three dimensional section of thickness, t, is more satisfactory. It still, despite Carr's objection, provides an intuitive indication that the strength of an ideal granular material is inversely proportional to the volume of the particles. If the fracture zone is three dimensional it can be thought of as a container, say a tobacco tin, the number of contacts depending on the number of spheres which can be placed in the tin and this depending on the size (volume) of the particles and thus on D^3 . In the original derivation of V. Smalley and myself, the interparticle force B was assumed to be independent of particle size and this is still a necessary condition.

Equation (6) of Douglas² is in agreement with the corresponding equations by Rumpfe and V. Smalley and myself3 in that the tensile strength was found to be directly proportional to B and this seems reasonable for real granular materials. The major problem still remains, as Morgan, showed so clearly for dry particulates, to relate D to σ for real materials.

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Received September 29, 1967.

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OBITUARIES

Sir Kenneth Lee

SIR KENNETH LEE, who died recently at Ewhurst in Surrey at the age of 88, was a firm believer in the need to apply science to industrial problems, and a good friend to science. He entered the firm of Tootal Broadhurst Lee as a young man; for 54 years he was a director, and for 30 of these chairman of the firm. He had the foresight and wisdom, not only to realize how important research could be in the cotton industry, but also to pose the problems for his researchers to solve. He appointed a physicist, the late Dr R. S. Willows, as head of his research laboratory and set him to find a way to produce a cotton fabric which resisted creasing. He had faith in Willows, and gave him unquestioning support for the many years during which Willows was struggling with the problem. I was a professor at Manchester during these years, and was able to follow the progress of the quest because Willows and I often played golf together on Saturday afternoons. I remember more than one occasion when Willows used the shelter of a convenient bunker to pull out and display to me some portion of his clothing which was getting nearer to the realization of his ideal, while indignant shouts of "fore" came from a distance. He succeeded in producing a polymer inside the tube of the cotton fibre, but at first it was unpopular with the ladies because it was too springy and would not drape. This was solved, but then it would not take dyes. As is well known, the final result was the famous and popular Tootal fabrics.

Kenneth Lee, though diffident about the fact that he had no scientific education, loved meeting scientists and talking about their discoveries. But he was a shrewd judge of character, and had no patience at all with the kind of industrial researcher who makes a noise like a scientist but gets no further with a practical problem.

When I retired from the Royal Institution, the problem arose of the future of the team engaged on protein research which I had gathered there, and the happy solution was found of its migration en bloc to the Zoology Department in Oxford. There were difficulties, however, in establishing fellowships for some members of the team. Kenneth Lee was one of two friends who came to my help. His contribution was characteristically given without any question or any delay. In spite of his age and increasing frailty, he made the business arrangements to ensure that the necessary covenant would continue to run if he died.

His many public services have been recorded elsewhere; his services to science are perhaps not so well known because he was so modest about them, and they should also be remembered.

W. L. Bragg

Professor K. S. Kirby

THE death at his home on November 10 of Professor Kirby, while still at the height of his career, will be a blow for cancer research in general and the field of nucleic acid chemistry in particular.

Ken Kirby was born in Yorkshire on January 16, 1918, and, although he lived there only for short periods after leaving for the University of Manchester in 1937, his determination and great sense of humour remained those of a Yorkshireman.

He gained a first class honours degree in chemistry in 1940, but war prevented his obtaining further academic qualifications at this stage. He was directed to work at the Wellcome Laboratories for Tropical Medicine where he first worked on chemotherapy of amoebiasis. He

became interested in the structure of alkaloids and this led to five papers in the Journal of the Chemical Society (1945–1950) on the structure of quinamine, a minor alkaloid of cinchona bark. The final two papers of this series were published in collaboration with the group of Sir Robert Robinson at the Dyson Perrins Laboratory in Oxford.

At the end of the war Kirby returned to the university and obtained his Ph.D. from Leeds in 1949. His thesis was concerned with the structure of tannins and his interest in this field continued when he joined the staff of the Forestal Research Laboratory in Harpenden. The results of his extensive studies into the complex field of tannin chemistry appeared between 1948 and 1954 in a series of six papers in the Journal Society of Leather Trades' Chemists. It was during this period that Kirby gained valuable experience of the techniques of chromatographic and counter current fractionations which he later applied so successfully in other fields.

Kirby's transfer from tannin chemistry to cancer research may seem strange, but he made it because he believed that the knowledge he had of the interaction and behaviour of polyphenolic substances could be applied in the isolation and study of cellular macromolecules. He was able to convince the British Empire Cancer Campaign of the value of his ideas, and it awarded him a five-year fellowship. He joined the staff of the Chester Beatty Research Institute at the Pollards Wood Research Station on October 1, 1953, and began the work which gained him wide international recognition.

At this time, nucleic acid chemistry had received great stimulus from the publication by Watson and Crick (Nature, 171, 737; 1953) of the structure of DNA. Kirby determined to apply the discipline of chemistry to the isolation of nucleic acids in a state of purity which would satisfy an organic chemist.

The first papers describing what soon became known as "the Kirby method" for the isolation of RNA and DNA appeared in the Biochemical Journal (64, 405; 1956, and 66, 495; 1957). He was always anxious to improve and extend his methods and this led to the publication of many papers, the most recent of which, describing the isolation of DNA from mammalian tissues (Biochem. J., 104, 254; 1967) and of RNA and DNA from bacteria (Biochem. J., 104, 258; 1967), appeared only shortly before his death.

The publications reporting his methods for nucleic acid isolation are fine examples of clear and explicit experimental detail, but many biochemists found them rather complex, for they employed a number of unusual chemicals, and to the uninitiated it was not always clear why a certain procedure was used. He was often asked how he arrived at the finally published methods, to which he always replied that he was sure that anyone who had applied a logical approach to the problem must have come up with the same answer. The truth was that he had clear ideas on the nature of the bonds between nucleic acids and proteins and his methods were designed to break these bonds and so allow the purification of the nucleic acids.

Having achieved his initial objective, Kirby wished to isolate a chemically homogeneous sample of DNA or RNA and so he devised a number of counter-current distribution systems for use with nucleic acids. He subsequently employed this technique to study and attempt to separate mRNA from mammalian tissues. His paper with Kidson (Cancer Res., 25, 472; 1965) reporting changes in the counter-current distribution pattern of the mRNA of rat liver during azo-dye carcinogenesis indicated the potential of this approach.

Kirby was aware of the possibilities of nucleic acid fractionation using centrifugation in density gradients, and, typically, he was not content to rely only on sucrose gradients but sought alternatives, such as sulpholane (Biochim. Biophys. Acta, 123, 202; 1966).

Kirby was unconcerned with personal advancement and had to be persuaded to submit his D.Sc. application which was awarded by the University of Manchester in 1964. He was appointed reader in chemistry by the University of London in March 1965 and the title of professor of cell chemistry was conferred on him in February 1967.

During the last three years of his life Kirby had to fight against illness, but his output of work continued—during 1966-67 sixteen papers appeared under his name.

Peter Brookes

University News:

Reading

DR P. A. HUXLEY, at present director of research in the Coffee Research Foundation, Kenya, has been appointed head of the Department of Horticulture on the retirement of Professor O. V. S. Heath.

Announcements

THE Robert Cormack Bequest Committee has awarded a Cormack Fellowship to Mr A. S. Houston of Glasgow. The fellowship is tenable for three years and will enable Mr Houston to pursue his research at the Royal Observatory in Edinburgh.

The Winter Gordon Research Conferences will be held from January 22-February 2, 1968, in Santa Barbara, California. From January 22-26 a conference on "Electrochemistry" will take place at the Miramar Hotel, and one on the "Chemistry of Ageing" will take place at Santa Barbara Biltmore Hotel. From January 29-February 2 a conference on "Polymers" will take place at the Miramar Hotel and one on "Science, Technology and Economic Growth" will take place at Santa Barbara Biltmore Hotel. The purpose of these meetings is to stimulate research in universities, research foundations and industrial laboratories and further information can be obtained from Dr W. George Parks, Director, Gordon Research Conferences, University of Rhode Island, Kingston, Rhode Island.

Meetings

Mossbauer Effect, December 12-13, Royal Institution, London (Secretary, The Faraday Society, 6 Gray's Inn Square, London WC1).

ACOUSTICAL Holography, December 14–15, Santa Monica (Douglas Aircraft Company, Incorporated, Santa Monica, California).

AMERICAN Physical Society meeting, December 18–20, Pasadena, California (Professor W. Whaling, California Institute of Technology, 1201 East California Street, Pasadena, California).

Solid State Physics, January 3-6, 1968, University of Manchester Institute of Science and Technology (The Meetings Officer, The Institute of Physics and The Physical Society, 47 Belgrave Square, London SW1).

VALENCE and Reactivity, January 9-11, 1968, Oxford (The Scientific Affairs Officer, The Chemical Society, Burlington House, London W1).

AGRICULTURAL Sciences and the World Food Supply, March 4-6, 1968, Wageningen (The International Agricultural Center, PO Box 88, Wageningen, The Netherlands).

EXPLOITABLE Molecular Mechanisms and Neoplasia, March 6-8, 1968, University of Texas M.D. Anderson Hospital and Tumor Institute at Houston, Texas (Dr Robert B. Hurlbert, 1968 Symposium Chairman, The University of Texas M.D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas).

Modern Chemistry in Industry, March 11-14, 1968, Eastbourne (The Honorary Secretary, Conference on Modern Chemistry in Industry, 14 Belgrave Square, London SW1).

RECENT Developments in Refrigeration Techniques and Equipment and their Range of Applications in the Process Industries, March 20-21, 1968, Grimsby (Mr P. Davies, Head of Group Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1).

STRUCTURE Analysis: Thermal Vibrations, Disorder and Phase Transitions, April 17–19, 1968, University of York (The Meetings Officer, The Institute of Physics and The Physical Society, 47 Belgrave Square, London SW1).

STEAM Generating and other Heavy Water Reactors, May 14-16, 1968, Institution of Civil Engineers, London (The Secretary, British Nuclear Energy Society, 1-7 Great George Street, London SW1).

COMMUNICATION Satellite Earth Stations, May 20-31, 1968, London and Post Office Earth Station at Goonhilly (Mr R. E. G. Back, UK Seminar on Communication Satellite Earth Station Planning and Operation, PO Engineering Department, WS2, 207 Old Street, London EC1).

ERRATUM. In the article "Linked Groups of Residues in Immunoglobulin × Chains" by C. Milstein (Nature, 216, 330; 1967) the following variants should be moved: Roy...Threonine from position 63 to 65; Ag...Phenylalanine from 65–67; and Rad...Glutamine from 64–66.

ERRATUM. In the article "Ice Nucleation and the Substrate-ice Interface" by Duwayne M. Anderson (Nature, 216, 563; 1967), reference 7 should refer to S. Taber and references 15 and 16 should be interchanged throughout. In line 13 of the third paragraph of page 566 absorbant should read adsorbate.

ERRATUM. In the communication "Solvated Electron Spectrum in Irradiated Ice" by K. Eiben and I. A. Taub (Nature, 216, 782; 1967), reference 2 is mentioned three times in lines 4 and 5. These should be references to work of Taub and Eiben which is being prepared for publication, and not to the reference 2 which appears in the footnotes.

Erratum. In the communication "Variability of Centaurus XR-2" by R. J. Francey et al. (Nature, 216, 773; 1967), it should be the Lawrence Radiation Laboratory, and not the Levermore Research Laboratory, which is mentioned in the second paragraph. In line 10 of page 774 "type II supernovae" should read "novae or supernovae".

CORRIGENDUM. In the communication "Relationships among Functional Properties in Californian Grasslands" (Nature, 216, 168; 1967), the regression coefficient for the regression of turnover time on productivity (Fig. 3) should read r=-0.531~(P>0.1). The conclusion concerning diversity function must therefore await further data. The other two conclusions are unaffected by this error.

CORRIGENDUM. The Gairdner Foundation has asked us to make the following amendments to its list of awards (Nature, 215, 1528; 1967): Dr D. H. Copp and Dr I. MacIntyre share equally a \$5,000 annual award, Dr Copp for introducing the "calcitonin concept" of endocrine control of hypercalcaemia, and for his discovery that high calcium perfusion of the thyroid-parathyroid complex caused release of a previously unrecognized calcium regulating hormone which he named "calcitonin"; and Dr MacIntyre for confirming Dr Copp's original observations and for demonstrating that calcitonin was produced by specific cells (parafollicular cells) in the mammalian thyroid.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 4

Institution of Electrical Engineers, (joint meeting with the Institution of Heating and Ventilating Engineers, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "The Commissioning of Engineering Services in Large Buildings", opened by Mr P. A. Flanigan and Mr W. C. Jeffreys.

SOCIETY OF ENGINEERS (at Burlington House, Piccadilly, London, W1). at 6 p.m.—Mr Brian J. Bell: "Reinforced Plastics".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, SW1), at 6.30 p.m.—Professor C. F. Cullis: "Recent Developments in the Controlled Oxidation of Hydrocarbons".

Tuesday, December 5

UNIVERSITY OF LONDON (at King's College, Strand, London, WC2), at 4 p.m.—Professor P. A. Bertocci (Boston): "The Cosmological Argument, Revisited and Revised".*

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP—PHYSICOCHEMICAL AND BIOPHYSICAL PANEL (at 14 Beigrave Square, London, SW1), at 5 p.m.—Mr J. B. Andrews: "The Nature of Rain"; Dr J. L. Monteith: "The Nature of Dew".

University of Aston in Birmingham (at Gosta Green, Birmingham, 4), at 5.30 p.m.—Professor S. L. Cook: "Science and Decision Making" (Inaugural Lecture).

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, SW7), at 5.30 p.m.—Professor J. G. Ramsay: "A Geologist's Approach to Rock Deformation" (Inaugural Lecture).*

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr E. T. O. Slater: "The Genetics of Schizophrenia". (Last of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1), at 6 p.m.—Annual General Meeting, followed by the Presidential Address of Major General Sir Leonard Atkinson, KBE.

INSTITUTION OF MECHANICAL ENGINEERS, MANIPULATIVE AND MECHANICAL, HANDLING MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Machinery for Oil Well Drilling".

Tuesday, December 5-Thursday, December 7

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2)—Conference on "Electrical Methods of Machining and Forming".

Wednesday, December 6

COLOUR GROUP (Great Britsin) (in the Physics Department, Imperial College, London, SW7), at 2.30 p.m.—Mr H. J. Charle and Dr F. W. Wood: "Colour and Food Colours"; Mrs M. H. E. Griffiths: "The Effect of Processing on Food Colours"; Mr I. F. Gaunt: "The Toxicity of Food Colours".

PLASTICS INSTITUTE, POLYMER PROPERTIES DISCUSSION CIRCLE (at 11 Hobart Place, London, SW1), at 2.30 p.m.—Meeting on "Phosphornitrils for Lubricants".

Sir Thomas Middleton Memorial Trust (at the Wellcome Building, 183 Euston Road, London, NW1), at 3.15 p.m.—Dr C. R. W. Spedding: "The Agricultural Ecology of Grassland" (Twelfth Middleton Memorial Lecture).*

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and ropical Medicine, Keppel Street, London, WC1), at 5 p.m.—Professor D. V. indiey: "The Choice of Variables in Multiple Regression".

PLASTICS INSTITUTE (at the Charing Cross Hotel, London, WC2), at 5.30 p.m.—Meeting on "The Future of the Independent Plastics Converter".

INSTITUTION OF MECHANICAL ENGINEERS. HYDRAULIC PLANT AND MACHINERY GROUP—FLUID POWER TRANSMISSION SECTION (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Fluid Power Pump and Transmission Testing Codes".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, WC2). 6 p.m.—Professor G. E. Fogg, FRS: "Food, Living Space and the Future

INSTITUTE OF FUEL (at Hatfield College of Technology, Roe Green, Hatfield, Herts), at 6.30 p.m.—Mr. R. E. Waller: "Atmospheric Pollution and Medical Statistics".

SOCIETY FOR ANALYTICAL CHEMISTRY, AUTOMATIC METHODS GROUP (joint meeting with Gas Chromatography Group of the Hydrocarbon Research Group of the Institute of Petroleum, in Lecture Theatre 1, Department of Physics, Imperial College, London, SW7), at 6.30 p.m.—Meeting on "Gas Chromatography in Process Control".

Thursday, December 7

BIOMETRIC SOCIETY—BRITISH REGION (at the Wellcome Building, Euston Road, London, NW1), at 2.30 p.m.—Annual General Meeting, followed by an Ordinary Meeting on "Statistical Methods in Archaeology". Dr F. R. Hodson: "Searching for Structure Within Multivariate Archaeological Data"; Mr James Doran: "Computers in Archaeology"; Dr C. B. M. McBurney: "Time Measurements and some Objectives of the Quantitative Approach." McBurney: Approach".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m.— Professor B. J. Harrison: "Biological Communication" (Civil Service Lec-

ROYAL SOCIETY (at 6 Carlton House Terrace, London, SW1), at 4.30 p.m.—Professor J. F. Enders, For.Mem.RS.: "Virus and Cancer: Problems of Relationship of Virus to the Transformed Cell and to Cancerogenesis as Illustrated by Experiments with the Oncogenic Agent Simian Virus 40" (Pariow Lecture)

University Of London (at Bedford College, Regent's Park, London NW1), at 5.15 p.m. Professor D. F. Cheesman: "Why are Lobsters Blue" (Inaugural Lecture.)*

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Institution of Electrical Engineers (at Savoy Place, London, WC2) 5.30 p.m.—Mr R. Cafruthers: "Technological Aspects of Fusion Power at 5.30 p.m.—Mr R. Carruthers: Generation".

COLOUR GROUP (GREAT BRITAIN), CALCULATIONS GROUP AND ENTIRON-MENTAL GROUP (in the Department of Electrical Engineering, Imperial College, London, SW7), at 6 p.m.—Meeting on "Concepts of Apparent Brightness—Designing for What We See",

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, SE1), at 6 p.m.—Mr I. M. D. Potter: "Design of Multi-Storey Cold Stores".

Institution of Mechanical Engineers, Process Engineering Geour (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Dynamic Sealing of Rotating and Reciprocating Shafts".

Society for Analytical Chemistry, Biological Methods Group (at "The Feathers", Tudor Street, London, EC4), at 6 p.m.—Twenty-third Annual General Meeting, followed by an Informal Discussion on "Automation in Microbiological Assays", opened by Mr J. W. Lightbown.

Institution of Electrical Engineers, London Graduate and Student Section (at Kingston College of Technology, Penrhyn Road, Kingston-upon-Thames), at 6.30 p.m.—Mr R. E. Gleadow: "Electric Cars".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at Bast Ham Technical College, High Street South, London, E6), at 6.30 p.m.—Mr A. G. North: "Room Temperature Curing Acrylic Systems".

Society for Analytical Chemistry, Atomic-Absorption Spectroscory Group (at the Pharmaceutical Society of Great Britain, 17 Biognosbury Square, London, WC1), at 6.30 p.m.—Third Annual General Meeting followed by Dr. J. Stupar: "The Use of Ultrasonic Nebulisers in Atomic Absorption Spectroscopy

Friday, December 8

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 10 a.m.—Colloquium on "Automatic Testing of Electronic Devices, Components and Circuits".

ASSOCIATION OF APPLIED BIOLOGISTS (at the Royal Society of Arts, John Adam Street, Adelphi, London, WC2), at 10.50 a.m.—General Meeting followed by Scientific Papers.

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albertale Street, London, W1), at 1 p.m.—Miss Angela Kelly: "Exciton marle Stree Processes".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the L.E.B., R.Ac.S. London Joint Group, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 5.30 p.m.—Mr R. F. Sims and Mr. R. L. A. McKenzie: "Aircraft A.C. Electrical Systems Using Change-over Contactors and Rapid Fault Clearance".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (in the Department of Mechanical Engineering, Imperial College, London, SW7), at 6.45 p.m.—Twenty-fourth Annual General Meeting, followed by a Discussion Meeting on "Oxygen Flask Techniques" introduced by Dr. B.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 9 p.m.—Professor S. A. Tobias: "Design and Development of High-Energy Rate-Forming Machines".

Monday, December 11

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP—FLUID MACHINERY SECTION (at 1 Birdeage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Giandless Pumps".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, WC2), at 6.30 p.m.—Sir Stanley Brown: Presidential Address.

Monday, December 11-Saturday, December 16

University of Cambridge, School of Clinical Research and Post-graduate Medical Teaching (at Churchill College, Cambridge)—Intro-ductory Course on "The Biology of Skin".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR TECHNICIAN IN THE DEPARTMENT OF GEOLOGY AND GEOGRAPHY—The Registrar, Kingston College of Technology, Penrhyn Road, Kingston upon Thames, Surrey (December 6).

LECTURER and an ASSISTANT LECTURER (suitably qualified candidates with a special interest in pharmaceutical microbiology) in the DEPARTMENT OF PHARMACEUTICS—The Clerk to the Council, The School of Pharmacy (University of London), 29/39 Brunswick Square, London, WC1 (December 8).

LECTURER or ASSISTANT LECTURER IN STATISTICS—The Secretary, The Queen's University, Belfast, Northern Ireland (December 9).

LECTURER or ASSISTANT LECTURER (medically qualified) in the DEPARTMENT OF BACTERIOLOGY AND VIROLOGY—The Registrar, University of Manchester, Manchester, 13 (December 9).

SENIOR OF PRINCIPAL BIOCHEMIST (with experience in protein biochemistry relating to immunology, and preferably with a Ph.D.) to be in charge of a new laboratory in the DEPARTMENT OF RHECMATOLOGY—The Group Secretary. The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shreyshire (December 16).

The Robert Jones and Agnes Hunt Orthopzedic Hospital, Oswestry, Shire (December 16).

LECTURERS/ASSISTANT LECTURERS (with at least a good honours degree or equivalent plus suitable teaching and research experience) in Chemistry at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Kuala Lumpur and London, December 17).

Lecturer of Assistant Lecturer (qualified in applied mathematics) in the Department of Mathematics—The Secretary, The University, Dundee, Scotland (December 23).

Assistant Experimental Officer (with some experience of chemical and/or biochemical analysis, HNC, AIMLT in biochemistry or an equivalent qualification) in a new analytical laboratory located within the Department of Biochemistry—The Secretary, Institute for Research on Animal Diseases, A.R.C., Compton, Berkshire (December 31).

Senior Lecturer, a Lecturer or an Assistant Lecturer in Geography at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, WC1 (December 31).

Research Fellow (with a Ph.D. or equivalent research experience) in the Department of Medical Chemistry, The John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University, to work on the synthesis and reactions of nitrogen heterocycles—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, January 1).

Lecturer in Medical Microbiology at Makerere University College, University of East Africa, Uganda—The Inter-University Council, 33 Bedford Place, London, WC1 (January 4).

Chair of Pathology at the University of Adelaide, Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia, January 12).

Lecturer in Physical Chemistry at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, January 15).

Lecturer (preferably with interests in teaching and research in land-

Lecturer in Physical Chemistry at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pali Mall, London, SW1 (New Zealand and London, January 15).

Lecturer (preferably with interests in teaching and research in landforms) in Geography at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, January 20).

Curator (with a degree in zoology and preferably a research degree particularly in the fields of marine, fresh water or land mollusca) of Molluscs to take charge of the Department of Malacology, Australian Museum, Sydney, maintain the collection and undertake research work—The Recruitment Section, New South Wales Government Offices, 56 Strand, London, WC2 (January 25).

Professor of Microbiology and Head of the Department of Microbiology, John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, January 31).

Assistant Lecturer (preferably with an interest in developmental biology or radiation biology) in Cell Biology—Professor L. Wolpert, Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School (University of London), Cleveland Street, London, W1.

Biochemistry Gasic Grade) to work in the Department of Chemical Instrumentation and measurement) in the Department, Middlesex Hospital Medical School, London, W1.

Physicist (with experience in clinical instrumentation and measurement) in the Department, Middlesex Hospital Medical School, London, W1.

Physicist (with experience and an interest in applying physics and mathematics to physiological and medical problems) to work on new uses of short lived isotopes from the experience and an interest in applying physics and mathematics to physiological and medical problems) to work on new uses

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REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Great Britain and Ireland

The Path to Vietnam: a Lesson in Involvement. By William P. Bundy. Pp. 23. (London: United States Information Service, 1967.) [119
Overseas Development Institute, 1967.) [119
Overseas Development Institute, 1967.) [119
Science and the Individual—Are They in Conflict? By Professor J. Z. Young. (Porty-sixth Earl Grey Memorial Lecture delivered in the University of Newcastle upon Tyne, 4th May, 1967.) Pp. 14. (Newcastle upon Tyne, The University, 1967.) 5s. [119
Scientific Proceedings of the Royal Dublin Society. Series A, Vol. 3, No. 2: The Influence of Pleistocene Glaciation on the Geomorphology of Eastern Murrisk, Co. Mayo. By John McManus. Pp. 17–32. 5s. Series A, Vol. 3, No. 3: A Note on Tournaisian Strata in Northern Ireland. By J. J. R. Sheridan, W. F. Hubbard and R. W. Oldroyd. Pp. 33–38. Series A, Vol. 3, No. 4: Ascariasis in Pig and Man. By James M. Barry and Fergus J. O'Rourke. Pp. 39–56. 5s. Series B, Vol. 2, No. 8: The Behaviour and Persistence of Rhizoctom solam in Irish Soils. By M. J. Downes and J. B. Loughnane. Pp. 67–74. 4s. Series B, Vol. 2, No. 9: An Improved Method for Extracting Leatherjackets from Soil and an Evaluation of the Maercks Method. By A. M. Feeney. Pp. 75–79. 2s. 6d. Series B, Vol. 2, No. 10: Results of Preliminary Survey for Virus Vector Nematodes in Ireland. By J. F. Moore. Pp. 81–86. 3s. (Dublin: Royal Dublin Society, 1967). [119

Other Countries

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United States Department of the Interior: Geological Survey. Bulletin 1230-J: Mineral Resources of the Pine Mountain Primitive Area, Arizona. By F. C. Canney, W. L. Lehmbeck and Frank E. Williams. Pp. vi+45+plates 1 and 2. \$0.65. Bulletin 1261-A: Mineral Resources of the Desolation Primitive Area of the Sierra Nevada. California. By F. C. W. Didge and P. V. Fillo. Pp. vi+27+plate 1. Water-Supply Paper 1608-G: Analysis of Aquifer Tests in the Punjab Region of West Pakistan. By Gordon D. Bennett, Ata-Ur-Rehman, Ijaz Ahmed Sheikh and Sabir Ali. Pp. lv+56+plate 1. \$0.55. Water-Supply Paper 1839-K: Ground-Water Resources of the Pascagoula River Basin, Mississippi and Alabama. By Roy Newcome, Jr. Pp. iv+36. \$0.20. Water-Supply Paper 1843: Geology and Ground-Water Resources of the Big Sandy Creek Valley, Lincoln, Cheyenne, and Klowa Counties, Colorado. By Donald L. Coffin. Pp. iv+49+plate 1. Professional Paper 542-E: The Alaska Earthquake, March 27, 1964: Effects on Communities—Sward, Alaska. By Richard W. Lemke. Pp. vii+43+2 plates. Professional Paper 542-F: The Alaska Earthquake, March 27, 1965: Effects on Communities—Kodiak and Nearby Islands. By Reuben Kachadoorian and George Plafker. Pp. vi+41+plate 1. \$0.75 Professional Paper 544-C: The Alaska Earthquake, March 27, 1964: Effects of Hydrological Regimen—Outside Alaska. By Robert C. Vorhis. Pp. vi+54. \$0.45. Techniques of Water-Resources Investigations of the United States Geological Survey. Chapter A: Measurement of Peak Discharge at Width Contractions by Indirect Methods. By Howard F. Matthai. (Book 3—Applications of Hydraulics.) Pp. viii+44. \$0.35. (Washington.) D.C.: Government Printing Office, 1967.)

United States Department of the Interior: Geological Survey. Water-Supply Paper 1885: Quality of Surface Waters of the United States, 1961. Parts 9-14: Colorado River Basin. Prepared under the direction of S. K. Love. Pp. xviii+677. (Washington, D.C.: Government Printing Office, 1967.) \$2.25.

Department of Agriculture, Fiji. Bulletin No. 47: An

1967.) \$2.25.
Department of Agriculture, Fiji. Bulletin No. 47: Annual Reports of Specialist Officers, 1962. Pp. 124. (Suva, Fiji: Department of Agriculture, 1967.) 4s.
National Research Council of Canada. NRC Review '67. Pp. 372. (Ottawa: National Research Council of Canada, 1967.) \$2.

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Parliament and the Research Establishments

FRESH from its encounter with the nuclear power industry, the Select Committee on Science and Technology of the House of Commons will, this coming parliamentary session, be tackling the problem of the defence research establishments operated directly by the British Government. Evidently the committee has decided not to restrict itself to easy problemsnext to the problem of how best to co-ordinate the making of policy on science and technology in Britain, that of knowing how best to deploy the great resources of the defence laboratories in civilian fields is the most urgent in sight. It is also an old problem. When the present government came to power in 1964, it firmly declared its intention of directing the efforts of the research establishments into more profitable fields. At one time there was talk of how the Ministry of Aviation might as a whole become a Ministry of Technology. But as things have turned out, the defence establishments remain firmly within the control of the Ministry of Defence. Collectively, they are afflicted by the kinds of troubles which have been obvious now for several years at the Atomic Energy Research Establishment at Harwell—they have often outlived the immediate task of supporting the development of military equipment and have time and spare capacity to use in other fields. But, like the AERE, they are understandably unwilling to put themselves out of business. In the circumstances, what the select committee will have to ask itself is how the defence research establishments can be reorganized in such a way as to make their great fund of talent available for other jobs. The committee should not be surprised if it finds itself covering ground which should be familiar from its inquiry into the condition of the nuclear power industry.

The committee will also have to contend with some difficulties which were not apparent in its first inquiry. For one thing, because a great deal of defence research is secret, it may be hard for the committee to operate for the most part in public. But although there is a great deal in what the civil service will argue about the need for private hearings, this is a temptation which the committee should resist as energetically as it can. So far, its influence has been enormously enhanced by the way in which evidence provided to the committee by official witnesses has been open to criticism and challenge from outside. In a sense, the publicity attending its proceedings has gone a long way to make up for the lack of expert counsellors of its own which is the most obvious of the committee's shortcomings in the past year. In the circumstances, it would be folly for the committee to allow itself to be kept behind closed doors while still not equipped with the kind of staff which could help it to design really penetrating questions. Congressional committees in the United States seem to be entirely successful in carrying out the most delicate inquiries on military matters without succumbing to the needs of secrecy. The British committee would do well to study the methods by which the military committees of Congress function before embarking on its new adventure. In the process, it will also learn how easy it is for committees to be bamboozled by a plethora of evidence. In the month-ahead, the committee should regard the appearance of memoranda which are unexpectedly voluminous with the gravest suspicion.

There remains the problem of deciding what questions to ask. The administration of particular research establishments will no doubt occupy a great deal of the time at the committee's disposal and this is not Establishments such as the Royal unreasonable. Aircraft Establishment and the Royal Radar Establishment are big spenders on a scale entirely comparable with the AERE. Like that establishment, they have also accumulated several functions not directly related to defence as well as what seems, on the face of it, to be unused capacity. This is why it would be entirely proper to ask whether some parts of these establishments would not be better placed outside the Ministry of Defence. Why not, for example, ask whether the radio astronomy at Malvern and the space research at Farnborough should not be transferred to some other organization, administratively if not physically? The problem is to balance the advantages of the present arrangements, which allow academic research to benefit from close contact with more practical activities and which give people engaged on applied research the stimulus of knowing that more distant goals are attainable, against the fact that basic research carried out at defence establishments deprives universities of funds and opportunities from which they could profit enormously. To begin with at least, it would make sense if the committee were to think of alternative arrangements under which some parts of the defence establishments could be transferred to other agencies -the Science Research Council, for example-without losing the physical services which the establishments can provide. Not merely would this increase the interchange between universities and the establishments but in the long run it would also provide more easy access by government scientists to the outside world—industry. for example.

This, however, is only the superficial part of the problem. A more serious question is the means by which the grand strategy of defence research is or should be determined. In the past, ever since the end of the Second World War, there has been no effective means by which the demands of the defence research establishments for manpower and money could be moderated by criticism from outside. It is true that there are many circumstances in which the needs of defence must be considered to be paramount, but this principle has often too easily become a means by which the defence establishments could grow without restraint and take on jobs which are not properly their business. With the reorganization of the Ministry of Defence in the past three years, it has become easier to keep the establishments properly in check. For one thing, explicit forward planning has become fashionable. More practically, central direction has become stronger. Yet there is a great deal still to be done if there is ever to be a built-in mechanism by means of which considerations which seem imperative to the Ministry of De-

fence do not distort the pattern of research and development in other fields as well. The Central Advisory Council on Science and Technology is the obvious way of striking a proper balance, but it has been less than fully effective since it was set up a year ago, partly at . least because the Ministry of Defence has responded with less than enthusiasm to suggestions that its research should be more fully co-ordinated with that of other agencies. To be fair, this unwillingness may also be prompted by unsureness about the future development of the Central Advisory Council-what kind of a body will it turn out to be? The Select Committee on Science and Technology could help a lot by helping to define more accurately the part which the Central Advisory Council should play. It may also think it profitable to consider other schemes for coordinating defence and civil research—one possibility, for example, would be an independent research council with powers to advise on and even direct the development of defence research as if this were any other public activity.

Good Start on Natural Environment

THE second report of the Natural Environment Research Council is a sober document but also a worthy one. In the short period of its existence, the council has evidently taken the line that it must begin slowly and concentrate on the creation of institutions. This is sensible enough. The council owes its existence to the report of the Brundrett Committee in 1964 which argued that there was a serious need of some comprehensive organization which could sponsor and encourage the development of research in a variety of fields, from oceanography to nature conservation. In the circumstances, it is only prudent that the council should wish to be assured of a firm base for operations. The search for a better organization for the Institute of Geological Sciences typifies the problems which the council quite properly seeks to solve. Regional offices responsible for the continuing geological survey of the United Kingdom, among other things, have to be rehoused, and there is also a need to make long term decisions about the siting of the headquarters. The difficulty here is that the institute must necessarily have a double-headed role—half of its work is academic and it should properly become a catalyst of much that happens in universities, but the institute also has administrative responsibilities for geology, mining and hydrology. It is therefore sensible that the council seems convinced that any new buildings there may be should be closely linked with universities, at least geographically. Whether it will be able to satisfy its ambition to find a headquarters outside London is another matter.

If the past year has been moderately successful, however, there is not much in the new report to suggest which way the council's institutes will develop in the years ahead. It is one thing to have a decent wish to work closely with the universities and quite

another to be energetic enough in the pursuit of such ideals. Indeed, for all its well-wishing on this subject, the council's latest report gives the impression that some of its establishments will remain self directing and essentially autonomous organisms with only the most arbitrary links with academic institutions, however closely interested these may be. This is why the council should ask itself whether there is not a case for duplicating some of its establishments in other environments. It would, for example, do no harm if some other university than Liverpool became so well endowed with facilities for research in oceanography that it could look the National Institute of Oceanography squarely in the face. There is also a case for asking that the dominant role of the Nature Conservancy in another field of operations should be somehow softened by the building up of well endowed centres of excellence at the universities. To say this, of course, implies no more than that competition between research establishments working in strictly academic fields is usually beneficial. And, in any case, anything which the council can do to strengthen its hold in the universities will help to strike a better balance within its budget, which at present seems to involve the spending of too great a proportion of its resources on institutions and too little on grants for supporting research projects.

Russian Eclipse

THE following description of the Russian preparations for the solar eclipse of September 22, 1968, has been supplied by Evald Mustel, chairman of the Astronomy Council of the USSR Academy of Sciences, and Nataliya Yegorova, an associate of the Astronomy Council of the USSR Academy of Sciences, through the Novosti Press Agency.

On September 22, 1968, a total solar eclipse will be observed on the territory of the USSR. A full phase band will start near Severnava Zemlya, North Land, will cross the Kara Sea and enter the mainland near Vorkuta. It will then go nearly straight to the south along the 62nd to the 64th meridians. Then, turning sharply to the south-east, it will pass slightly north of Alma-Ata, west to west China and will end north of Lobnor Lake.

Although the 1968 eclipse is unfavourable for observations because of the short length of time for which it will be visible—a maximum of 42·7 seconds—and the low height of the Sun above the horizon—a maximum of 18·7°—many Soviet astronomers are going to observe it. It is proposed to place nearly all expeditions near the railway station of Kargopolye, on the Sverdlovsk–Kurgan line, where the maximum duration of the eclipse and the maximum height of the Sun above the horizon are combined with relatively favourable meteorological conditions.

In the optical range, all traditional types of eclipse observations of the corona, chromosphere and photosphere will be conducted. Associates of the astrophysical observatory of the Georgian Academy of Sciences, as well as the astronomical observatories of Kiev, Lvov and Ural Universities, intend to obtain large-scale photographs of the inner, middle and outer coronas important for studying coronal structural Kiev and Lvov Universities are planning to observe the degree of polarization of coronal emission, while the observatory at Lvov will undertake colorimetric observations of the corona. The spectrum of the corona will be photographed by the Abaetumani Astrophysical Observatory of the Georgian Academy of Sciences, and by the astronomical observatories of Kiev, Ural and Kharkov, as well as by the Sayanskaya Solar Observatory.

The observatory of Kharkov University is planning to take direct photographs of the chromosphere at the wavelength of the hydrogen a-line. The sun department of the Institute of Terrestrial Magnetism, the Sayanskaya Solar Observatory (with the Echellete spectrograph) and the main astronomical observatory of the Ukrainian Academy of Sciences are planning to study the chromospheric spectrum, which requires great skill in observations and subsequent thorough processing. The programme of the Sternberg Astronomical Institute includes taking pictures of the chromosphere and the corona with the Fabry-Perot standards which make it possible to evaluate the distribution of the temperature and the turbulence velocity around the solar disk.

In addition, the programme of the Sternberg Institute and the main observatory of the Ukrainian Academy of Sciences includes investigations of the photospheric spectrum on the solar limb, and the behaviour of spectral lines near the limb. Ural University will do similar studies using a stationary horizontal solar telescope. The Sternberg Institute will also be making photoelectric records of fluctuations from the centre of the disk to the limb in individual portions of the photospheric continuous spectrum.

Finally, the main astronomical observatory of the USSR Academy of Sciences and the observatories of Leningrad and Kiev Universities are preparing to record solar emission during the eclipse. These recordings will include investigations of the fine struc-

ture of local sources of the slowly varying component of the solar emission at 4 cm, and of the distribution of radio brightness of local sources on the Sun, and on the limb of the quiet sun. Investigations will also be made of the degree of inhomogeneity of the corona, and of the radio sizes of the solar disk in the centimetre wavelength range.

Transport Arranged

THE Minister of Transport, Mrs Barbara Castle, seems now to have completed most of her planning for the development of transport in Britain in the years ahead. The last of four white papers on transport policy was published earlier this week, and deals with the organization of public transport (Cmnd 3481, HMSO, 3s. 9d.). The outstanding proposal among those contained in the White Paper is that responsibility for public transport systems should be transferred to public transport authorities set up in the various regions of the United Kingdom. On some occasions, this will mean that municipal buses are nationalized. Because most transport undertakings are at present in public ownership, however, the principal effect of the new proposal would be to transfer to regional authorities responsibility which is at present centred on the central transport commission.

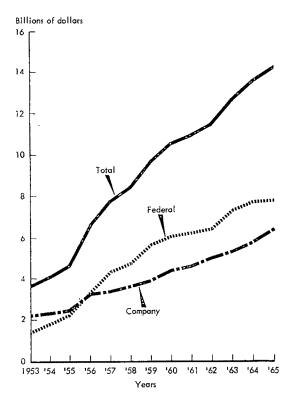
Mrs Castle hopes that her plan will commend itself to those who argue that transport systems should be planned in such a way that they are closely integrated with city planning, and she is no doubt correct in that assessment. It is also reasonable that, if the regional transport authorities are to be subsidized, the injection of money should be used to pay for capital resources rather than for running costs. Some difficulties stand out, however. For one thing, there is no assurance that within the framework of the proposals now put forward it will be possible to find room for the kinds of technical developments in transport which are necessary in modern cities. Monorails may be out of fashion, but there is always a possibility that a regional transport authority would be more anxious to guard the sanctity of its existing stock of buses than to introduce small road transport vehicles, for example.

American Industrial Research

Industry in the United States seems to be playing a decreasingly important part as a performer of research and development. It still does the lion's share—70 per cent in 1965—but this represents a decline from the peak reached in 1957, when 78 per cent of all research was done by industry. The growth in the industrial sector, 5 per cent between 1964 and 1965, is the slowest of all. These figures, published by the National Science Foundation in a report called Basic Research, Applied Research and Development in Industry (US Government Printing Office, 65 cents), show that despite the change in emphasis, industry in the United States still does a greater proportion of the work than does industry in Britain. In 1964–65, British industry was doing about 60 per cent of all research and development work.

The slower growth recorded by industry in the US seems to have been a consequence of a deceleration of federal support; industry's own contribution has continued to rise at the same rate for several years.

Between 1953 and 1959 the federal contribution to industrial research expenditure increased sharply, and overtook the industrial contribution in 1956. Since then the scales have begun to tip the other way. American industry in 1965 financed some 45 per cent of the research work which it carried out, while federal support made up the rest.



The changes in distribution are naturally overshadowed by the sheer growth of research expenditure since 1953. Total expenditures have increased since then from \$3,630 million to \$14,197 million. Companies in the aircraft and missile business accounted for more than a third of this, some \$5,120 million, while research in the electrical equipment and communication business amounted to \$3,167 million. Just under 90 per cent of the aircraft and missile research was supported by federal funds, as was more than 60 per cent of the electrical and communication research. At the other end of the spectrum, the chemical and allied products industry found 85 per cent of its research expenditure from its own pocket.

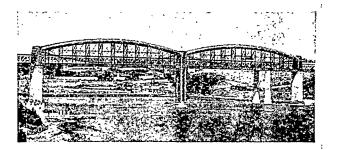
Overwhelmingly the advantage lies with the big battalions. The eight companies with the largest research and development expenditures accounted for 11 per cent of net sales, 13 per cent of total employment, and 47 per cent of all federally supported research. There were some 13,400 companies conducting research in the United States in 1965, but 87 per cent of the research was done by about 3 per cent of the companies. Among large companies, 29 had research and development programmes costing more than \$100 million and made up \$9,000 million of the total expenditure.

More than half the scientists and engineers working in industrial research worked in the aircraft, missile, electrical and communications industries. Together with chemicals and allied products, machinery, motor vehicles and other transport equipment, these industries employed 81 per cent of the qualified manpower. In January 1966, the total number of scientists and engineers employed in research and development was 358,900.

Monumental Masonry

In 1955 Mr Michael Rix invented the name industrial archaeology. Although some say that industrial monuments are too recent to be considered archaeological relics, Mr Rix points out that an eighteenth century ironworks, the model T Ford and the crystal set are really as out of date as a Stone Age axe. In a pamphlet called Industrial Archaeology, published by the Historical Association (3s. 6d.), Mr Rix makes a convincing case for industrial archaeology and, in particular, for preserving the monuments of the Industrial Revolution before it is too late. This is especially important in Britain, of course, where the Industrial Revolution began and where there are many of its relics.

The pamphlet makes encouraging reading, and the increase in the number of industrial museums, the founding of the journal Industrial Archaeology and the encouragement and indirect support of the Government point to the rapid expansion and interest in industrial archaeology. The most immediate problem is to assess the importance of sites and items. Nobody knows exactly what relies of the Industrial Revolution are still left scattered about the country. A national survey of industrial monuments is being made under the auspices of the Council for British Archaeology, which receives some support from the British Government. This survey is being co-ordinated by Mr Rex Wailes, who, before joining the Council for British Archaeology eighteen months ago, acted as a consultant to the Ministry of Public Building and Works. Mr Wailes collects information and presents proposals for the preservation of industrial monuments to the council. If they are agreed upon, the council makes representations to the Government asking for a preservation order.



Railway bridge across the Saltash, built by I. K. Brunel for the Great Western Railway. The modern suspension bridge can be seen in the background. (British Rail photograph.)

At the same time, a national record of industrial monuments is being maintained at the Centre for the Study of the History of Technology at Bath University of Technology. But progress is slow and depends almost entirely on the work of individuals and local archaeology societies. In a progress report in the

current issue of *Industrial Archaeology*, Dr A. Buchanan, the director of the centre, says he has adequate records for only three counties—Hertfordshire, Somerset and Staffordshire.

Many monuments have been preserved. The New Lanark industrial estate, which incorporates the technology of Arkwright with the social planning of Robert Owen, and Coalbrookdale, where Abraham Darby first successfully smelted iron ore with coke, are obvious and famous examples. But these are exceptional and the list of industrial monuments which have been destroyed is depressingly large and growing steadily. As things are, the relics of the transport revolution, canals, turnpike roads and railways, with their magnificent bridges, are more than ever threatened.

Oxford on Iron Rations

from our Oxford Correspondent

This week the governing bodies of British universities will be examining the details of their Quinquennial Grants from the University Grants Committee, but few of them, perhaps, with as little delight as Oxford. Yet at first sight, Oxford's grant seems generous. In 1965–66, of Oxford's total income of £8.0 million, the UGC provided £5.25 million, with a further million pounds of government money coming from research councils and other public bodies. By comparison, the recurrent grants for the next five years are:

1967-68 1968-69 1969-70 1970-71 1971-72 £6·1 m. £6·2 m. £6·4 m. £6·6 m. £6·8 m.

Equipment is to be provided for separately from these grants.

In fact, the increase in the grant since 1966 will do little more than cover increases in salaries and prices during the period so that, according to the Hebdomadal Council, there will be no additional funds available for new expenditure next year. At the same time, universities are having to repay sums to the Grants Committee in compensation for the increase in their incomes from the higher fees of overseas students. Oxford, however, refused to increase these fees, so that £63,000 will have to be paid from the university's own funds, with none of the income for which it would have been a compensation. These payments are expected to increase over the five years.

Even if the Quinquennial Grant had been less parsimonious, the devaluation of the pound would doubtless have forced economies on most university faculties, especially on the science side. Ironically, devaluation will harm those university departments the growth of which the committee had hoped to encourage by The Bodleian Library, for increased allocations. example, has long been short of funds; in 1963-64 its total income was only \$1.75 million (at the old rate) compared with nearly \$6 million for Harvard Univer-The Quinquennial Grant contains sity Library. provisions for increasing library expenditure by 20 per cent per student. How far this will be vitiated by devaluation and the increased cost of some books, it is too early to say. In the same way, the growth of Middle Eastern and Latin American studies will be slowed down and these departments, as well as the science faculties, will be waiting earnestly to hear that the government will compensate for the fall in the value of the pound.

There is therefore little hope that Oxford will be able to expand its activities, even though it receives public money from a variety of sources. Increasing college fees—paid for in the main by local authorities—is no light matter and, even if tuition fees are increased. colleges will be able only to appoint a few new tutors to help out penurious university departments. Even so, the majority of college tutors depend in part on university emoluments, paid for by the UGC. Finally, whatever elasticity of this kind there may be in arts faculties, there is far less in science, where the expense falls almost entirely on the Grants Committee and the various research councils. These latter will shortly be issuing their own estimates; if they are equally disappointing, then Oxford will indeed have to live on rather sparser fare than might have been hoped.

Money for Harvard

HARVARD UNIVERSITY is trying to raise nearly \$50 million for an ambitious (some might say, luxurious) programme to make science more exciting for undergraduates at Harvard College. Of the total sum. \$14.5 million will be used to build a new science centre, with laboratories, computers and a library, in which undergraduates will be encouraged to do research. Their efforts to do this now are inhibited by the demands of graduate and post-doctoral research. Another \$12.6 million has been budgeted for a new centre for research and teaching in biochemistry and molecular biology (fields in which Harvard has four Nobel Laureates). A project is also indicated to endow five professorships, in astronomy, biology, engineering, mathematics and physics.

More than one fourth of the several thousand undergraduates at Harvard take degrees in science. The others, who read humanities or social sciences, must spend at least a small fraction of their time at Harvard studying science or the history of science. They, as well as the science undergraduates, will benefit from "a programme for science" as it is being called. Harvard says that it is conscious of its responsibility to give all of its students, especially those who will become teachers, some acquaintance with what modern science is about.

Physics Directory

The ninth edition of the Directory of Physics and Astronomy Faculties has been published by the American Association of Physics Teachers and the American Institute of Physics for the period 1967-68 (\$3). The purpose of the directory is to list those universities in America, Mexico and Canada which offer physics courses and the members of staff who teach these courses. The information which has been collected is part of the procedure used to improve coverage of the National Register of Scientific and Technical Personnel which is maintained by the American Institute of Physics under the sponsorship of the National Science Foundation.

Also listed this year are departments of astronomy and astrophysics, but this section is incomplete and it is hoped that better coverage will be given next year. The directory is divided into seven parts: part one provides a geographical listing of personnel and institu-

tions in the United States; part two includes a geographical list of personnel and institutions in Canada; and part three provides a similar list for Mexico. Parts four, five and six are delegated to an alphabetical index of colleges and universities in the three countries, and part seven is an alphabetical index of personnel.

Progress in Time Sharing

In August 1966, the Computer Board was set up under the chairmanship of Professor B. H. Flowers to carry out, on the basis of planned development, the proposals for providing computers in British universities and research councils in the light of the Flowers Report of January 1966, and for ensuring that the facilities provided were fully used. The five Board members are Professor C. E. H. Bawn of Liverpool University, Professor Gordon Black of the National Computing Centre, Professor D. J. Black of Edinburgh University, Mr J. K. Steward of ICI, and Lord Halsbury; Mr L. S. Rutterford is secretary to the Board.

In accordance with the recommendations of the Flowers Report, which suggested the expenditure of a total of £17.68 million over a period of five years for the provision of computer hardware in universities, a regional centre is now being set up in Edinburgh. This is one of the three suggested centres—London, Manchester and Edinburgh—at which large computers with special facilities are to be installed for the general use of universities in the areas. The concept of a multiple access time sharing system at Edinburgh is, however, an additional feature which was not visualized at the time of the report. In comparison with the actual cost of the hardware, additional costs of consoles and software to furnish the multiple access systems are small and will not represent a major item of expenditure above that recommended by the Working Group. The University of Edinburgh will soon be taking delivery of a British computer, the English Electric 4/75. Several agencies have contributed to the cost of the computer and the software: these include the Computer Board, the Ministry of Technology and various research councils, particularly the Agricultural Research Council. The cost has not yet been settled, but the total so far committed is about three-quarters of a million pounds. Development of the system is being shared by the university and the manufacturers. Whether or not multi-access systems will be established at the two other centres has not yet been decided.

The University of Newcastle upon Tyne has already taken delivery of an American IBM 360/67 multi-access computing system which was installed in October and is currently undergoing tests. When operational, use of the machine will be shared by Newcastle and Durham Universities. £575,000 of the total cost of the computer was provided by a grant from the Computer Board. Communication terminals will be available in various departments of both universities and satellite computers can be attached to the central computer.

Research workers in two universities are developing their own multi-access facilities by building on to existing computers already at their disposal. Thus a smaller computer providing these facilities has been developed at Edinburgh University under Professor D. Michie for application to medical research work. This is based on the Elliott 41/20 model which was delivered

in early 1966. The software has been produced in the university, but the cost of salaries and expenses was covered partly by the MRC, partly by the SRC and partly by the university itself. There are twenty consoles connected to the system, any eight of which can be used simultaneously. In addition there are two remote consoles which are attached by telephone: one is at the National Institute for Medical Research at Mill Hill and the other is at the Department of Social Medicine at Edinburgh. The system involving the twenty consoles has been operational since this summer, but the two remote consoles will not become operational until the end of the month.

Serious work on multi-access systems at Cambridge began at the end of 1964 with the design of a multiplexor to attach to the Titan computer. Development of the hardware was a joint exercise between the university and International Computers and Tabulators, Ltd. The cost of the consoles and multiplexor was met by the SRC while the disc store, which represented the major item of expenditure, was ultimately paid for by the Computer Board. By November this year the multi-access computing service was running at more than a hundred hours a week. At present there are about twenty-five consoles and between sixty and seventy regular users: most of the consoles are in the mathematical laboratory, but there are others in the engineering laboratory, the chemical engineering and the medical psychology laboratories. Workers at Cambridge maintain that the multi-access system has greatly increased the productivity of its users, and has materially diminished the demand for specially rapid service through the ordinary computing system.

Indeed, multiple access computing systems are very much a subject of objective research and exploration in leading British universities concerned with computer science. Applications for the time sharing systems are being considered by the Computer Board, which has in mind the linking up of computer systems in various universities, particularly in the south-west—to provide a complex computer network.

Despite the advantages of a multi-access system which gives a large number of users immediate access to the large computing system and which allows intimate contact between the worker and the tool as well as providing—in principle—quick and easy programme development, several problems have to be overcome. For example, considerable difficulties have arisen at Newcastle in connexion with the software and the system is far from satisfactory. Similar difficulties have been encountered in the USA with the same model. Evidently, the most economic arrangement has yet to be worked out.

Environmental Research

THE Natural Environment Research Council enters its second year in a better state of organization than in its first. The report of the council for the year ending March 31, 1967 (HMSO, 11s.), describes what has been happening. Broadly, the council seems to have been digging itself in as a permanent part of the Civil Service, by setting up a headquarters (from which it has since moved), establishing specialist committees, and defining the details of recruitment and employment of staff. Scientifically, the council is making an attempt to originate policies of its own, and organize

more cohesively the research programmes it is responsible for. Already there are some signs that the divisions between disciplines which the council inherited have softened, although the establishment of separate committees to serve the separate arms of the council's activities is bound to preserve some of the distinctions.

The council is responsible for research in the United Kingdom which is concerned with the environment. This covers geology, oceanography, the Nature Conservancy, the Antarctic Survey, hydrology and forestry, and each of these broad areas has been provided with an advisory committee to establish policy and make recommendations about grants. The total income of the council over the year was just over £5½ million; the table shows how this income was distributed among the competing interests:

Institute of Geological Sciences	£1.34 m	25.60 per cent
Nature Conservancy	£0.96 m	18.40, ,, ,,
National Institute of Oceanography	£0.65 m	12.40 ,, ,,
University grants	£0.68 m	13.04 ,, ,,
University training awards	£0·44 m	8.33 ,, ,,
Meteorological Office	£0.08 m	1.46 ,, ,,
Hydrological Research Unit	£0.08 m	1.45 ,, ,,
Grant aided laboratories	£0.70 m	13.30 ,,
Administration	£0·17 m	3.32 ,, ,,

The main force of reorganization has so far been felt in the Institute of Geological Sciences (formerly the Geological Survey). Here a much wider research programme has been agreed, and the institute will be responsible for geological investigations of the continental shelf. The discovery of natural gas in the North Sea, and the increasing importance of the exploitation of sand and gravel, have stimulated this new development. During the summer of this year the first investigation was made, in the north Irish Sea, and preliminary work has also been done off Scotland and in the Moray Firth. There is a good chance that the institute will be moving out of London at some time in the future, although so far it has been impossible to find anywhere suitable. Difficulties of accommodation in London have proved "the most hampering factor for smooth progress during 1966" the report says, but the situation has been eased by the leasing of another building in Princes Gate, not far from the museum.

The Nature Conservancy has also undergone a complete reorganization since the coming of the council. The research has been regrouped into eight groups which the council has decided to call Habitat Teams. Each is responsible for a different habitat—Monks Wood Research Station looks after lowland grasslands and grass heaths, and wildlife in the agricultural environment, for example, while hill grasslands are the responsibility of the group at Bangor, and wetlands that of the group at Edinburgh. One of the council's principal preoccupations since the Torrey Canyon incident has been the study of coastal ecology, and this will be studied by the team at Furzebrook until the research station at Norwich is complete. One interesting conclusion reached by the Unit of Grouse and Moorland Ecology during the year was that grouse stocks can be considerably improved by burning small fires all over the grouse moors. The survey shows a direct correlation between the number of fires started and the grouse stocks, and the report suggests that by better management many of the Scottish grouse moors could be brought back to their former glory.

The most fashionable part of the council's work is The Ministry of Technology, that in oceanography. the report says, "is concerned to identify technological developments which can make use of any spare capacity of the Atomic Energy Research Establishment (Harwell) that may arise in the near future". The report then goes on to suggest that the ministry is thinking of turning Harwell's attention towards the technology of the sea and the sea-bed, and that this was the reason for the conference on the subject at Harwell in April this year. But the report makes clear that it intends to remain the prime co-ordinator of marine scientific research in Britain and to encourage the ministry to plan the commercial exploitation of the research findings. In other words, if oceanography is to be promoted to a major research industry by the British Government, the council has no intention of being outmanoeuvred by Harwell. It is clear, though, that more research vessels are going to be needed; the council recommends that a new vessel primarily intended for biological research, and for use by the Scottish Marine Biological Association and the universities, should be built. The Institute of Geological Sciences will need a new dual-purpose vessel, again to be used in conjunction with the universities, and two smaller vessels will be needed for coastal work. Finally, the council has made provision for a two-man submersible, capable of operating at 100 fathoms, with a support vessel. It would be surprising if the development of just such a craft by a British firm (see Nature, last week, 216, 845; 1967) can have been entirely coincidental.

Apart from the ecology and geology of the coastline, and the geology of the continental shelf, the other major new development described in the report is in the hydrology and biology of inland waters. This will be done, the report says, by building up the Hydrological Research Unit so that it can carry out a comprehensive programme of research into all the factors affecting water balance in catchments, and develop an effective means of co-ordinating work in hydrology being done elsewhere. As if to demonstrate that this is more than a fine phrase, the council points out that the research effort of the unit has more than doubled since the council took over responsibility for it two years ago. And the council now intends to treble the present research effort by 1970.

All this has not been done without a marked expansion of the administrative side of the council. When the report went to press the staff was some seventy-five, and since then a further expansion to almost 100 has occurred. As a result of this expansion, the council was forced to move out of its offices in State House, and has now taken over eight floors in Alhambra House in Charing Cross Road. Even this, however, seems to be barely adequate for the council's purposes.

Research for Industry

THE Ministry of Technology is to set up industrial units in British universities to help industry by providing commercial consultancy and research services. For a start, the units will be established in four universities, at the College of Aeronautics at Cranfield and at the Risley establishment of the Atomic Energy Authority. The ministry has made a launching grant of about £1 million to get the units started, on the condition that

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each appoints a full time manager to look after the commercial activities. Once the centres are operating successfully, it is expected that they will be self supporting, and the money will be freed to start new centres at other universities.

The first centres reflect the British Government's interest in tribology—the study of interacting surfaces in relative motion, including friction, wear and bearing design. The universities of Leeds and Swansea and the establishment at Risley will be concerned with tribology. The University of Strathclyde has a general brief covering the whole of engineering; Cranfield will look after precision engineering and Bangor will be responsible for instrumentation and control engineering. Each unit will be undertaking contract research for industry and the project is intended to be a thoroughly commercial one. But the ministry foresees no conflict between the units and the universities proper, and the minister himself goes near to saying that the commercial pressures will be a good thing for all concerned. "The universities are going to sell themselves to industry," Mr Benn declares.

In approving the centre at Risley, the minister seems implicitly to have rejected the recommendation of the Select Committee on Science and Technology, which reported last week. The committee said that the authority should not undertake work on subjects not inextricably linked with its primary task. Mr Benn said that the policy of diversification is "well established".

The emphasis on tribology, and the name, derive from a working party set up by Lord Bowden when he was Minister of State at the Department of Education and Science in 1964. The committee, under the chairmanship of Mr H. Peter Jost, was the originator of the now celebrated claim that industry could save £500 million a year by the better use of lubrication. In support of these figures, Mr Jost has quoted the experience of East Germany, where training in lubrication has been made compulsory, and qualified lubrication engineers have been appointed to state owned works. Within a few years, Mr Jost said, the East German steel industry has saved £2-£3 million, which would have meant £21 million in Britain. One of the conclusions of the Jost report was that inadequate training in tribology would be a major stumbling block-but Mr Benn's new proposals make no direct provision

Glass Reinforced Plastics

A HAPPY accident in 1940, when catalysed polyester resin was spilt over several layers of glass fibre, produced the first glass reinforced plastic (GRP). Since then the highly satisfactory structural properties of this material have been studied and it can now be considered alongside the more conventional building materials of concrete and steel. The development, properties and design qualities of glass reinforced polyester resins were described by Mr Brian J. Bell at a meeting of the Society of Engineers on December 4.

Polyester resin has the great advantage of polymerizing at room temperature and low pressure. GRP can therefore be moulded (layed-up) by hand, and can be used for moulds of any size and shape. It is resistant to weather and many chemicals. These advantages are somewhat offset by the slowness of production

compared with injection moulding methods, and skill is required to produce a material of consistent strength. Glass fibre for reinforcement and the resin itself are expensive, but no costly equipment is required for production of GRP. Fillers can be added which alter the gelation time, and mechanical, thixotropic and self-extinguishing properties of GRP and the form of reinforcement can also be chosen to suit the required design properties. The most common form of reinforcement is the chopped strand mat in which 2 in. random strands are bonded together with resin to form a mat which weighs from 1 to 2 oz. per square foot.

GRP can be produced by contact pressure and by low or high pressure moulding. Each method has variations which are introduced to suit the size, shape, dimensional tolerance, cost, strength and quantity of the required product. In all cases a release agent is required for releasing the product from the mould. Mr Bell gave examples of the general properties of various laminates that can be made from GRP, and compared its properties with those of mild steel. Volume by volume GRP can be almost twice as strong as mild steel, while weight for weight it can be thirteen times stronger. But sometimes it lacks rigidity, and variations can occur in mechanical properties because of differences in manufacturing conditions or in composition. Mr Bell described ways in which the limited span of GRP can be extended, particularly by folding the material—despite the unsolved problem of prestressing, GRP obviously has a great future.

Another Food Supplement

LAST week the American University of Beirut announced that a high protein food supplement, called "l'aubina", had been developed in Beirut under the direction of the Columbia University Institute of Nutrition Sciences and financed by the United States National Institutes of Health. It is the latest addition to the growing list of food supplements developed from local products in various parts of the world in an attempt to combat local malnutrition with locally grown produce. "L'aubina" is made from chick peas and parboiled wheat-both are grown in the Middle East, North Africa, India and Pakistan—with small amounts of bone ash and skim milk. It is easy to make-apparently any food manufacturer could make it with existing machinery—and cheap. In tests, the nutritional health of children was maintained by a daily supplement of 7 ounces of "l'aubina" which would cost only about 2s. 1d. (25 cents) to produce. It contains 17.1 per cent protein-about the same amount as roast leg of lamb—adequate amounts of vitamins A and D and some vitamin B and calcium.

The question now is whether a commercial producer can be found to produce "l'aubina" in bulk and, even more important, whether it will be accepted by those who are suffering from malnutrition. Past experiences are not at all encouraging. In Peru, a supplement called "Peruvita", which is made from the local plant quinoa, was developed over a period of five years but abandoned last year because it was not accepted. Similarly in Guatemala, "Incaparina". made from cotton seed and sorghum, has not been accepted. It is still produced but only reaches 2 per cent of the children in the country. So great is the prejudice against these supplements that even

when they are offered free they are not used. Giving them away often increases suspicions. Anything which is free must be no good, is the feeling. Recent events in India suggest that some people would rather starve than change from eating one cereal to another. It may be encouraging that in experimental tests with "l'aubina", the children seemed to like it and their mothers have accepted it.

Controlling Pollution

More than £30 million is spent each year in Britain on the treatment of sewage and industrial waste water, according to the report of the Water Pollution Research Laboratory of the Ministry of Technology for 1966 (HMSO, 15s. 6d.). About half of the laboratory's resources are concerned with various methods of treatment of wastes, but other work includes a study of the effects of pollution on rivers and estuaries and the During 1966, the last of these was the coastline. largest single project; the object was to study the influence of environmental factors on the rate of dispersion of sewage discharged into the sea by coastal authorities. To do this, physical dispersion of liquid sewage has been followed using a radio-tracer, bromine-82. Bacteriological examinations of sea water samples have also been undertaken. In this way, it is hoped to provide an improved basis for choosing sites for discharge of sewage into the sea and for deciding on the degree of treatment required before discharge.

The control of aquatic weeds such as Elodea canadensis, Myriophyllum spicatum and Zannichellia palustris costs river authorities some £300,000 each year; the use of a proprietary herbicide is now being investigated for this purpose. An important and continuing line of work at the laboratory is the determination of the effect of pollution on fisheries—the toxicity of zinc to trout eggs, for example. Results of research have shown that sudden rises in temperature increase the sensitivity of trout to phenol and toxicity of phenol to rainbow trout also increases with salinity.

Studies designed to improve the performance of unit processes used in the treatment of domestic and industrial waste water have formed an important part of the laboratory programme. In addition to conventional methods, a new method of treating sewage has been developed which involves wet oxidation at high pressure. The contribution which protozoa make in the activated sludge treatment of sewage has also been followed; it seems that these organisms bring about a marked clarification of the effluent.

The treatment and disposal of sludge present more difficulties than almost any other waste treatment operation. In the 1965 laboratory report, it was pointed out that in some sewage the concentration of synthetic detergents had risen to a level which caused acute inhibition of sludge digestion: digestion can now be restored by the addition of a long-chain amine which causes precipitation of the detergent, and an experimental study of twenty-two new detergents has been carried out.

Aid by Modelmaking

THE development of econometric models has offered economists a better chance of making the right decisions. By designing quantitative models which

behave like the economy of the country concerned, it should be possible to assess more precisely the effect of small changes—in tax structure, age distribution. birth rate, or the supply of trained manpower. The history of the past three years of British economic policy indicates the sort of errors it might then be possible to avoid.

The Organization for Economic Co-operation and Development has now attempted to apply the same sort of reasoning to development assistance (Quantitative Models as an Aid to Development Assistance Policy. OECD, 1967). The sorts of questions which need answering are concerned with the effects of decisions about aid on the development of the country concerned. Would the development plan be overturned by a 10 per cent reduction in aid? Should the transport sector be developed at the expense of primary production How would changes of birth rate or migration influence economic progress? One difficulty, of course, is that econometric models are only as good as the data that go into them, and most underdeveloped countries are short of reliable statistics-often, the OECD expert group reports, the records include a quantity of miscellaneous information and statistics, without combining them into any coherent pattern. Despite this, the expert panel went on to assess how valuable the models might be.

It is clear from the start that the notion of a comprehensive model which could provide an answer to every question is unrealistic. The solution to this, according to Professor Chenery of Harvard University, and a member of the panel, is not to make the model more complicated, but to design instead a set of related sub-models to carry out the more detailed work. The group considered two models of the Pakistan economy. one prepared by Professor Tims at Harvard, and the other by Professor Chenery. The models take as one fixed point the desire by the Pakistan government to end foreign aid by 1982. Interestingly, the model reaches different conclusions from the planners in Pakistan; they assumed that aid would fall steadily as a proportion of GNP from 8 per cent in 1965 to 1 per cent (representing enough to cover external interest and dividend payments) in 1985. But the model shows an increase in aid in the early period, to a peak of 10 per cent in 1975, and then a fall to zero by 1982. This is because in the early period heavy investment is needed to produce rapid growth in the economy: demand for capital therefore exceeds what can be supplied from home resources, although 24 per cent of the growth in GNP would be saved. Demand for foreign capital therefore increases in the short run. After 1976, the emphasis would shift to the task of import substitution, or increasing exports, either of which would have the effect of improving the balance of payments. Total investment falls, and can thereafter be financed from Pakistan's own resources. After 1983, the model predicts self-sustaining growth, with sufficient investment to prevent imports from outrunning exports.

Industrial Biology

THE Institute of Biology last week held a conference on "Improving the contacts between industry and university". More than a hundred people settled into the comfortable orange seats of the Royal Society's

large lecture hall to hear ten speakers and some lively discussion, which frequently echoed the kind of discussion familiar in physical science since the publication of the Swann Report. Professor M. M. Swann himself introduced the morning session with the thought that for a long time biology had been "small, cheap and neglected", but that industrial biology is now growing rapidly. But it is not clear whether or not there is a shortage of biologists in industry, and no official analysis of the situation has yet been made. From remarks made by industrialists and academics, however, there appears to be room for more communication and co-operation. Some university departments, for example, had found that industrial budgets are so tied up that negotiations at board level are required to obtain small sums of money for a research project which in the long run could save thousands of pounds for the company. Some industrialists, on the other hand, when allotted money to pay for research in outside bodies such as universities, could find nobody who would take on particular projects. If graduates are reluctant to enter industry, some academics think it is because industry is not attractive enough, while some industrialists believe the universities are giving their students the wrong ideas.

Sandwich courses are one answer to this problem. Professor L. Broadbent of Bath University of Technology favours the thin sandwich, when the students spend alternate periods of six months in university and different industries, thereby gaining a wide variety of experience before coming to decide on a future career. Employers favour thick sandwiches, as they can give the student a worthwhile project during the year, but this long time away from university can easily disrupt academic studies.

Mr F. R. Reavell from Unilever described the difficulties of recruiting suitable biologists for industry. In 1966 there were 1,500 biologists in industry, most of them specialists. Mr Reavell saw the problem in terms of training "the right number of the right specialists for the few jobs". He wondered if the Institute of Biology could run a comprehensive appointments service.

Speaking of industrial biologists as honorary lecturers, Dr D. J. D. Hockenhull of Glaxo Ltd. said the difficulty came in "knowing what the students know", but lectures by industrial biologists can be of great value in communicating enthusiasm for the subject. Professor E. B. Chain of Imperial College discussed the question of academic biologists as consultants to industry. general," he said, "the situation is fairly satisfactory, and is improving." He believes the idea that fundamental and applied research are different is "a red herring". In his view there are only two types of research—"useful and useless". He suggested that a biological committee should be set up, possibly within the Science Research Council, to discuss and select long term research projects of industrial interest. Academics could then be approached and government money could be used to put the ideas into action. Dr A. Spicer of the Rank Research Centre described how he uses consultants in his research projects. Biologists are involved in the "marketing and economic implications" of a scheme and are therefore part of the team, instead of merely being outside observers.

Some of the difficulties faced by staff in moving between industry and university were mentioned by Professor P. W. Brian. The two groups are too often "mutually suspicious", and cherish illusions as to the way of life of the other. Dr W. F. Jepson brought in the practical factors involved when such a move is made. Security of tenure in university posts was weighed up against higher pay in industry. There would be more movement between industry and universities if pensions were transferable.

Parliament in Britain

Libraries

LORD WINTERBOTTOM, Parliamentary Secretary, Ministry of Public Building and Works, acknowledged that the National Reference Library for Science and Invention formed part of the British Museum Library, and that its future arrangements would therefore be examined by the small independent committee which the government was setting up to investigate the national libraries. Alternative sites in the Central London area were being discussed, to see if they could provide whatever is needed after the committee reports. On November 30, in the House of Commons, the Secretary of State for Education and Science, Mr P. Gordon Walker, said he hoped to announce the composition of the committee soon. He hoped the committee would recommend that the library should be sited in Central London, but added that the government's decision on the Bloomsbury site was final. He would resist any attempt to move the National Lending Library for Science and Technology to London or to the Home Counties. (Oral answer, House of Lords, November 28.)

Skylark

MRS S. WILLIAMS, Minister of State at the Department of Education and Science, said that the Science Research Council's Skylark sounding rockets which were launched at Woomera as part of the jointly financed Anglo-Australian programme cost the United Kingdom £175,000 in 1966-67. The cost in this country of the rockets, including research and development, was £531,000 in 1966-67. (Oral answer, November 30.)

Food Additives

MR S. HAY, Joint Parliamentary Secretary at the Ministry of Agriculture, Fisheries and Food, gave details of the Food Additives and Contaminants Committee (chairman, Professor R. A. Morton) and the Pharmacology Sub-Committee (chairman, Professor A. Kekwick). Mr Hay said that the Minister of Agriculture and the Minister of Health asked the Food Additives and Contaminants Committee for advice when additives were used or proposed for use in food. This committee, in conjunction with the Pharmacology Sub-Committee, then took into account all the data available in this country and internationally. (Written answer, November 29.)

Wash Barrage

MR A. GREENWOOD, Minister of Housing and Local Government, said that he was considering very carefully the suggestion of the Water Resources Board for a desk study to assess the scope for water conservation in the Wash and to appraise the case for a full feasibility study. Mr Greenwood asserted that the Wash Barrage proposition would cost about £90 million more than conventional methods, but added that the question would not be considered in isolation. (Oral answer, November 28.)

Cost of Working

A new study has defined the rate at which research expenditure is increasing.

A DETAILED study of the continually increasing cost of scientific work at several scientific laboratories, some public and some at universities, has now been published by the secretariat of the Council for Scientific Policy. The document, The Sophistication Factor in Science Expenditure (HMSO, 6s.) is the first of a series of studies commissioned by the council. Although the study seems to have shown that the variation of the rate of increased costs from one kind of establishment to another is far too great for there to be a rule of thumb applicable right across the board, with the result that the making of science policy cannot be left to men with slide rules, the analysis of expenditure which makes up the bread and butter of the report will certainly provide the heads of university departments with a useful yardstick for deciding whether they are spending too much-or too little.

The study has been concerned with a total of sixteen laboratories—nine research stations operated by the Ministry of Technology, the National Institute for Medical Research, the Rothamsted Experimental Station, the Atomic Energy Research Establishment at Harwell, the Central Veterinary Laboratory of the Ministry of Agriculture, the chemistry department at one university and the geology and zoology departments at another. The study begins by pointing out how difficult it must be to disentangle the true cost of increasing sophistication of equipment used in research from other causes of variation from one year to another—changes in the proportions of staff of various grades, natural growth and even such phenomena as inflation.

To allow for these and other factors contributing towards the total cost of a laboratory, the study begins with the statement

F/N = B + W + A

where F is the annual budget, N the number of scientific researchers, and B, W and A the cost per individual researcher of buildings, wages and apparatus respectively. One cause of increasing costs from year to year is the "desire of workers to be better accommodated" as well as the usually increased cost of housing complicated equipment. It must also be reckoned that the salary bill of a predominantly youthful establishment will tend to rise quickly because of the effects of built-in salary increments. For each of the three terms on the right-hand side of this equation, the study has assumed a separate sophistication factor. The last of the three terms, for example, is written as

$$A = A_0 I_A R_A$$

where A_0 is the cost of apparatus per year per worker at the beginning of some period, I_A is a factor which represents growth due to monetary inflation and R_A is the sophistication factor representing the increasing complexity of scientific equipment.

The chief conclusion of the report is that the increasing cost of equipment is the principal cause of rising budgets in the universities and the public laboratories. For institutions in which exceptional considerations do not inflate costs unpredictably, and where there is no natural growth, the cost of research (for each scientifically qualified man and woman) may be

expected to increase by between 2 and 5 per cent a year. The report emphasizes that this range of figures is merely a pair of brackets, and that some of the laboratories included in the survey have revealed rates of growth outside these limits.

Universities seem to have experienced the most rapid increase of growth in the cost of equipment, at least if the three university departments included in the survey are a reliable guide. For one chemistry department, for example, the staff grew at 7.2 ± 0.4 per cent a year during the 8 years from 1958, but the cost of major equipment (each item exceeding £100) increased by an average of 27 per cent a year during the same period. The corresponding figures for the public laboratories range between essentially zero (for the National Engineering Laboratory) to 27 per cent (for the National Institute for Medical Research). The rate of growth of expenditure on major items of equipment in the zoology department of the second university worked out at 8.9 per cent, but the geology department had increased its budget for major items of equipment by 25 per cent a year over the same period. These figures are at current prices—in other words, they are not corrected for inflation.

The correction for inflation necessarily entails that the survey should rely on published information about the general increase of costs in the construction industry, for example. The average annual increase of construction costs, for example, was taken as 2.3 per cent. It is calculated, on the basis of pay scales in the scientific Civil Service, that the inflation of salaries during the period 1958-65 worked out at 6.5 ± 0.5 per cent a year. At the same time, the effect of inflation on prices of equipment and machinery seems to have increased by between 3.0 and 3.5 per cent a year. These figures are intended merely to represent increases of money costs arising from monetary inflation. They are, in other words, the basis of the corrections which must be applied to the annual increase of the budget of a laboratory before the true cost of sophistication can be calculated. It is nevertheless interesting and important that the buoyant trend of scientific salaries should have been so conspicuous in the past few years.

When inflation is allowed for, the increasing sophistication of the scientific labour force, chiefly expressed by the tendency for the comparative numbers of supporting staff to increase, seems to have varied widely from one laboratory to another, but to have been not too different from 1 per cent a year. According to the report of the survey, the effect on real costs arising from the way in which a youthful labour force wins rapidly increasing increments is more important. and may account for an annual increase of real costs of about 2.5 per cent a year.

The report emphasizes that this conclusion is tentative, as is the statement that "such evidence as there is suggests a sophistication factor of 2.5 per cent a year for construction costs". Minor items of equipment are considered to cost an extra 3.0 ± 1.0 per cent a year in real terms, but the secretariat of the Science Research Council has shrunk altogether from attempting to define a sophistication factor for major items of equipment. In the period covered by the review,

expenditure under this heading seems to have varied markedly from one laboratory to another. Where the rate of expenditure on major items of equipment seems to have been about £100 per head per year, the rate of spending for major equipment may have been as much as 23 per cent. Where expenditure worked out at three times as much, however, the annual increase of expenditure might typically be about 9 per cent a year.

Although the authors of the report have been quite properly concerned with the way in which the changing character of scientific research is likely to bring in its train increases of costs, it is inevitable that those who read the document will pay at least as much attention to the absolute costs of running various departments. The "nine 'establishments within the Ministry of Technology and the Atomic Energy Research Establishment at Harwell will thus inevitably excite the interest and the envy of those who work elsewhere. On paper, at least, the AERE is the most lavishly endowed, no doubt because of the cost involved in building and refurbishing equipment associated with nuclear reactors. In 1965-66, the cost of the establishment worked out at £14.57 million, equivalent to £19,663 for each scientifically qualified member of the staff. In the nine stations transferred to the Ministry of Technology from the old DSIR, the average cost of a year's work by a scientifically qualified researcher worked out at £15,148. These figures are much greater than those applicable in other kinds of laboratories. some of which are referred to in Table 1.

Table 1. Costs of running british research establishments in 1964-65 in £ per qualified researcher

Establishment	Salaries	Equipment	Buildings	Total
Min-Tech labs.	8,096	2,660	4,167	15,148
NIMR	6,701	1,260	583	8,590
Rothamsted	4,860	542	1,219	6,888
AERE*	10,439	5,796	3,428	19,663
	* For 1	965–66.		

The figures in the report which indicate university expenditure on research and development are not comparable with these, chiefly because of the difficulty of deciding how to apportion the cost of buildings and services to the research which is carried on in university departments. It is also important that the cost of salaries attributable to the research in different public laboratories does not reflect great differences in the individual salaries paid to research workers. Although some laboratories seem to pay better salaries than others, the most marked difference between them is the extent to which the efforts of research workers are supported by the efforts of others. Thus the ratio of total staff to scientific staff varies from 4.65 at the National Institute for Medical Research and 3.77 at Rothamsted to 8.22 at the Atomic Energy Research Establishment at Harwell. The Central Veterinary Laboratory at Weybridge boasts of 2.6 technical staff for each researcher, but the record of the university departments is even less cheerful, even when research students are not counted among the academic researchers—the convention followed in the report.

Although the amount of money being spent on running the laboratories has increased absolutely over the years, and although the cost of equipment has often increased quite sharply, it does appear that the greater part of the increasing cost of operating scientific laboratories has been swallowed up by inflation. Table 2

shows the way in which the percentage rates of growth in recent years are almost entirely offset by the percentage increases in each year which must be attributed to inflation.

	Table 2	2	
	Apparent growth rate per scientist (%)	Inflation (%)	Real growth rate (%)
Min-Tech labs.	6.0 ± 0.5	4.9 ± 0.5	1.0 ± 0.7
NIMR Rothamsted	$\begin{array}{c} 8.6 \pm 1.3 \\ 9.6 \pm 0.8 \end{array}$	$\begin{array}{c} 5.9 \pm 0.5 \\ 5.9 \pm 0.5 \end{array}$	$2.5\pm1.4 \\ 3.5\pm0.9$
AERE	$4 \cdot 3 \pm 0 \cdot 6$	$5 \cdot 0 \pm 0 \cdot 5$	0.6 ± 0.8

Precisely what implications these figures will have for the development of policy in science it is too soon to know. The report of the survey itself says that there is a great need to accumulate further information on this and other subjects. Long before that has been done, the British Government may choose to interpret information like that contained in Table 2 as a proof that, if only government departments are tough enough, even the most powerful laboratories can be made to draw in their horns.

The report will also be followed carefully by those seeking evidence of the trend of university expenditure in the past decade or so. The three university departments covered by the survey seem to have kept successfully ahead of the government laboratories during most of this period. The department of geology included in the survey, for example, increased its staff from 8 to 15 between 1955 and 1964, and the numbers of research students on the books increased from 5 to 14 during the same period. Technical staff increased in numbers even more rapidly, from 5 in 1955 to 18 in 1964. This implies an annual rate of growth of the research force at the laboratory of 10.9 per cent a year, but average salary during the same period increased from £1,051 per researcher per year to £1,837 per researcher per year. These figures include the cost of technical assistance (but research students are counted as researchers). The most rapid increase in the department's salary bill is attributable to the salaries of technical assistants—a three-fold increase of the labour force was accompanied by a six-fold increase of salary.

Much the same pattern seems to have been followed at the zoology department at the same university, although this has not grown as quickly in numerical terms. One striking feature of the figures which are provided in the survey is the modesty of the sums available for travel by the academic staff. zoology department, for example, spent £239 on the travel of 27 research people in 1955 and £675 on the travelling expenses of 49 people a decade later. Although the report on sophistication has made no explicit attempt to work out the sophistication of these smaller components of the budget, it is hard to believe that the rates of increase implied by the figures obtained from two university departments can have allowed the academics concerned to keep pace with the growing cost of travel, let alone with the growing need among research people to attend research conferences in ever more distant places. Academic readers of the report will also be impressed with the comparatively modest scale on which university departments appear to be able to provide themselves with equip-

NEWS AND VIEWS

New Era for Salk Institute?

THE appointment of Mr Joseph Slater as president of the Salk Institute for Biological Research has been welcomed inside the institute as well as in the wider world. For one thing, Mr Slater has won a distinguished reputation in his work with the Ford Foundation in the past few years, and has in particular been concerned with a great many of the schemes which the foundation has devised for the creation in Europe of new kinds of institutions. One of his associates the other day described him as a splendid intellectual entrepreneur, and it seems commonly to be agreed that Mr Slater is an easy person to work with. All of this suggests that he may be just the man to give the Salk Institute what it has most conspicuously lacked in the past two years—a strong sense of direction, a firm administration and an air of confidence, which is not the same thing as smugness. His appointment, which becomes effective in the New Year, also raises important questions about the functions of independent research institutes like that which Dr Jonas Salk has built up in California. What place is there in the modern world for establishments like this which are not directly linked with universities?

It is important to separate the troubles which have assailed the Salk Foundation from the more general questions of how independent institutions should be organized and co-ordinated with the rest of the intellectual community. The most obvious problems in the short history of the institute have been money troubles, and most of these derive from the way in which the institute elected to sink more than it could reasonably afford on the superb buildings which now grace La Jolla in southern California. By all accounts, these edifices have cost the best part of £20 million. More seriously, they have consumed much of the capital fund originally provided by the National Foundation as an endowment intended to provide the institute with a regular income big enough to keep the laboratories ticking over. In these circumstances it has been understandably difficult for people to plan ahead.

How will these circumstances be changed by the arrival of Mr Slater? To begin with, he seems firmly convinced that the institute can resume the growth which was expected when the new buildings were planned only a few years ago. Moreover, he plans to graft on to the work now carried out at La Jolla, most of it in fields somehow related to molecular biology, some work which may be considered more properly to belong to the social sciences. The likelihood that the institute will increase its stake in neurophysiology in the years ahead has been talked of seriously for some time. Now there is also a chance that it will find itself engaged in a much wider field of activities related to the interaction between people and their environment. It is only proper to acknow-

ledge that these developments are entirely in line with the original conception of the institute which Dr Salk outlined at the foundation of his establishment, and that Dr Salk will continue as director of the institute after Mr Slater arrives. The difference now, however, is that Mr Slater may be better placed than anybody else at the Salk Institute to recruit the kinds of people who are capable of distinguished work in fields like this. On all these counts, it is entirely understandable that his transfer to the Salk Institute will be welcomed.

There remains the issue of how institutions like the Salk Institute can be expected to contribute to intellectual life, in the United States and elsewhere. That they provide an opportunity for distinguished scholars to pursue their own activities is beyond dispute, but there is a constant danger that they will serve to isolate the same people from what happens at the universities and elsewhere. Badly managed, ivory towers like this can even protect the work of some research groups from the fierce blast of criticism which is elsewhere found to be a necessary assurance of quality. The problem is how to strike a balance between the benefits and the hazards of isolation. The Salk Institute is fortunate in that it is placed within the thriving intellectual community at La Jolla. The system whereby a number of part-time fellows, all of them distinguished people, serve both as intellectual catalysts and talent scouts, is another protection against isolation. Yet these are mechanisms which demand constant attention. The danger at La Jolla is that the passage of time would erode the determination of the men in charge to keep the institute on its chosen course. In the circumstances, it will be a benefit and not a handicap if Mr Slater can somehow engage the institute more fully in matters which go beyond the boundaries of its present work.

Machine Tools

The modern investigation of machine tool problems did not start until 1880, although Henry Maudsley had earlier been concerned with perfecting the production of screws. This was one view introduced by Mr K. R. Gilbert on November 29 when he spoke to the History of Science discussion group at the Royal Institution. Maudsley, who was born in 1771 and who set up his own workshop in London in 1797, realized that an accurate screw is fundamental to the production of accurate machine tools. Maudsley's masterpiece was a screw five feet long, two inches in diameter and with a total of three thousand turns. On it was screwed a nut twelve inches long with six hundred threads. It was used in an apparatus for dividing scales for astronomical and other metrical purposes. Maudsley favoured a mechanical method for producing

screws, and his beautifully made apparatus provided with fine adjustment for helix angle and knife and cutter feed still exists.

The most significant development in the control of machine tools this century is hydraulic control. The idea of controlling a machine tool hydraulically had been put forward in the United States in 1893 by C. M. Conradson, but no great advances were made until the work of the Oilgear Company in the mid 1920s. The advantages gained over mechanical control were flexibility, infinitely variable speed, avoidance of shock on reversal of motion and constant pressure on the cutting tool. Hydraulic control also led to the application of servo mechanisms to machine tool control. Watt's steam engine centrifugal governor is the earliest example of this. Techniques of servo control have been applied to the most advanced system of machine tool control-numerical control. This permits continuous path machining and works straight from the dimensions and curves specified by the designer. The value of numerically controlled machine tools is in batch production: indeed, numerical control was conceived as a means of simplifying medium and small run production such as that encountered in the aircraft industry. This research was carried out over a period of five years starting in July 1949 at the Servomechanisms Laboratory of the Massachusetts Institute of Technology by Professor Gordon S. Brown and twenty-six co-workers.

Hyperbaric Oxygen

from our Microbiology Correspondent

Much has been written on the subject of oxygen toxicity since the classic studies of Bert in the 1870s, and many micro-organisms are now known to be affected adversely by increased oxygen concentrations. Aerobic bacteria can frequently tolerate partial pressures of exygen of up to 2-10 atmospheres, but raising the hydrostatic pressure drastically increases their sensitivity to oxygen. The growth of aerobic bacteria is little inhibited at pressures of 50-100 atmospheres provided that the medium contains normal levels of oxygen, that is, less than 10 µg/ml. At higher pressures, however, the lag phase and generation time are prolonged and the maximum population is depressed. Furthermore, with the exception of barophilic species, increasing the pressure to 400-600 atmospheres inhibits growth, and extended compression often sterilizes the culture. ZoBell and Hittle (Canad. J. Microbiol., 13, 1311: 1967) have found that hyperbaric oxygen concentrations (35-105 $\mu g/ml$.) are similarly lethal at pressures as low as 4 atmospheres. Even barophilic marine bacteria such as Pseudomonas perfectomarinus, which will grow at pressures of more than 600 atmospheres if the medium is low in oxygen, are susceptible to hyperbaric oxygen at a pressure of 5 atmospheres. ZoBell and Hittle reiterate that a disturbed redox balance, oxidation of thiol or disulphide enzymes and an accumulation of toxic oxidation products are among the factors contributing to hyperbaric oxygen toxicity.

Recent work with euglenoid algae has defined more precisely some of the repercussions of hyperbaric oxygen on metabolism. Bégin-Heick and Blum (Biochem. J., 105, 813; 1967) discovered that cell division in Astasia longa was inhibited when culture

aeration with air + carbon dioxide (95:5) was replaced by an oxygen+carbon dioxide (95:5) mixture. Growth was resumed at the normal rate after re-gassing with air + carbon dioxide and both the initial inhibition and the post-oxygen recovery occurred very rapidly. In this system the toxic effect of oxygen is obvious even at atmospheric pressure. Assay of mitochondrial enzymes showed that succinate-cytochrome C oxidoreductase, NADH-cytochrome C oxidoreductase, succinate dehydrogenase and the succinate oxidase system had greatly reduced activities compared with preparations from cells grown in air+carbon dioxide. The oxygen effect can be related, therefore, to a mitochondrial lesion, and the 75 per cent fall in succinate dehydrogenase activity is entirely responsible for the reduced activities of the succinate-cytochrome Coxidoreductase and the succinate oxidase system. The fact that NADH-cytochrome C oxidoreductase is also inhibited by oxygen is indicative of a second site of action in the electron transport chain.

Blum and Bégin-Heick (Biochem. J., 105, 821; 1967) have made the interesting observation that the closely related Euglena gracilis var. bacillaris is only sensitive to hyperbaric oxygen when grown in a lowphosphate (20 μ M) medium with ethanol as the carbon source. Euglena under these conditions showed a decreased rate of respiration and an inability to resume growth when phosphate was replenished. This oxygen toxicity was far less marked when the alga was grown on glucose. Like Astasia, oxygen sensitized Euglena also had decreased activities of succinate- and NADHcytochrome C oxidoreductases and succinate dehydrogenase when compared with cells grown in a highphosphate (20 mM) medium. Although the differentiating action of hyperbaric oxygen plus low phosphate on cells grown with ethanol and glucose may reflect different sensitivities of endogenous and exogenous oxidative pathways, the mechanism of the sensitizing effect of low-phosphate as such still has to be resolved.

Proteins and Ligands

from our Molecular Biology Correspondent

THE study of the behaviour of ligands has been a promising approach to the difficult problem of observing and interpreting localized conformational changes in proteins, associated, for example, with enzyme activity. Ligands may in some cases compete directly with a substrate at an active site, or they may stabilize one conformational state of the protein with respect to others for which they have lower affinities.

An interesting example of the latter phenomenon-comes from Markus et al. (J. Biol. Chem., 242, 4402; 1967), who have studied the binding of anionic dyes to-serum albumin—a protein chiefly noted for its propensity to bind an extraordinary variety of small molecules of different kinds. The albumin with and without these ligands was then used as a substrate formitive different proteolytic enzymes, and in all cases the bound ligand was found to inhibit digestion. From the stoichiometry of the binding, it appears that onlytwo dye molecules need to be attached to produce the full effect. There are also appreciable changes in the distribution of the products of limited proteolysis. The most attractive explanation is that the protein exists in a state of equilibrium between two or more

conformational isomers, and that the equilibrium is displaced by the added dye in favour of the form to which it most avidly binds. In the present case, this form is evidently one in which the susceptible peptide bonds are relatively unavailable, or in which the structure is comparatively rigid and inflexible. Markus and his co-workers have previously recorded a case in which the binding of a ligand renders a protein more prone to proteolysis, and the converse presumably holds in their latest work.

Several examples of the binding of dyes at or near the active sites of enzymes have recently been reported. The latest of a number of studies by Glazer (J. Biol. Chem., 242, 4528; 1967) concerns the binding of the anionic dye, Biebrich scarlet, to chymotrypsin. It has proved generally true, as revealed notably by X-ray studies on enzymes, that active sites are situated in crevices, giving on to the predominantly non-polar interior of the molecule. Equally, most organic ligands which are capable of binding strongly to a protein evidently seek an essentially non-polar environment (as shown, for example, by dyes which exhibit fluorescence only in substantially non-aqueous solvent systems, and which become fluorescent when they bind to proteins). Thus it is not surprising that a pattern is beginning to emerge of binding of many such small molecules specifically at active centres. Several cationic dyes have been found to bind at the active centre of chymotrypsin, and it is interesting that Biebrich scarlet, being anionic, binds with comparable affinity. The binding can be followed by changes in the absorption spectrum of the ligand. The dye competes with, and is displaced by, substrates and competitive inhibitors. It does not bind to chymotrypsinogen, which suggests that here the active site is occluded.

Another application of dye-binding is due to Koshland, who uses a covalently attached chromophore as a so-called "reporter" for events involved in the binding and conversion of substrates. Hille and Koshland (J. Amer. Chem. Soc., 89, 5945; 1967) have bound a compound containing the highly medium-sensitive nitrophenyl chromophore to methionine-192 of chymotrypsin, which is three residues along the chain from the serine of the active site. The activity of the enzyme is preserved, and one can then observe the perturbation of the nitrophenyl spectrum, when for example the pH is changed. The magnitude of the perturbation, in fact, follows a titration curve, with a pK of 7. Addition of substrate eliminates this effect, and it is suggested that the bound chromophoric group is pushed out of the way. The speculation is that the ionizing group is the active-site histidine, and if this is true its pK is thereby established. It must be hoped that a comparison of data of this kind with the environment of the residues in question in the crystallographic structure will ultimately lead to general correlations about their properties, and that this will help in interpretation of the mechanism of catalysis.

Chromosome Breakage

from our Cytogenetics Correspondent

Radiation and many chemicals used in cancer chemotherapy and as antibiotics cause chromosome breakage. Breakage is a result of the disruption of DNA synthesis or some other disturbance of chromosome organization and so interest in induced chromosome breakage lies in what this can say about the structure and chemistry of chromosomes. One important question is whether chromosomes in different phases of the cell cycle are equally sensitive to damage. In both animal and plant cells there is evidence that chromosomes are more sensitive to certain agents during the G_2 phase of interphase (that is, between the DNA synthetic stage, S, and mitosis) than during the S or pre-S (G_1) phases of Methyl coumarin has been shown by Ronchi and her colleagues (Mutation Res., 4, 615 and 791; 1967) to cause most breaks to bean and onion chromosomes during G_2 as well as to prolong this phase. They also showed that exposing roots previously treated with methyl coumarin to chloramphenicol causes an increase in the number of breaks, and they interpret this as evidence that protein synthesis is required to repair breaks. Breaks are more numerous in the presence of chloramphenicol because many of the breaks induced by methyl coumarin have not been allowed to heal. The requirement of protein synthesis for healing breaks is not a new idea, but it does pose some obvious questions. Is it a repair enzyme or a protein of chromosome structure that is synthesized? And if it is an enzyme, can differences in its activity account for differences in apparent sensitivity of chromosomes during the cell cycle?

Scott and Evans have also shown (Mutation Res., 4, 579; 1967) that chromosomes of bean at G_2 are most sensitive to X-rays and, further, that chromosomes at different points in the G_2 phase differ in sensitivity. Similarly, Dewey et al. (Int. J. Radiat. Biol., 12, 597; 1967) showed that Chinese hamster chromosomes sustained 2.5 times more chromosome damage in G_2 than in S. In their experiments the chromosome damage was self inflicted as the chromosomes were allowed to incorporate a high level of radioactivity in the form of tritiated thymidine.

Breaks in chromosomes could be caused by interference with nucleic acid metabolism rather than a physical shattering of the chromosome thread. This at least seems to be one of the effects of 9- β -D-arabino-furanosyladenine (ara-A). Its triphosphate has been shown by Furth and Cohen (Cancer Res., 27, 1528; 1967) to be able to inhibit DNA polymerase of mammalian cells (but not that from cells of the bacterium Escherichia coli) and may also inhibit the nucleotide reductase system. But ara-A is able to cause breaks at G_2 ; could it be that there is some DNA synthesis not detectable by conventional methods during G_2 that all these agents inhibit?

What of induced chromosome breakage in meiotic cells? Westerman (Chromosoma, 22, 401; 1967) describes the effects of irradiating meiotic chromosomes during spermiogenesis in male locusts. He found that cells irradiated during the S phase of meiosis showed chromosomes with a lowered chiasma frequency at the subsequent metaphase, while cells irradiated at diplotene or in the S phase of premeiotic mitosis showed an increase in chiasma frequency. The chiasma frequency was most changed in the long chromosomes while that of the short chromosomes was hardly changed at all. It is tempting to speculate on the role of chromosome breakage on the time and mode of chiasma formation, but only further experimental work will allow concrete fact to replace speculation in this perplexing problem.

Superconducting Magnets in High Energy Physics

P. F. SMITH
D. B. THOMAS

Rutherford High Energy Laboratory, Science Research Council, Chilton, Didcot, Berkshire Magnets play a large part in the study of elementary sub-nuclear particles. Advances in the technology of superconducting magnets suggest that these magnets will be an important factor in the design of future accelerators, and their associated experimental equipment.

In recent years, in the high energy physics laboratories of the world, a large number of elementary sub-nuclear particles have been discovered by studying the interactions produced when suitable targets are bombarded with beams of charged particles from giant accelerators. These accelerators and their associated equipment use experimental techniques of the utmost sophistication, and magnetic fields are the basis of most of these techniques, because the transverse force on a charged particle moving in a magnetic field constrains it to describe an arc of a circle.

This simple effect is employed in a variety of ways. For example, during acceleration in a proton synchrotron the particle beam is confined to a closed circular orbit by a ring of powerful electromagnets. After acceleration, the primary beam can either be extracted from this circular orbit and directed at a target, or made to impinge on a target in the machine. In both cases, secondary particles are produced and these have to be transported to experimental equipment often situated one or two hundred feet from the accelerator. Here again, magnetic fields play a prominent part, for, in beam optics, magnetic lenses provide the necessary focusing, and bending magnets can steer the particles along the desired paths. In many experiments, separated beams, consisting only of particles of the desired type and momentum, are selected from the wide spectrum of secondary particles. For such beams a bending magnet with collimating slits provides the momentum selection in an analogous fashion to a glass prism in an optical spectrometer. In the nuclear experiments themselves, the momenta of particles created by interactions within the experimental apparatus can often he determined by measuring the actual curvature of the paths of the particles in a known magnetic field. An elegant example of the type of apparatus is the bubble chamber, where the curved tracks of charged particles can be seen as lines of minute bubbles which the particles have nucleated in a superheated liquid, for example, liquid hydrogen. The entire liquid volume is immersed in a high magnetic field, and measurement of stereophotographs of the curved tracks makes it possible to calculate the momenta of participating particles. There are also a number of smaller scale applications which use a magnetic field in a fundamental manner, such as measuring the magnetic moments of particles and the polarization of protons in a target.

It is clear that any technological breakthrough in the generation of higher or more stable magnetic fields would have a substantial impact on the instrumentation of high energy physics. This would be particularly true if economies also resulted either in terms of reduced capital outlay or running costs, for in recent years elementary particle physics has become so expensive that it can only be pursued by the larger countries or by groups of smaller countries collaborating in the funding of international laboratories. So far, virtually all the requirements for magnetic fields have been fulfilled using conventional electromagnets consisting of water-cooled copper conductors, usually wound on steel yokes to reduce power consumption and to shape the magnetic field. The value of the magnetic field is usually in the range 10–20 kgauss;

above this, the steel saturates and power requirements increase sharply. Because of this, fields in the region 30-200 kgauss, although technically feasible, have not hitherto been generally available (except in very small volumes) for purely economic reasons.

In 1961 a spectacular advance occurred with the discovery of several new superconducting alloys which, at very low temperatures, would carry very high steady currents in high magnetic fields with no dissipation of electrical power whatsoever. Previously known superconductors would only exhibit zero electrical resistance in magnetic fields typically less than a few kgauss. Using the new materials, large volumes of magnetic field of up to 100 kgauss and above could in principle be provided, free from the power limitations of conventional magnets. The potential significance of this led to development programmes of superconducting magnets being initiated in many nuclear physics laboratories, and elsewhere in connexion with other possible applications in fields such as plasma physics, magneto-hydrodynamic power generation, power transmission and energy storage for space research. The transition from this stage of promise to that of working hardware has been somewhat protracted and this has been caused largely by a number of unexpected technical difficulties which will be discussed. Considerable progress has now been made in overcoming these difficulties and it has now become feasible to construct large d.c. superconducting coils.

Already, a liquid helium chamber, 10 in. in diameter, equipped with a pair of superconducting coils giving a field of over 40 kgauss, has been used in nuclear physics experiments at Argonne National Laboratory in America, and a polarized target with a superconducting magnet has been used in experiments at the Cambridge Electron Accelerator, Massachusetts. The largest superconducting magnet yet to be successfully tested was built in connexion with a magneto-hydrodynamic programme by the Avco-Everett Research Laboratory, Massachusetts. This magnet gives a transverse field of 40 kgauss across acylindrical volume of approximately 2 ft. inner diameter and 10 ft. length. The highest field so far achieved withas superconducting magnet is 140 kgauss, in a 6 in. bore coil constructed by the Radio Corporation of America.

A measure of the general confidence invested in these new techniques can be gleaned by examining the many magnets now under construction. The largest of these is a superconducting magnet to provide a field strength of almost 25 kgauss over a cylindrical volume 15 ft. if diameter by 9 ft. high. This magnet, intended for a bubble chamber application at Argonne National Labora tory, should receive its initial test in the second half o 1968.

Present Status

In this article, a brief summary is given of the presenstate of superconducting magnet development togethe with a survey of the expected impact on high energ, physics in both the immediate future and, speculatively in the more distant future.

A superconducting magnet is essentially a coil of wir made from a superconducting alloy which is maintaine. at a temperature a few degrees above absolute zero. The current density attainable in a particular superconductor decreases with both increasing magnetic field and increasing temperature; for practical purposes it is most convenient to operate at a temperature of 4.2° K by simply immersing the coil in liquid helium. The size and shape of the coil and its low temperature enclosure vary considerably according to the application, but the principles are the same in all cases. With modern advances in liquid helium refrigeration and cryogenic engineering, making coils is now relatively straightforward even up to the larger sizes. The principal difficulties encountered to date all arise from the nature of the materials themselves.

There are, firstly, the manufacturing problems. superconductor with the highest known critical field (of over 200 kgauss) is niobium-tin, but this is a brittle compound which cannot be drawn into a wire. It has taken several years for manufacturers to develop reliable techniques for producing suitable conductors from this material, for example, by vapour deposition of a thin layer on a stainless steel tape, or by diffusing tin into niobium tape. Somewhat easier to handle are the ductile alloys niobium-zirconium and niobium-titanium which are suitable for fields up to 80 to 90 kgauss. These can be made into strong wires but require special sequences of mechanical working and heat treatment to achieve the required high current densities.

A more fundamental problem encountered with these new materials was the unexpected discovery that they behaved in an unstable manner when wound into coils. Many early magnets reached only a fraction of their design current before reverting to a resistive state. Initially this was thought to be caused by imperfections in the material arising from the manufacturing difficulties; eventually, however, the effect was shown to be caused by the discontinuous penetration of magnetic flux into the superconductor, resulting in transient heat pulses which were sufficiently large to destroy the superconducting state. In this way, small resistive regions were formed which grew rapidly and resulted in a complete collapse of the magnetic field in a few msec, often damaging the coil

by internal voltage breakdown or local overheating. This phenomenon is still not fully understood, but a variety of ways of stabilizing the materials are known in principle and are under investigation. Of these, the best understood technique is that of "full stabilization" in which the superconductor is bonded to a parallel length of low resistance normal metal, such as copper, which provides a temporary alternative path for the current in the event of a local resistive region appearing in the superconductor. The heat dissipated in the copper is carried away by the liquid helium (for which channels are provided to allow it to permeate the whole coil), so allowing the system to recover to its original state. The amount of normal conductor necessary to achieve this degree of stability, however, can be as much as ten or twenty times the amount of superconductor; this considerably decreases the current density and increases the size and weight of the coil, making it a technique only suitable for very large magnets.

More elegant "partial stabilization" techniques are being studied, in which relatively small quantities of normal metal are used to absorb the heat released by flux motion; small coils can be designed in this way simply on the basis of experience, but thorough quantitative understanding will be necessary before these principles can be applied with confidence to larger magnets. Another promising idea is to increase stability by subdividing the superconductor into finer strands, and by this means it should be possible in the future to achieve very compact high

current density coils.

Finally, there are several major mechanical and electrical engineering problems to be faced in the design of large superconducting coils. Perhaps the most significant of these is in the containing of the large electromagnetic

forces on the windings at high magnetic fields, and the necessity for protection circuits to extract from the coil most of the magnetic stored energy, in the event of an accidental transition to the normal (resistive) state.

Because of the many technical problems, and their attendant financial risks, the construction of superconducting coils is still proceeding rather cautiously. convenient indication of the size of a magnet is given by the stored energy in the magnetic field. For example, a 20 kgauss, 1 m internal diameter, coil would have a stored energy of about 10° Joules, and a 100 kgauss, 3 m diameter coil would store about 10° Joules. At the present time the largest coils built and operated have energies in the region 10⁶ to 10⁷ Joules, and cost up to about £100,000. Recent design studies, associated in particular with proposed new bubble chambers, have shown that coils in the range 10° to 10° Joules are now feasible, and several magnets of this size are likely to be completed within the next 5 yr.

So far the demand for superconducting alloys has been relatively small and has yet to justify really large scale production. Processing costs are high, with the basic metals representing only a small fraction of the overall price of stabilized conductors. As superconducting magnet technology becomes established, however, the increasing demand will decrease both conductor and engineering costs, and even larger and more powerful magnets will become possible.

Beam Transport Magnets

As already mentioned, two kinds of magnet are of particular importance in beam optics. The first, generally known as a bending magnet (Fig. 1a), provides a channel of uniform field and is used to change the direction of a particle beam and also to select particles of the required

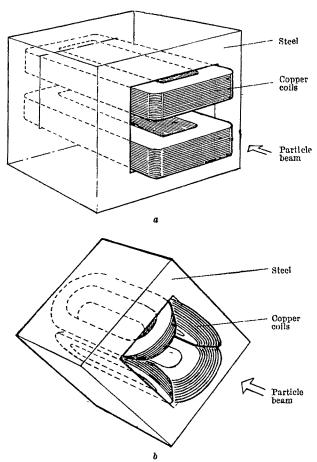


Fig. 1. a, Conventional fron-cored bending magnet; b, conventional iron-cored quadrupole focusing magnet.

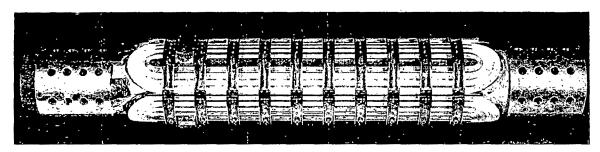


Fig. 2. Superconducting quadrupole constructed at the Brookhaven National Laboratory, USA. It is designed to produce a peak field in the range 40-60 kgauss in a channel 60 cm long by 10 cm diameter.

momentum; the second, known as a quadrupole (Fig. 1b), provides a special distribution of magnetic field to focus the particle beam.

Typically these magnets are 50 to 200 cm long, with a 10 to 20 cm aperture for the beam; costs are in the region £5,000 to £15,000, and power consumption 30 to 100 kW, for each magnet. A large accelerator laboratory requires about 150 magnets of this type, with individual stabilized power supplies. Proposed accelerators of even higher energy would require conventional beam transport magnets up to 400 cm long and 40 cm aperture, multiplying the cost and power requirements by a factor of four.

The increasing size of these magnets is caused principally by the fact that the magnetic field is limited (by saturation of the steel) to about 10-20 kgauss, and the length of magnet required to produce a given deflexion increases in proportion to the particle momentum. In this situation the use of superconducting magnets offers two possible advantages. First, the higher fields and field gradients would allow the magnet lengths and apertures to be reduced. As an example, a 10 cm aperture, 1 m long, 40 kgauss superconducting quadrupole would be equivalent to a 40 cm aperture, 4 m long, 10 kgauss iron-cored quadrupole. These more compact magnets would have many purely practical advantages, for example, in situations where a large beam deflexion is required in a restricted distance, or when it is necessary to focus a beam of short-lived particles. Second, preliminary estimates suggest that, if produced in large numbers, these smaller superconducting elements should eventually have a lower capital cost than their conventional counterparts, and should also give a considerable saving in running cost.

The practical advantages may be more beneficial at existing laboratories, where space is limited, and the increasing use of superconducting magnets should considerably widen the scope and improve the efficiency of experiments. The potential economic advantages are more relevant to future accelerator laboratories, and in any such proposals serious consideration will be given to the possibility of making the entire beam transport system superconducting, perhaps with all the magnets linked to one central helium refrigerator.

The development of these magnets is still at a very early stage. As yet, most superconducting coils have been of the simple cylindrical type, whereas for bending and focusing magnets much more complicated coil shapes are needed, to give the required field distribution accurately together with access for the particle beam. accelerator laboratories are constructing prototype versions of these magnets; Fig. 2 shows the coils of one of a series of quadrupoles constructed at the Brookhaven Laboratory in the USA. A very compact design is achieved by using high current density niobium-tin tape. Fig. 3 shows a bending magnet to be constructed at the Rutherford Laboratory during 1968. This uses a fully stabilized composite conductor of niobium-titanium and copper. Both of these will be used in actual particle beams, and will provide valuable experience in the engineering, instrumentation and operation of this type of magnet. Con-

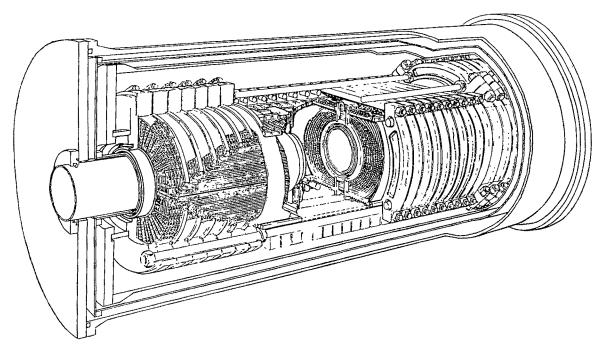


Fig. 3. Artist's impression of a superconducting bending magnet under construction at the Rutherford Laboratory. The coils are shaped to produce a very uniform field of 40-45 kgauss in a channel 140 cm long by 14 cm diameter.

siderable effort will be made during the next few years to study and simplify the design of these types of magnet so that they can be produced reliably and economically in large numbers.

Bubble Chamber Magnets

The new generation of hydrogen bubble chambers, now under design or construction, will all be equipped with superconducting magnets. It is the advent of this type of magnet which has made possible chambers of the size now contemplated, because the cost of powering conventional coils to produce magnetic fields of the required magnitudes and volumes would be prohibitive. It is for this reason that a paradoxical situation exists in which, in spite of the present high unit cost of superconductors, it is the largest and most expensive magnets which have the strongest economic justification.

At the time of writing there are two such large magnets under construction in the USA. Several others are at an advanced stage of planning in Europe, America and Russia. The principal parameters of the individual designs are given in Table 1. It should be noted that, while the iron yoke makes an important contribution to the central field strength in the Argonne magnet, in the CERN and Rutherford Laboratory designs the contribution is small, amounting to only a few per cent, the iron being included simply for magnetic shielding of the surrounding apparatus. An artist's impression of the proposed 1.5 m diameter high field chamber for the Rutherford Laboratory is shown in Fig. 4.

In addition to new chamber proposals, the replacement of conventional coils on many existing bubble chambers is being actively considered in order to increase the available magnetic field strength and thus increase the precision with which the momentum of the various elementary particles under study can be determined.

With these large coils, the engineering problems, which dominate the design, are principally those connected with the mechanical constraint of the massive electromagnetic forces generated by the interaction between the magnetic field and the current producing the field. In an infinitely long coil, these forces would all be directed radially. In practical coils, which often have lengths comparable with their diameter, large axial forces are also generated, particularly at the extremities of the coil where the largest radial components of field exist. Furthermore, in a bubble chamber, to allow access for the beams of particles, it is necessary to use a pair of coils separated typically by 20 cm, and these must be held apart against the powerful attractive force which exists between them. Some idea of the size of the forces is given by the following example. The pair of coils of the bubble chamber illustrated in Fig. 4 attract each other with a force of 10,000 tons, and the radial electromagnetic forces produce tensile "hoop" stresses perpendicular to the rectangular crosssection of the coil amounting in total to almost 4,000 tons for each coil.

In addition to the stabilized conductor it will therefore usually be necessary to incorporate into the coil a stronger material, such as stainless steel, to support the high stresses. In the Rutherford Laboratory's proposed 70 kgauss coil, for example, strips of stabilized superconductor will be wound into flat "pancake" coils together with stainless steel strengthening tapes, interturn insulation,

and spacers to allow helium penetration. The conductor will probably consist of superconducting strands embedded in a matrix of copper, of rectangular section about 5 cm by 0.3 cm, and will carry a fully stabilized current of 7,000 amps. Thirty-six of these pancakes will be stacked together to form two complete coil assemblies of approximate total weight 100 tons. A schematic drawing of a section of one pancake is shown in Fig. 5.

In lower field coils, such as for the Argonne bubble chamber, it is possible to omit the stainless steel altogether and rely on the copper to carry the internal forces. Furthermore, sufficient thermal stabilization can be provided by exposing only the edges of the conductor to the liquid helium; interturn helium channels are therefore unnecessary, and a more rigid coil structure can be achieved.

In the design of these large coils particular attention must be paid to the compatibility of the materials used from the standpoint of thermal contraction. In cooling from 300°-4° K, appreciable mechanical stresses can arise from local differences in thermal contraction or by uneven cooling of the whole coil. For this reason cooldown times for these coils are intended to extend over four or five days. The closed loop refrigerator must then produce several thousand litres of liquid helium in which to immerse the coils. There are suggestions, however, that stabilized coils might run satisfactorily in cold helium gas and this is being investigated. During charging of these coils to their full field, power dissipation occurs in the conductor because of induced eddy currents; because of the limited refrigeration capacity available, charging times of several hours are envisaged. In the event of an extended refrigerator failure, measures must be taken to remove the energy stored in the magnetic field and dissipate it in an external resistance. Apart from the possibility of physical damage to the coils if this energy were all dissipated locally in the coils, the overall temperature rise would be such that a further cool-down period of perhaps one day would elapse before the coil could become operational again.

All of these problems are being intensively studied at the present time; no unsurmountable obstacles have been encountered, and it can be confidently predicted that several coils of this size will be in operation by the early 1970s.

Future Possibilities

The most obvious target for speculation is the particle accelerator itself. Although many types and varieties of accelerator have been developed, the only one which seems suitable for construction on a very large scale is the alternating gradient proton synchrotron. The principal feature of the machine is an underground tunnel housing a ring of magnets which maintain the particles in a circular orbit during acceleration. The individual magnets in the ring are quite small, and are similar in type to the beam transport magnets discussed previously, except that the deflecting and focusing properties are usually combined in each magnet. The largest existing accelerators at Brookhaven and CERN give particles with energies of up to 30 GeV and have magnet rings about half a mile in circumference. A new accelerator is to be built in the United States with an energy of 200 GeV, and there is a proposal for a European accelerator of

Table 1. MAIN PARAMETERS OF HYDROGEN BUBBLE CHAMBER MAGNETS UNDER CONSTRUCTION OR DESIGN

Laboratory	Diameter of bubble chamber	Central field (kgauss)	Internal diameter	Magnet coil dimensions External diameter	Overall height of pair of coils	Iron yoke or shield	Expected completion date
Argonne National (USA) Brookhaven National (USA) CERN (Switzerland— European collaboration)	12 ft. 7 ft. 14 ft.* 3-7 m	18-25 20 30 35	15 ft. (4·6 m) 8 ft. (2·4 m) 16‡ ft. (4·9 m) 4·7 m	17 ft. (5·2 m) 9 ft. (2·7 m) 19½ ft. (5·9 m) 5·7 m	9 ft. (2·7 m) 7; ft. (2·3 m) 18; ft. (4·2 m) 4·2 m	Yes No No Yes	1969 1968 1972 1971
Rutherford (GB) Serpukhov (USSR)	1.5 m* 5 m*	70 30–40	1-9 m 5-6 m	3·4 m 7·2 m	2-3 m 4-5 m	Yes No	1973 1973

^{*} Projects awaiting financial approval: quoted parameters subject to adjustment.

300 GeV. The latter would have a magnet tunnel 4.5 miles in circumference, containing about 900 precisely aligned individual magnets, each 6 m long. The magnets alone will cost £16 million, and the estimated capital cost of the whole project is £148 million.

Whether the use of superconducting magnets could significantly reduce the cost of accelerators is still an unresolved question. The potential cost gain arises principally from the decreased size of the ring (because of the higher magnetic field), but because something like 25 per cent of the circumference has to be occupied by equipment other than magnets it is not yet clear how much it would really be possible to reduce the diameter. Assuming the diameter could be reduced to less than about one-quarter of that for conventional magnets, preliminary estimates suggest that there could be a significant gain in overall cost. Perhaps a more attractive idea, however, is

the possibility of converting a conventional accelerator to a higher energy. The possibility has been considered of converting the Rutherford Laboratory 7 GeV synchrotron "Nimrod" to an energy of perhaps 35–40 GeV by replacing the present 14 kgauss conventional magnet with an 80 kgauss superconducting magnet, although the competing requirement for a higher beam intensity may make this conversion difficult. As a more extreme example, a 300 GeV machine built in the near future would necessarily have to use conventional magnets; but in tensifier years' time, with superconductor technology in a more advanced stage, these might be replaced by high field magnets, thus converting the accelerator to perhaps 1,500 GeV at only a fraction of the cost of a completely new accelerator of this energy.

A serious obstacle to the development of this type of magnet is the a.c. loss in the superconductor. A synchro-

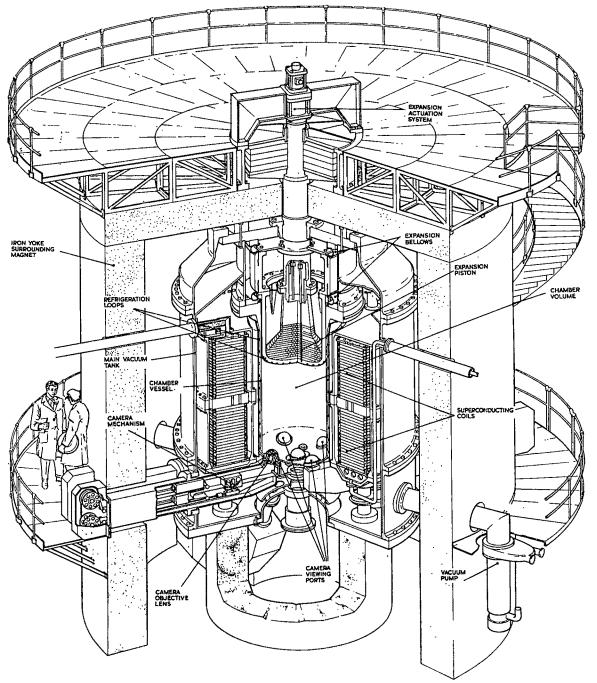


Fig. 4. Artist's impression of proposed new hydrogen bubble chamber for the Rutherford Laboratory. A pair of cylindrical colls produces a field of 70 kgauss, uniform to a few per cent, over the working volume of the chamber 1.5 m diameter by 1 m deep.

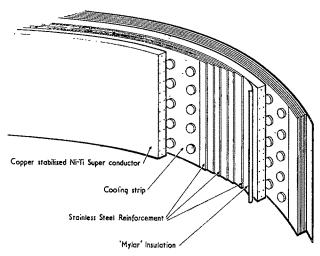


Fig. 5. Possible conductor configuration for bubble chamber coil, showing stainless steel reinforcement and interturn cooling channels.

tron magnet is pulsed, the field rising to a maximum and falling back to zero every few seconds. A fluctuating magnetic field causes heat to be dissipated in the superconducting material, and with existing conductors this would increase the refrigeration requirement to an un economic level. One possible solution is to produce a conductor in which the superconducting alloy is subdivided into filaments each about $10-20\mu$ in diameter; it can be shown theoretically that this should reduce the heat dissipated to a tolerable level. Development work along these lines has already commenced, but it is evident that it will be some years before the feasibility and long term reliability of pulsed superconducting magnets can be established with the required certainty.

A significant related problem is that a high field superconducting magnet, although physically smaller, would probably have at least four times the stored energy of a conventional magnet. It is this peak stored energy which governs the size of the power supply for a pulsed magnet system, and the feasibility of conventional motor-alternator systems supplying energies up to perhaps 10° Joules per pulse is in some doubt. It is possible, however, to envisage a completely new type of power supply in which large superconducting coils are used to store and transfer the magnetic energy. The simplest version of this idea would consist of a pair of superconducting coils mounted on a

common shaft and rotating inside a larger coil, equivalent to a mechanically coupled motor and dynamo; if the parameters are chosen correctly, such a device could be used to transfer energy repetitively between a superconducting energy storage coil and a synchrotron magnet with negligible loss. Moreover, despite the size of the coils (in the region of 3 to 6 m diameter for 10° Joules), the cost of such a system is likely to be less than that of a conventional power supply.

A rather more straightforward and less speculative accelerator application would be the use of superconducting magnets in particle storage rings. In this technique, beams of particles from an accelerator are fed into two intersecting rings of d.c. magnets; the two beams of particles circulate continuously, and, when a sufficiently high density of particles has built up, an appreciable number of head-on collisions occur in the intersection regions, the effective energy in such collisions being very much higher than in the usual case of a simple beam hitting a stationary target. (For example, two colliding 30 GeV beams are equivalent to a single beam of energy 2,000 GeV.) The magnet rings are similar in type and size to that of a particle accelerator, but do not have the problems of pulsed operation, so that the use of superconductors will obviously be seriously considered for any large installations of this type built in the future

Finally, we consider what is perhaps a more urgent future problem—the construction of even larger bubble chambers to partner the new accelerators now planned. Diameters of 10 to 25 m have been mentioned for these hydrogen chambers. At this sort of size the surrounding magnet coils would have to be fabricated in situ, and this might involve the development of entirely new conductor configurations and constructional techniques. One obvious extension of existing technology would be to develop larger cross-section superconducting cables, carrying perhaps hundreds of thousands of amperes; a more attractive possibility, however, might be to deposit layers of superconductor on prefabricated surfaces of the required shape and mechanical strength by a process such as plasma spraying. This technique is already possible on a small scale and could be used in principle to form coils of indefinitely large size. Although it is not possible to foresee which of these, and many other possibilities, will eventually prove successful, it is evident that substantial advances in superconductor technology will be necessary before these very large magnets become economically realistic.

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Recognition of Syntactic Structure by Computer

by P. BRATLEY H. DEWAR J. P. THORNE

A computer program has been designed to simulate the process by which humans recognize the syntactic structure of sentences.

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An essential part of the capacity to understand utterances lies in the ability to recognize syntactic structure. Over many centuries, grammarians have devised a special vocabulary for describing these structures. This vocabulary includes terms such as word, sentence, noun, verb,

subject and object. Unfortunately, grammar is usually taught in a way which obscures an interesting and important fact about grammatical analyses—that they are statements about what every native speaker knows about his language. It is therefore necessary to emphasize the point. Every

English speaker, irrespective of whether he has ever heard of, or understands the meaning of, terms such as noun and verb, possesses the information contained in statements such as the following: in the utterance The cat sat on the mat, cat is a word and catsa is not; the word the occurs twice; cat is the same class of word as mat and a different class from sat, the and on; the cat is a phrase but cat sat is not. A speaker could not understand the utterance unless he possessed this information.

It is important to notice that only some of this information-how much is disputable-is derived directly from what the speaker hears. For example, there is nothing in the pronunciation of cat which suggests the word shares characteristics with mat which it does not share with sat. Recognizing the syntactic structure of a sentence is not just a matter of hearing it. It is also a matter of knowing the syntactic structure of the language.

A grammar is a set of statements about what the native speaker knows about the structure of his language. The scientific study of grammars dates from the publication in 1957 of Chomsky's book Syntactic Structures¹. Chomsky, for the first time, laid down the formal conditions which a grammar must satisfy if it is to count as a reasonable description of the native speaker's knowledge of the structure of his language.

Chomsky shows that what is required is a set of effectively computable rules, which must be finite (because it is a model of knowledge "internalized" by a human being) but which is capable of generating an infinite number of sentences together with their analyses (because the number of well formed sentences in any natural language is infinite). Chomsky was also the first to set out clearly the empirical conditions which a grammar of a natural language must satisfy. The most important is that the grammar should generate all and only the well formed grammatical") sentences of the language. The conditions also include the requirement that in the case of an ambiguous sentence such as I dislike climbing plants, the grammar should reflect the ambiguity by assigning two analyses—that is to say, the grammar must contain two sets of rules for generating the sentence.

Chomsky also points out that, for many kinds of sentences, not all the syntactic information about them available to the native speaker relates to elements that he actually hears. There is, for example, the sentence The girl I liked left. Any English speaker hearing this sentence possesses the information contained in the statements that the girl I liked is the subject of the sentence and left is the predicate, and that the is a definite article, girl a noun, etc. This Chomsky calls information about the "surface structure" of the sentence. But any English speaker also knows that in this sentence, the girl is not only the subject of left but also the object of liked, even though, of course, the correct surface form of the sentence is The girl I liked left not The girl I liked the girl left. Because we derive this information from the sentence without making any reference to the context-as here where it is used merely as an examplethen it is clear that we derive this information not from the context (as is sometimes suggested) but from our perception of the structure of the sentence, even though, as we have seen, this part of the structure is not actually realized in the surface form of the sentence. The information forms part of what Chomsky terms the "deep of the sentence. If a grammar is to be a complete statement of the native speaker's knowledge of the syntactic structure of his language, it must assign both deep and surface structure analyses to all wellformed sentences of the language.

Many of the structural characteristics of natural languages are still not properly understood. But enough progress in developing models of the native speaker's knowledge of the syntactic structure of his language has been made in recent years to lead some linguists and psychologists to begin work on a further problem—the

problem of how human beings employ this knowledge in recognizing the syntactic structure of the utterances they Constructing a computer program of the kind described here to simulate the process is one approach to the problem. Because very little progress has so far been made in automatic speech perception, the program has been designed to work with an orthographic input, which means—because it is already divided into words—a partially analysed input. Even so, the problem of constructing an analyser which will provide both deep and surface structure information about arbitrary English sentences submitted to it in orthographic form is not trivial.

Most work on automatic syntactic analysis has been undertaken in the context of information retrieval, translation and other text-processing systems and has not aimed at simulating human behaviour. The chief differences between the analysers used in these systems (for example, the Harvard Predictive Analyser2, the MITRE Analyser³ and the FAP String Analysis Program4) and the analyser developed at Edinburgh5 are attributable to the fact that a syntactic analyser which is intended to model the way in which human beings recognize the syntactic structure of sentences must satisfy certain a priori conditions.

First, such an analyser must not be dependent on looking up every word of an input sentence in a dictionary. Other automatic parsers of English (or Russian and other languages) operate on the assumption that they will eventually incorporate a dictionary containing every word in the English (Russian, and so on) language, listing the parts of speech to which each word can be assigned. It seems unlikely that a speaker of the language needs to internalize and employ a dictionary of this kind in order to be able to recognize syntactic structure. On the one hand, everybody can recognize the syntactic structure of sentences which contain words we have never heard before (He has gone to shoot a grison, He is going to disple his mother-in-law). On the other hand, the knowledge that a word such as iron, say, may be a noun, an adjective or a verb is of no help in deciding its role on any particular occasion (He ruled with an iron hand, Strike while the iron is hot, I will iron your shirt tomorrow). It is because we are able to recognize the structure of these sentences that we know that iron is an adjective in the first example, a noun in the second, and so on.

This is not true of all words. Some, such as pronouns, conjunctions and prepositions, have fixed syntactic functions, and play an essential part in the recognition of sentence structure. Syntactic information is also provided by certain suffixes such as -s, -ed and -ing. Our program is designed to require access to a dictionary containing information only about those words with fixed syntactic functions (the closed-class words) and about suffixes of the type already mentioned. This dictionary is very short, approximately a thousand words. Nevertheless, with this dictionary alone, infinitely many English sentences can be analysed.

The second constraint our program must satisfy is that it must process each sentence in a single left-to-right pass, analysing each part of the sentence once and once only. Very many sentences are syntactically ambiguous. Nearly all sentences contain elements which, taken in isolation, are ambiguous. In view of the efficiency with which human beings recognize the syntactic structure of utterances, it seems reasonable to assume that in listening to utterances all possible analyses are usually considered simultaneously. The program is therefore designed so that, if an input sentence is ambiguous, the program will work on all the possible analyses simultaneously. In other words, the program does not follow through one analysis and then backtrack to see if others are possible. The progress of the analysis procedure is represented by a continuously growing data structure. When the end of the sentence is reached, each possible analysis is to be found represented as a complete path through this structure.

It seems equally reasonable to require that, if all ambiguous items are treated simultaneously, elements of a sentence which are not ambiguous should not be analysed more than once. For example, in the sentence He rolled up the bright red carpet, the phrase the bright red carpet is either the object of the phrasal verb roll up, or else the object of the preposition up. The analysis of the bright red carpet as a noun phrase is, however, the same for both interpretations. In a case such as this, the program will analyse the noun phrase once only and the structure representing this analysis will become part of two paths, representing the two possible analyses of the whole sentence.

Third, it seems necessary that the program should work in a predictive fashion. There is a good deal of evidence to suggest that the efficiency with which human beings recognize the syntactic structure of sentences is to some extent a result of their ability, having heard part of a sentence, to predict the structure of the remainder. It seems probable that having heard, say, the subject of a sentence, people then predict in some sense the occurrence of a verb to go with that subject. In the two examples He ruled with an iron hand and I will iron your shirt tomorrow, it is obvious that the kinds of words which can follow He ruled with an ... and I will ... are different. Roughly speaking, in the first case we are predicting a noun or an adjective, while in the second case we are

ture grammar1; the base grammar embodied in the analyser is a somewhat simpler type—a regular or finitestate grammar6—for which it is possible to describe the transformational rules more conveniently and economically. In outline, the base grammar specifies sequences of category-names. An example of such a sequence is PRONOUN VERB PRONOUN ADVERB, which would be instantiated by sentences like She visited him yesterday. It is possible to substitute for certain category-names not simply individual words but transforms which are themselves (modified) realizations of base strings. Thus He asked who admired Descartes is a possible realization of the string PRONOUN VERB INDIRECT-QUESTION, where the part who admired Descartes, which is substituted for INDIRECT-QUESTION, is derived from a realization of the string PRONOUN VERB PROPER-NAME. Associated with each element in a string there is also a syntactic relation marker (SRM) which describes tho syntactic role of the element in the overall structure of the string.

The output produced by the analyser reflects the form of the grammar. Each complete analysis of an input sentence is displayed in a series of numbered levels, the level structure corresponding to the transformational structure of the sentence. Within each segment of a level, the parsing of the individual units is indicated by the attachment of SRMs either to individual words or to the names of transforms. Thus for the two sentences quoted, the output would be

```
      1
      SE: STAT
      2
      SU: she
      AV: visited
      OB: him
      MO: yesterday
      2

      1
      SE: STAT
      1
      SU: he
      AV: asked
      OB: INDQ
      2

      3
      SU: who
      AV: admired
      OB: Descartes
      3
```

(See next page for a list of the abbreviations used)

predicting a verb. After the next word of the sentence, we can check that it fulfils at least one of our predictions and, on the basis of the predictions satisfied and our knowledge of the language, new predictions can be made. Accordingly, the operation of the analysis program is a process of making and checking predictions about the syntactic structure of a sentence in which the predictions made at any stage are governed by the grammar which is incorporated in the program.

This grammar is a transformational grammar. A transformational grammar consists of a base component and a transformational component. The base component specifies a set of strings which correspond roughly to the

Special conventions are used to indicate the analysis of sentences involving inversion and co-ordination. Inversion is exemplified by such interrogative and relative constructions as:

Has he informed the police? (inversion of auxiliary) The apples which they bought were rotten

(inversions of object of bought)

Which book do you want?

(inversion of both auxiliary and object). In the printout the inverted unit is labelled with a dummy SRM (OO) and the position from which the unit is inverted is marked by a genuine SRM attached to an asterisk. Thus for the sentences quoted above the output would be

```
1
              SE: QUES
                                                                                         1
              00: has
                           SU: he
                                               AV: informed
                                                               OB: CNP
         \mathbf{2}
                                      ATT:*
         3
                                                               DE: the
                                                                           HE: police
                                                                                         3
    SE: STAT
    SU: CNP
2
                                                                         AV: were
                                                                                    CO: rotten
                                                                                                   2
                                                                                                  3
3
                            AT: REL
    DE: the
              HE: apples
                            OO: which SU: they
                                                    AV: bought OB:*
                                                                                                   4
            SE: QUES
                                                                                           1
       1
       2
            OO: CNP
                                      00: do
                                                SU: you
                                                            AU:*
                                                                     AV: want
                                                                                  OB:*
                                                                                           2
            DE: which
                         HE: book
```

simple declarative or kernel sentences of the language. The transformational component accounts for complex sentences by deriving them from one or more basic underlying strings. In most formulations of transformational theory the base component is a context-free phrase-struc-

More generally, an asterisk following an SRM indicates the non-realization of some deep structure element, as in the case of imperative sentences, which lack a surface structure subject. Thus the analysis produced for the sentence Watch him would be

```
1 SE: IMP
2 SU:* AV: watch OB: him 2
```

	ABBREVIA	TIONS	
	Syntactic relation markers	C	ategory names
SE SU AV OB MO AU DE HE AT IN LI PO PA OO	sentence] subject active verb object modifier auxiliary determiner head (of noun phrase) attribute indirect object link (preposition or conjunction) prepositional object particle inverted element	STAT QUES IMP INDS INDQ SUBC GER REL PREC CNP	statement question imperative indirect statement indirect question subordinate clause gerund relative prepositional clause complex noun phrase

In the case of sentences containing co-ordinate parts coupled by conjunctions such as and, but and or, the scope of the conjunction is indicated in the printout by brackets. For example, the two analyses for the ambiguous sentence The policeman stopped and questioned him would be

used for the program and its data. In addition to the analysis routines the program contains routines to accept the grammar and dictionary and set them up in the required internal format. The closed-class dictionary is small enough to be kept entirely in core, so that the

```
SE: STAT
2
    SU: CNP
                              [AV: stopped and
                                                                               2
                                                 AV: questioned]
                                                                   OB: him
3
               HE: policeman
                                                                               3
    DE: the
1
    SE: STAT
    SU: CNP
                                                                               2
                              [AV: stopped and
                                                 AV: questioned
                                                                   OB: him]
3
    DE: the
                                                                               3
               HE: policeman
```

The analysis program is written in Atlas Autocode, a language akin to Algol, and runs on the KDF9 at the Edinburgh Regional Computing Centre. 16K 48-bit words of core are available, about 2K of which are taken up by the operating system, the remainder of the space being

program will run without backing store. Sentences to be analysed are submitted in free format on paper tape and the output is produced on the high-speed printer. The time taken to analyse each sentence is printed out with the analyses.

Examples of Output

The boy who kissed the girl laughed uproariously

(time taken: 1.281 seconds)

```
      1
      SE: STAT

      2
      SU: CNP

      3
      DE: the HE: boy AT: REL SU: who AV: kissed OB: CNP DE: the HE: girl

AV: laughed MO: uproariously 2

3

4

5
```

The boy who the girl kissed laughed uproariously

(time taken: 1.294 seconds)

```
1 SE: STAT
2 SU: CNP
3 DE: the HE: boy AT: REL
OO: who SU: CNP AV: kissed OB:*
DE: the HE: girl

1 AV: laughed MO: uproariously 3
```

When did John say he would come?

(time taken: 1.294 seconds)

```
SE: QUES
2
               OO: did
                         SU: John
                                   AU:*
                                          AV: say
                                                    MO:*
                                                            OB: INDS
                                                                                            2
   00: when
3
                                                            SU: he
                                                                                            3
                                                                    AU: would
                                                                                AV: come
   SE: QUES
               00: did
                                           AV: say
   00: when
                        SU: John
                                   AU:*
                                                     OB: INDS
                                                                                            2
3
                                                     SU: he
                                                             AU: would AV: come
                                                                                    MO:*
                                                                                            3
```

```
He rolled up the bright red carpet
```

```
(time taken: 1.363 seconds)
```

```
1
    SE: STAT
                                                                                            2
2
    SU: he
                             MO: PREC
               AV: rolled
3
                                                                                            3
                                       PO: CNP
                             LI: up
                                                                                            4
4
                                       DE: the
                                                   AT: bright
                                                                 AT: red
                                                                            HE: carpet
                                                                                            1
    SE: STAT
1
                                                                                            2
2
    SU: he
               AV: rolled
                                        OB: CNP
                             PA: up
                                                                                            3
3
                                       DE: the
                                                   AT: bright
                                                                 AT: red
                                                                             HE: carpet
```

I dislike playing cards

(time taken: 0.951 seconds)

```
1
    SE: STAT
                                                        2
              AV: dislike
                            OB: CNP
2
    SU: I
3
                            AT: playing
                                           HE: cards
                                                        3
SE: STAT
 SU: I
         AV: dislike
                       OB: GER
                                                            2
                                                            3
                       SU:*
                                AV: playing
                                              OB: CNP
                                              HE: cards
                                                            4
```

Playing cards intrigues me

(time taken: 1.045 seconds)

2

3

4

```
      1
      SE: STAT
      1

      2
      SU: GER
      AV: intrigues
      OB: me
      2

      3
      SU:* AV: playing
      OB: CNP
      3

      4
      HE: cards
      4
```

Playing cards intrigue me

(time taken: 1.026 seconds)

```
      1
      SE: STAT
      1

      2
      SU: CNP
      AV: intrigue
      OB: me
      2

      3
      AT: playing
      HE: cards
      3
```

The queen's sister's husband took good photographs

(time taken: 1.521 seconds)

```
SE: STAT
                                                                                               1
   SU: CNP
                                                                  OB: CNP
                                                       AV: took
                                                                                              3
                                        HE: husband
                                                                             HE: photographs
3
   DE: CNP
                                                                  AT: good
   DE: CNP
                           HE: sister's
                                                                                               4
                                                                                               5
   DE: the
             HE: queen's
```

She handed John a pear and Mary an apple

(time taken: $1.\overline{322}$ seconds)

```
1 SE: STAT
2 SU: she AV: handed [IN: John OB: CNP and IN: Mary OB: CNP ] 2
3 DE: a HE: pear DE: an HE: apple 3
```

An analyser which simulates the process by which human beings recognize the syntactic structure of sentences in certain obvious ways is interesting only in as far as it serves as a source of hypotheses about aspects of this process which are not obvious. For example, it could be that the sequence of operations in the program will throw some light on the structure of the human performance. One specific line of research is an investigation of the extent to which the processing time of the analyser can provide a measure of the complexity presented by sentences to the human hearer. Another is to study in which ways the structure of the program has to be changed in order to produce output which would reflect features of various language disorders. A major problem

in all these cases is to distinguish those features of a model which are significant from those which are merely a consequence of the fact that the model is a computer program.

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Angular Measurements of the Structure of 3C 446 and Other Quasars with High Red-shifts

by

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Measurements of the radio source 3C 446 show that it is elongated in the east—west direction and may well be a double source. 3C 9 also seems to be a double source, and PKS 1116+12 and 4C 31:38 have angular dimensions <0·1 and <0·2 sec arc, respectively, in at least one direction.

LITTLE is known about the angular dimensions of quasars with large red-shifts. Although there are now more than twenty quasars with red-shifts greater than 1.4, only the structures of 3C 270·1, 3C 298 and AO 0952+17 have been published¹⁻³. Three others, 3C 191, 3C 454 and PKS 0106+01, showed high fringe visibilities when observed with interferometers of baseline sixteen or twenty thousand wavelengths^{1,4}, while 3C 446 is known to have an angular dimension less than 0.3 sec are at 67 cm wavelength⁵. Finally, PKS 1148-00 and 3C 446 both lie in the category of radio sources with an angular dimension smaller than about 0.05 sec arc at 11 cm listed by Palmer et al.6. Their communication gave a preliminary account of observations of forty-six radio sources with an interferometer between Jodrell Bank and the Royal Radar Establishment, Malvern, with a baseline 127 km long: the wavelengths of observation were 21 cm, 11 cm and 6 cm. We present here a full analysis of the 21 cm measurements on the quasar 3C 446, showing that it is significantly resolved at this wavelength. We also give the results of further observations at 18 cm and 73.5 cm on three additional quasars with high red-shifts.

3C 446

For interferometric measurements, the radio source 3C 446 was observed continuously at 21 cm for 5 h on September 2, 1966, and the resultant variation of fringe visibility with hour angle is shown in Fig. 1. In calculating the visibilities the 21 cm flux density was assumed to be 5.9×10^{-26} Wm⁻² c/s⁻¹, this value being taken from the Parkes survey⁷ and the work of Conway, Kellermann and Long⁸. The method of analysis and the calibration pro-

cedure will be discussed in detail elsewhere. The error bars include errors caused by calibration and uncertainty in the flux, but no allowance has been made for possible variability.

The dependence of the effective resolution vector on hour angle for 3C 446 at both 21 cm and 11 cm wavelengths is shown in Fig. 2. Because of the low declination of this source the magnitude of the north-south resolution does not change greatly as the source moves through the sky. This increases the difficulty of fitting a model brightness distribution to the visibility curve. Although a fit can be obtained for a single elliptical Gaussian model, other quasars the structure of which has been resolved consist characteristically of two separated regions of

radio emission. The 21 cm results were therefore fitted with a double model having two circular Gaussian components of equal diameters but unequal fluxes. The computed best fit (least mean square deviation between model and observed results) was obtained with components of half power width 0.15 ± 0.04 sec arc, separated by 0.37 ± 0.15 sec arc in position angle $83^{\circ} \pm 16^{\circ}$ with flux ratio 0.2 ± 0.1 . The visibility curve of this model is shown by the broken line in Fig. 1. A double model with a similar separation and position angle in which the weaker component is a point source fits the data equally well.

 $3\bar{C}$ 446 was also observed for a short time at a wavelength of 11 cm when its visibility increased from 0.70 ± 0.04 to 0.81 ± 0.04 between hour angles 21.5 and 23.5 (private communication from W. Donaldson). It is difficult to reconcile the behaviour of the visibility functions at 21 cm and 11 cm with a frequency-independent model of the source. It seems probable that $3\bar{C}$ 446 is double, that the spectra of the two components differ and that the flux inequality is greater at 11 cm. A less probable explanation of the results is that $3\bar{C}$ 446 has a single elongated component the size of which increases with wavelength.

Some further evidence on the angular structure of 3C 446 comes from scintillation measurements made with the Mark I telescope at Jodrell Bank during February and March 1967. During this period 3C 446 approached within 4° of the Sun. Strong scintillations were observed at a wavelength of 73.5 cm out to solar elongations of 25°. The intensity from the source was sampled at a rate of twenty-five samples per sec, and the auto-correlation function was calculated during the observations by an

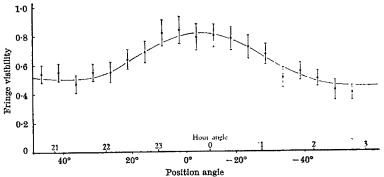


Fig. 1. Variation of fringe visibility with hour angle for 3C 446 at 21 cm. The line shows the best fitting double Gaussian model.

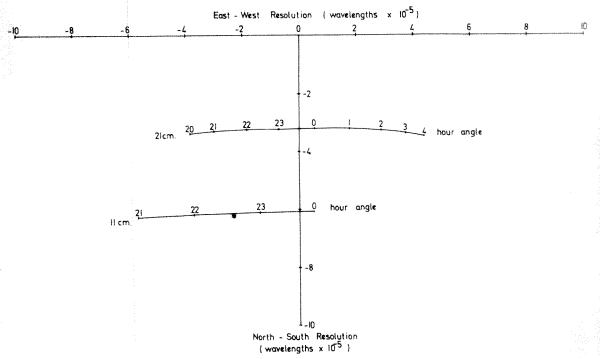


Fig. 2. Variation of the resolution on 3C 446 with hour angle, using Jodrell-Malvern baseline.

on-line computer. The rate of scintillation is measured by the width of this auto-correlation function, and the method of analysis is similar to that used by Cohen et al.⁹.

An extended source produces a reduced scintillation index and, in addition, the measured auto-correlation function is broadened by an amount depending on the angle between the solar wind and the source axis. An elliptical Gaussian brightness distribution has been assumed for the source, and the measured auto-correlation function was compared with that predicted using the two dimensional diffraction theory of Little and Hewish¹⁰. The predicted auto-correlation function is $\exp(-t^2/\tau^2)$, where the time scale of the scintillations is given by

$$\tau^2 = \{a^2 + 2L^2 (\theta_0^2 \cos^2 \alpha + \varphi_0^2 \sin^2 \alpha)\}/v^2$$

where a is the scale of the point source diffraction pattern which is assumed isotropic and moving radially out from the Sun at a velocity v; L (= 1 astronomical unit) is the distance to the diffracting medium; the 1/e radii of the source are θ_0 and ϕ_0 ; and α is the angle between the solar wind and the major axis. Measurements were made of the scintillation index and time scale, \u03c4, as the Sun moved past the source. The properties of the diffracting medium are functions of solar elongation and may vary with time. For this preliminary analysis, however, a simplifying assumption has been made that the scale of the pattern, a, and velocity, v, are functions of elongation only. On both February 17 and March 6 the solar elongation was 10° , but the angle, α , differed by 60° . The respective values of scintillation index were 0.4 and 0.3 which agree with the assumption of no large changes in properties of the solar wind, and are somewhat smaller than might be expected for a point source. The values of τ for these

dates were measured as 0.4 sec and 0.8 sec. Using the expression for τ , the limits for the half power widths of an elliptical Gaussian model are $\gtrsim 0.34$ sec are by $\lesssim 0.19$ sec arc, with the major axis at a position angle of $94^{\circ}\pm30^{\circ}$. These preliminary results also agree well with the double model fitted to the interferometric data at 21 cm wavelength.

Although the results from both techniques leave considerable latitude in possible models of source structure, there is little doubt that 3C 446 is elongated in the east—west direction, in which it has an angular extent of about 0·3 sec arc.

PKS 1116+12 and 4C 31.38

In January 1967, after a series of galactic OH line observations¹¹, a few continuum measurements of quasars were made at 18 cm. Fringes were obtained for about 1 h from each of the quasars PKS 1116+12 and 4C 31-38, both of which have high red-shifts. Using 3C 287 for calibration, the fringe visibilities were 1.3 ± 0.3 and 0.9 ± 0.3 , indicating that these quasars have angular dimensions <0.1 sec arc and <0.2 sec arc, respectively, in at least one direction, as shown in Table 1.

3C 9

In September 1967 the quasar 3C 9 at a wavelength of 73·5 cm was observed with the Mark I telescope at Jodrell Bank and the new Mark III telescope which is situated near Nantwich. The length of this baseline was 31,000 wavelengths. A complete analysis of these observations together with details of the instrument will be given later, but 3C 9 seems to be a double radio source with a separation of about 8 sec arc, as shown in Table 1.

Table 1. NEW ANGULAR INFORMATION ON SOME QUASARS WITH HIGH RED-SHIFTS

	Source	Red-shift $z = \frac{\lambda_0 - \lambda_c}{\lambda_c}$	Wave- length observed λ ₀ (cm)	Wave- length emitted λ_{ϵ} (cm)	Position angles observed	Effective baseline (wavelengths)	Angular structure	Corresponding linear dimensions?
3C 4C	KS 1116 + 12 19 131·38 1446	2·118 2·012 1·557 1·403	18:0 73:5 18:0 11:0 21:0 73:5	5·8 24·5 7·0 4·6 8·7 30·6	20° -50° to $+80^{\circ}$ 60° -10° to 40° -45° to $+45^{\circ}$ Scintillation	5.6×10^{3} 1×10^{3} to 3×10^{3} 6.3×10^{5} See Fig. 2 See Fig. 2 observations	<0·1 sec Double separation ~8 sec* <0·2 sec ~0·3 sec by \$0·15 sec. Elongated in east—west direction. Possibly double	< 300 parsec ~ 25,000 parsec < 700 parsec ~ 1,000 parsec by ~ 500 parsec

^{*} Provisional interpretation. † Assuming z purely cosmological and $q_0 = \sigma_0 = 1$.

Scintillation measurements were also made on this source at the same wavelength giving a scintillation index of < 0.1, which indicates that it has little structure smaller than 0.3 sec arc.

Information about the linear dimensions of the sources is also given in Table 1 for the case where the red-shifts are purely cosmological in origin. At these large red-shifts, the linear sizes are greatly influenced by the geometry of the Universe, and a cosmological model in which $q_0 =$ $\sigma_0 = 1$ has been used (where q_0 is the acceleration factor and σ_0 the density parameter). Factors for conversion to other models are given by McVittie¹². The difference between the dimensions of 3C 9 and PKS 1116+12 (both of which have approximately the same red-shifts) is nearly two orders of magnitude, which indicates that even at these large red-shifts the intrinsic dispersion in the dimensions of quasars would mask any cosmological effects.

For 3C 446 an angular dimension of 0.3 sec are would correspond to a linear dimension of about 103 parsec if the red-shift is cosmological, or about 10 parsec if 3C 446 is situated at a distance of 10 Mparsec. These values may be compared with the upper limits of 10-3 parsec on the size of the regions emitting the optical continuum radiation, calculated from the time scale of the large variations in the light received from 3C 446 during 1966 (ref. 13).

Apart from 3C 9, the quasars in Table 1 all have comparatively flat radio spectra as well as small angular dimensions, both properties characteristic of variable radio sources. 3C 446 is known to be variable at 1.96 cm observed wavelength¹⁴ (0.82 cm emitted wavelength); it would prove valuable to observe PKS 1116+12, 4C 31-38 and also PKS 1148-00 for possible variability. The magnitude of variations in the radio output of quasars has been shown to be greater at short wavelengths 15, but because of their high red-shifts it would be valuable to monitor the fluxes of these quasars at comparatively long observing wavelengths.

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Molecular Hydrogen in Pre-galactic Gas Clouds

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During the collapse of pre-galactic gas clouds through a density of about 10^4 particles/cm³, hydrogen molecules are produced and dominate the subsequent cooling.

In a plausible picture of galaxy formation, proto-galaxies are produced by the gravitational clustering of many gas clouds^{1,2}. Various origins for these clouds have been discussed by Lifshitz³, Bonnor⁴, Saslaw⁵ and Harrison⁶ among others. In this article, the important, and probably dominant, part that molecular hydrogen plays in the early evolution of large clouds is first investigated, and then the possibility of detecting the radiation they produce is discussed.

The era examined occurs in the conventional big-bang cosmology after matter and radiation have decoupled and before star formation has begun. During this period, $z\lesssim 1,000$, the recombination time scale for hydrogen exceeds the Hubble time, and about one hydrogen atom in 10⁵ remains ionized⁷. Then, by an unknown process, gravitationally bound condensations are assumed to establish themselves. At first, they will expand rather more slowly than the rest of the universe. Later, at a time determined by the ratio of their mass to the Jeans mass, they will contract. For the detailed cosmological model, that of Einstein and de Sitter is assumed, although this is not critical.

Hitherto, H₂ has not been considered a component of the pre-galactic medium. This was because the time scales for appreciable amounts to form in three-body reactions or in radiative association are always greater than the Hubble age (for example, ref. 8), and there are no grains to speed the reactions. Any H₂ formed in the uniform background is dissociated by the radiation, until the density is too low to produce it. We find, however, that later, in the condensations, charge transfer reactions produce enough H2 to radiate most of the thermal energy of contraction. General references to the chemistry of charge transfer reactions may be found in ref. 9. Here we consider only those relevant to H2 (P. Solomon, private communication)

$$e^- + H^+ \rightarrow H$$
 (1)

$$\mathbf{H} + \mathbf{H}^+ \rightarrow \mathbf{H}_2^+ + hv$$
 (2)
(0.7 eV at 2,000° K)

$$\mathbf{H}_{2}^{+} + \mathbf{H} \rightarrow \mathbf{H}_{2} + \mathbf{H}^{+} \tag{3}$$

$$H_2 + H \rightarrow 3H$$
 (4)

If helium is present, small amounts of HeH+ and related compounds may form. But these, as well as H-, are not likely to be important. Evidently reactions (2) and (3) form a cycle and one proton may produce many hydrogen molecules. This cycle competes with hydrogen recombination and H2 dissociation, and it is necessary to turn to a detailed computation of their rivalry.

In a contracting cloud model, we begin with a spherical cloud of pure hydrogen at its moment of maximum expansion. We assume that its density is comparable with the average density of the universe at that time (an assumption which does not affect the important results seriously) and that its mass is sufficiently greater than the Jeans mass to enable free fall to prevail. The equations which determine the chemical composition are

$$\dot{N}_{+} = \frac{1}{V} N_{0} N_{2+} < \sigma v >_{2} - \frac{(N_{+} + N_{2+}) N_{+}}{V} < \sigma v >_{0} - \frac{N_{+} N_{0}}{V} < \sigma v >_{2+}$$
 (5)

$$\dot{N}_{2+} = \frac{1}{V} N_0 N_+ \langle \sigma v \rangle_{2+} - \frac{1}{V} N_0 N_{2+} \langle \sigma v \rangle_{2}$$
 (6)

$$\dot{N}_2 = \frac{1}{V} N_{2+} N_0 \langle \sigma v \rangle_2 - \frac{1}{V} N_2 N_0 \langle \sigma v \rangle_{\text{dissoc}}$$
 (7)

$$N_0 + N_+ + 2N_{2+} + 2N_2 = N \tag{8}$$

where N_+ , N_2^+ , N_2 , N_0 are the total numbers of particles of \mathbf{H}^+ , \mathbf{H}_2^+ , \mathbf{H}_2 , \mathbf{H} in a volume V, N is the total baryon number in V and the $\langle \sigma v \rangle$ s are rate coefficients for reactions (1) to (4). We also need the equations for energy conservation and the velocity of free fall

$$\left(\frac{3}{2}NkT\right)^{\bullet} = -P\dot{V} - \Lambda V = -NkT\frac{\dot{V}}{V} - \Lambda V \qquad (9)$$

$$(\dot{L})^2 = 2 GM \left(\frac{1}{L} - \frac{1}{L_0}\right) \tag{10}$$

where T is the temperature, k is the Boltzmann constant, P is the pressure and Λ is the radiation rate in erg cm⁻³ sec⁻¹. The total mass, initial radius and the time dependent radius of the sphere are M, L_0 and L, respectively. The pressure term in equation (9) indicates that the radiated energy is essentially the P d V work done by the collapsing cloud.

Three approximations promote simplicity with little loss of accuracy. First, although og is not accurately known, it most probably has a value of about 10-15 cm² which is typical of charge exchange reactions above threshold, and is therefore much greater than σ_{2+} . Thus an H; ion is converted to H, as soon as it is formed and the H₂ concentration never becomes large. As a result of this large cross-section and also the low electron concentration, dissociative recombination of H₂ is negligible compared with reaction (3). Second, dissociation of H₂ is negligible below about 6,000° K. We therefore omit it because—as will be found—the temperature does not rise sufficiently high for dissociation. Finally, nearly all particles will be in the form of hydrogen atoms. Thus we have $N_+ + N_{2+} \approx N_+$; $N_0 \approx N$; $N_{z+} + N_2 \approx N_2$ (after the short initial H_2^+ production). If we combine equations (5) and (6), (6) and (7) and change the notation, we have

$$\frac{\mathrm{d}m}{\mathrm{d}y} = -\frac{A - m^2}{\sqrt{g} \sqrt{y - 1}} \tag{11}$$

$$\frac{\mathrm{d}n}{\mathrm{d}y} = B \ m \ g^2 \frac{y^5}{\sqrt{y-1}} \tag{12}$$

$$\frac{\mathrm{d}g}{\mathrm{d}y} = -\frac{2}{3} \frac{t_f}{\mu_0 k T_0} \frac{\Lambda}{y^7 \sqrt{y-1}} \tag{13}$$

in which we define $m=(N_++N_{2+})/N\approx N_+/N$, $n=(N_{2+}+N_2)/N\approx N_2/N$, $T=T_0gy^2$, $y=L_0/L$, $A=\mu_0t_f<\sigma v>_0^0$, $B=\mu_0t_f<\sigma v>_0^2$, and $t_f=(8\pi Gm_p\mu_0/3)^{-\frac{1}{2}}=$ free fall time. The initial total number density and temperature at the start of free fall are μ_0 and T_0 , respectively, and $<\sigma v>^0$ denotes a rate constant at $T=T_0$. The rate constants are

$$\begin{aligned} &<\sigma v>_0\approx 6\cdot 3\times 10^{-11}~T^{-\frac{1}{2}}~{\rm cm^3/sec}~({\rm ref.}~10) \\ &<\sigma v>_{2+}\approx 5\times 10^{-24}~T^2~{\rm cm^3/sec}~({\rm ref.}~11) \end{aligned}$$

where T is in °K. Their temperature dependence has been inserted into equations (11) and (12). It is more convenient to use y than t for the independent variable. The function g is just the ratio of the actual temperature to the temperature the cloud would have had if its collapse were adiabatic.

Takayanagi and Nishimura¹² have calculated the radiation function Λ and we fit it to an approximate formula

$$\Lambda = 2.5 \times 10^{-31} T^{2.4} \mu_{H^{2/3}} \mu_{2} \text{ erg cm}^{-3} \text{ sec}^{-1}$$

where μ_H and μ_2 are the number of densities of H and H_s. This fit is accurate to about a factor of two in the range $500^{\circ} < T < 5,000^{\circ}$, $10 < \mu_H < 10^4$. Outside this range, it overestimates the cooling. Equation (13) now becomes

$$\frac{\mathrm{d}g}{\mathrm{d}y} = -1.67 \times 10^{-31} \frac{g^{2.4}y^{2.8}}{k\sqrt{y-1}} \mu^{2/3} t_f T^{1,4} n \tag{14}$$

It should be noted that these equations do not depend on the size of the cloud as long as it is large enough for the free fall approximation to be valid. In effect the only free parameters are the average matter density in the universe now, the initial number fraction of H⁺ and the red-shift when the collapse begins.

We have numerically integrated equations (11), (12) and (14) using the initial conditions y=1, n=0, g=1. The values of the parameters are $\rho_{\text{now}} = 2 \times 10^{-29}$ g/cm³, $m_{\text{initial}} = 10^{-5}$, $z_0 = 5$; z_0 is the value of red-shift when the collapse starts, $1 + z_0 = (t_{\text{now}}/t_0)^{2/3}$ where t_{now} is the present age of universe and t_0 is the age when collapse starts. Given z_0 , values of μ_0 and T_0 are found from the formulae for the background gas which expands adiabatically and conserves baryons: $T_0 = T_{\text{now}}(1 + z_0)^2$, $\mu_0 = \mu_{\text{now}}(1 + z_0)^3$ with $T_{\text{now}} = 3 \times 10^{-3}$ °K.

The results are shown in Fig. 1. To understand them qualitatively, return to equation (9) and integrate it to find

$$E_{(t)} \equiv \int_{t_0}^{t} \Lambda V dt = \frac{3}{2} Nk \left[T_{(t)} \log \frac{1}{g} + T_0 \int_{u_t}^{\mu(t)} \frac{dT}{d\mu_{\text{tot}}} \log g \ d\mu_{\text{tot}} \right] \tag{15}$$

where E(t) is the total energy emitted up to time t. We see that this depends explicitly only on T(t) and $\mu_{tot}(t)$, the total density at time t. A numerical computation for $z_0 = 20$ (that is, higher initial density) shows that T does not depend on the starting time, but only on μ_{tot} . This is reasonable because the radiation is essentially a consequence of the work done on the gas and this is appreciable only after contraction to a fairly high density. Similarly, we conclude that the energy radiated is essentially independent of the initial concentration of H+. For suppose the initial H+ concentration were less, then the cloud would contract more before forming H2, and its maximum temperature would be higher. Then the cloud once again would cool and, after the temperature had dropped to a few hundred degrees, the net work would be about the same as before. This argument breaks down,

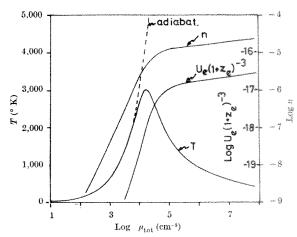


Fig. 1. The temperature, T, fraction of hydrogen, n, and the "invariant" energy density, $U_1(1+Z_c)^{-3}$, are plotted as a function of the instantaneous total density, μ_{-1} , of the collapsing cloud. The dashed extension of T gives the temperature if the cloud does not radiate. The initial conditions are $z_0=5$, $m_0=10^{-5}$, ρ_{210} , ν_{110} , $\nu_{110}=\nu_{110}$, $\nu_{110}=\nu_{110}=\nu_{110}$, as similar plot with $z_0=20$ differs from these curves by less than 10 per cent.

of course, when there is so little H+ that the cloud heats adiabatically and ionizes before appreciable H₂ can be

Now that we have found that H2 prevents the cloud from heating, we may ask if its radiation can be detected. From the total energy radiated by a single cloud, we want to find the average radiation flux striking a detector now. First, the average radiation energy density produced by these clouds is computed, assuming that all the matter of the universe is in clouds and collapses instantaneously. Then this is multiplied by the fraction of matter that actually collapses between t and t+dt. Then dt can be converted to a spread in wavelength by using the cosmological Doppler formula, and finally the result is multiplied by the speed of light to find a flux density between λ and $\lambda + d\lambda$.

All the matter in the universe, if it emits at a red-shift of z_e , produces an energy density

$$U_e = \frac{\mathcal{H}E}{V_u} (1 + z_e)^3 \tag{16}$$

where \mathcal{M} is the total number of clouds, V_n is the volume of the universe now and E is given by equation (15). Because most of the radiation comes out in a short burst (about 1 per cent of free fall time), E(t) is approximately constant after the density becomes greater than about 10⁵ cm⁻³, well past the peak of emission (see Fig. 1). It can be seen from equations (15) and (16) that $U_e(1+$ $(z_e)^{-3}$ is independent of (z_e) and is proportional to the present number density of particles. The value of this "constant", as determined from equation (15) and the results in Fig. 1, is

$$\frac{\mathcal{H}E}{V_B} \approx 1.5 \times 10^{-17} \text{ erg/cm}^3$$
 (17)

The flux between λ and $\lambda + d\lambda$ is then given by

$$F_{n\lambda} d\lambda_n = \frac{c}{4\pi} U_e (1 + z_e)^{-4} G_{(te)} dt_e \text{ erg cm}^{-2} \text{ sec}^{-1} \text{ ster}^{-1}$$
 (18)

where λ_n is the wavelength of interest now, $G_{(t_e)}dt_e$ is the fraction of clouds which emit between t_e and $t_e + dt_e$, and the factor $(1+z_e)^{-4}$ converts from emitted to present energy density. Next dt_e is converted to $d\lambda_n$ by the cosmological Doppler formula

$$\lambda_n = (1 + z_e)\lambda_e = \lambda_e (t_n/t_e)^{2/3}$$
(19)

$$|dt_e| = 1.5t_n \lambda_n^{-1} (1 + z_e)^{-1.5} d\lambda_n$$
 (20)

Finally, from equations (16), (17), (18) and (20) we obtain $\lambda_n F_{n\lambda} = 5 \times 10^{-8} t_n G_{(t_e)}(t_e/t_n)^{5/3} \text{ erg cm}^{-2} \text{ sec}^{-1} \text{ ster}^{-1}$ (21)

The radiation is emitted by collisionally excited rotational lines of H₂. At the temperatures given in Fig. 1, there are three strong lines: the $l=8\rightarrow6$, $6\rightarrow4$, and $4\rightarrow2$ transition lines with relative intensities of approximately 1:1:0.5. Their rest wavelengths are 4.4, 5.6 and 7.6μ , respectively. Actually there are also lines between these which correspond to the rotational transitions between odd l values for hydrogen in the ortho spin state; although Takayanagi and Nishimura considered only parahydrogen, including orthohydrogen, would not change the cooling rates or total radiation appreciably, but would distribute the energy over more lines. In our case, however, the cloud emission function $G_{(t_e)}$ would smear the lines into a con-Thus the relevant wavelength is a weighted average of the emitted lines which is about 5µ in the rest frame.

As a favourable numerical example, suppose that the clouds radiated when the age of the universe was between 1 and 2×10^9 years. Then using $G_{(t_e)} \approx 10^{-9} \text{ yr}^{-1}$ and $t_e \approx 1.5 \times 10^9 \text{ yr}$, $t_n = 10^{10} \text{ yr}$, $\rho_n = 2 \times 10^{-29} \text{ g/cm}^3$, we obtain $\lambda_n F_{n\lambda} = 2 \times 10^{-8} \text{ erg cm}^{-2} \text{ sec}^{-1} \text{ ster}^{-1}$. The peak is near $\lambda_n \approx 20\mu$ and has a full width of about 10μ . Total intensity is proportional to the baryon number of the universe. In

this calculation, the most uncertain quantity is G. Not enough is known about the physics of self gravitating gas to rule out possible collective effects which may increase G and $F_{n\lambda}$ substantially. It should be mentioned that there is some evidence, from the radio source counts¹³, for an appreciable decrease of radiation emission for $z \gtrsim 4$ $(t \approx 10^{\frac{1}{9}} \text{ yr})$. This could indicate that our value of G may not be overly optimistic.

Because of atmospheric absorption, there are no detailed observations of the 15-30µ background. Partridge and Peebles^{14,15} have estimated approximate upper limits to the chief background contributions in this band, from extragalactic Ne+ emission, metallic interstellar grains, and galactic free-free emission, to be about 2×10^{-6} , $4 \times$ 10^{-7} , and 2×10^{-7} erg cm⁻² sec⁻¹ ster⁻¹, respectively. Integrated starlight from the galaxy, perpendicular to the plane, may also be about 10⁻⁷. In addition, the integrated infrared radiation from distant galaxies may be comparable. These numbers are about a factor of ten to a hundred times larger than our reasonably optimistic estimate for H2 emission. But because they are so uncertain, the H₂ emission we have discussed could be an important contributor. If so, it could be distinguished from galactic radiation by its lack of angular dependence, and from Ne+ by its smoother spectrum. The shape of the H_2 spectrum is a direct measure of $G_{(t_0)}$, the cloud emission function, and we would not expect this to rise monotonically with time and then abruptly stop at some epoch, as would be required for the H2 spectrum to mock that of Ne+.

In conclusion, we find that if the clouds quickly contract to a density above about 104 particles/cm3, they will produce enough H₂ to cool very rapidly. Under optimum conditions it may be possible to detect this radiation. The further collapse, if spherical and not stopped by rotation or magnetic fields, continues until the optical depth becomes large. This does not occur until densities considerably greater than those necessary to produce H_z are reached. A massive cloud then continues to collapse until the H₂ dissociates and the hydrogen atoms ionize. Thus it faces two more cooling instabilities, both of which would promote fragmentation and further collapse. A small opaque cloud contracts gradually. In any case, at the high densities reached we would expect stars to form. This could produce enough ultraviolet radiation to ionize the remaining gas and expel it from the cloud. Although the resulting object probably resembles a globular cluster, we would not wish to maintain that this describes their mode of formation without further detailed

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Theory of Universal Primary Interactions

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This theory originates from observations that the theory of particle interactions becomes much simpler and more capable of correlating apparently unrelated phenomena if it is postulated that electromagnetism and weak interactions are not primary properties of the nucleons but are acquired by virtue of their interaction with vector and axial vector fields.

TEN years ago, Marshak and I analysed the then existing experimental data on weak interactions and concluded that all the experiments could not be consistently interpreted in terms of a general theoretical scheme¹. We singled out four crucial experiments which should be remeasured with different results if a satisfactory theory of weak interactions was to be developed. We also showed that the only possible universal theory would be a vectoraxial vector interaction. This was satisfying because the principle of chirality invariance for the interaction, already valid for electromagnetism, unambiguously led to this interaction. Almost immediately Feynman and Gell-Mann² developed the same theoretical scheme and re-introduced the notion of a conserved vector current3 to show why the vector coupling constant was not re-normalized. The chirality invariant theory, which we formulated a decade ago1,2, has been accepted as the theory of universal Fermi interaction. Even the small departure of the ratio of the vector and axial vector coupling constants from unity by a factor of approximately $\sqrt{(25/18)}$ can be computed on the basis of the original formulation of the theory4.

Several new experimental developments, however, make it desirable to re-examine the theoretical basis for the universal four-fermion interaction. Within the realm of weak interactions, it has been found that the leptonic decays of kaons and hyperons are slower, by a factor of about ten, than the predictions of the universal fourfermion interaction extended to strange particles. There is now unmistakable evidence for a small violation of CP invariance in weak interactions leading to the neutral long lived kaon. Among strong interactions, the existence of a whole collection of vector mesons has been experi-mentally established. These mesons are strongly coupled to baryons and their contribution to low energy pionnucleon scattering and the electromagnetic properties of the nucleons make them essential to an understanding of particle interactions. In fact, soon after the first vector meson resonances were experimentally identified, following a method suggested by us, Sakurai pointed out the qualitative explanation of some features of strong interactions based on the hypothesis of a universal vector meson coupling to hadrons (for discussion see refs. 7-10). Evidence has been accumulating for the validity of such a universal coupling. Finally, there has been some evidence for axial vector meson resonances, although they cannot yet be considered experimentally established.

Interaction Types and Families of Particles

In the present theory, I distinguish four families of particles and their associated fields. These four families have their characteristic interaction properties and are shown in Table 1.

The pseudoscalar and pseudovector mesons are both quanta of the same axial vector field, and may be written

$$A_{\lambda} = B_{\lambda} + \xi \frac{1}{m\pi} \partial_{\lambda} \varphi_{\pi}; \ \partial^{\lambda} B_{\lambda} = 0$$
 (1)

where φ_{π} is the pion field and B_{l} is the transverse part associated with the pseudovector particles. As a consequence

$$\partial^{\lambda} A_{\lambda} = \xi \cdot \frac{1}{m_{\pi}} \cdot \Box^{2} \varphi_{\pi} = -\xi \cdot m_{\pi} \cdot \varphi_{\pi}$$
 (2)

where the second member is strictly valid only when the pion is on the "mass-shell". On the other hand, the vector field has only a vector particle because it is required that

$$\partial^{\lambda} V_{\lambda} = 0 \tag{3}$$

The nucleon field is denoted by N and the electromagnetic field by a_{λ} . It is now possible to formulate the fundamental primary interactions. For simplicity, in the first instance, the strange particles are ignored.

(i) Electromagnetism

The electrons and muons are coupled to the Maxwell field, a_{λ} , according to the usual form

$$-e \left(\overline{\mu}\gamma_{\lambda} \mu + \overline{e} \gamma_{\lambda} e\right) a^{\lambda} \tag{4}$$

The nucleons are not directly coupled to the Maxwell field, but the neutral vector fields ρ and ω are coupled according to

$$- (e/g) (m_{\varrho}^2 \cdot \rho_{\lambda} + m_{\omega}^2 \cdot \omega_{\lambda})$$
 (5)

where g is a strong coupling constant, the value of which is specified later.

$(ii) \ Strong \ Interactions$

The leptons do not have any strong interactions. The strong interactions are completely specified by the Yukawa coupling

$$\frac{1}{2} g \overline{N} \left\{ \gamma^{\lambda} \mathbf{\tau} \cdot \mathbf{\rho}_{\lambda} + (g'/g) \sigma^{\lambda \nu} \stackrel{1}{=} \mathbf{\tau} \cdot \mathbf{\rho}_{\lambda \nu} \right.$$

$$+ (g_{0}/g) \gamma^{\lambda} \omega_{\lambda} + (g'_{00}/g) \sigma^{\lambda \nu} \stackrel{1}{=} \phi_{\lambda \nu}$$

$$+ (f/g) \gamma^{\lambda} \gamma_{5} \mathbf{\tau} \cdot \mathbf{A}_{\lambda} + (f'/g) \sigma^{\lambda \nu} \gamma_{5} \stackrel{1}{=} \mathbf{\tau} \cdot \mathbf{A}_{\lambda \nu}$$

$$+ (f_{0}/g) \gamma^{\lambda} \gamma_{5} E_{\lambda} + (f'_{00}/g) \sigma^{\lambda \nu} \gamma_{5} \stackrel{1}{=} D_{\lambda \nu} \right\} N$$
(6)

where the symbols stand for the respective vector and axial vector fields. By an argument related to the symmetry properties of the non-relativistic limit of this interaction¹¹ the ratios

$$(g'/g) = 5/3; (g'_{00}/g) = (g_{0}/g) = 1;$$

 $(f/g) = (f'/g) = (5/3\sqrt{2});$ (7)
 $(f_{0}/g) = (f'_{00}/g) = 1$

are derived.

Given these ratios, by virtue of the coupling of expression (5) the electric charge and magnetic moment of the proton and neutron can be calculated. It is found that the proton charge is +e and the neutron charge is zero. For the proton and neutron magnetic moments

$$(\mu_p/\mu_n) = -\{1 + (g/g') \ (m_g/m)\} = -1.49;$$

 $(\mu_p - \mu_n) = 1 + (g'/g) \ (2m/m_g) = 5.1$ (8)

are obtained.

The observed values are -1.46 and 4.7. The predicted and magnetic form factors will have a fall-off with momentum transfer governed by the vector meson mass. This is in qualitative agreement with experimental results.

(iii) Weak Interactions

The purely leptonic weak interactions are of the form

$$\frac{G}{\sqrt{2}} \left(\overline{6} \gamma_{\lambda} \left(1 + \gamma_{5} \right) \nu_{e} \right) \left(\overline{\mu} \gamma^{\lambda} \left(1 + \gamma_{5} \right) \nu_{\mu} \right)^{+} \tag{9}$$

with possible terms involving the electron covariant or the muon covariant quadratically. The hadrons do not couple directly to leptons or to each other. The hadron weak interactions are the consequence of the vectoraxial vector field coupling to lepton covariants. This interaction is

$$(-G/g) \cdot (m_{\varrho} \, \rho^{\lambda} + m_{A}^{2} \, A^{\lambda}) \, (\overline{\mu} \, \gamma_{\lambda} \, (1 + \gamma_{5}) \nu_{\mu} + \overline{\Theta} \, \gamma_{\lambda} \, (1 + \gamma_{5}) \nu_{e}) \quad (10)$$

By virtue of the strong interaction of expression (6), there is an effective nucleon–lepton four-fermion coupling. For small momentum transfers it can be approximated by the familiar beta decay interaction^{1,2}

$$\frac{G}{\sqrt{2}} \left(\overline{N} \gamma_{\lambda} \left(1 + g_{A} \gamma_{5} \right) \tau^{+} N \right) \left(\overline{\Theta} \gamma^{\lambda} \left(1 + \gamma_{5} \right) \nu_{e} \right) \tag{11}$$

Here the Fermi coupling constant is the same as the one occurring in the muon decay interaction in expression (9). According to expression (7) (for alternative derivation see refs. 13 and 14), the ratio of Gamow-Teller to Fermi interaction is

$$g_A = (-G_A/G_V) = (f/g) = (5/3\sqrt{2})$$
 (12)

The effective V-A four-fermion interaction is thus recovered as in the original formulation^{1,2} but with the correct ratio of the Gamow-Teller and Fermi coupling constants. When the momentum-dependent terms are considered the familiar induced pseudoscalar term¹⁵ and the weak magnetism term¹⁶ with the usual values are obtained.

Because of the electromagnetic coupling in expression (5), the fundamental principle of electric charge-current conservation demands that the neutral components of ρ and ω remain divergence-free. Thus no neutral lepton currents are expected to be present in weak interactions. None is, of course, found.

There is a degree of time reversal and CP violation in the strong interaction in expression (6) and thus in the effective nuclear beta decay interaction. This effect seems to be beyond the present experimental accuracy; in a typical beta transition the energy release is a fraction of an MeV. But even for 1 MeV energy release, the amplitude of the CP violating term is

$$(f'/f) (m_e/m_A) \simeq 10^{-3}$$
 (13)

of the normal (CP conserving) amplitude.

Using the observed value of the charged pion and muon lifetimes and by virtue of expressions (1) and (10), a relation for the absolute value of the parameter ξ and the

strong vector coupling constant g can be deduced. From expression (10), the effective pion decay interaction is

$$\frac{G\,m_A^2\,\xi}{g}\,\,\cdot\,\,\frac{1}{m_\pi}\,\,\cdot\,\,\partial^\lambda\varphi_\pi\,\,\overline{\mu}\,\,\gamma_\lambda\,(1+\gamma_5)\,\,\nu_\mu \eqno(14)$$

From the muon lifetime of 2.198×10^{-6} sec

$$G = 2.43 \times 10^{-7} \ m_{\pi}^{-2} \tag{15}$$

From the pion lifetime of 2.551×10^{-8} sec and expression (14)

$$(\xi/g) = 1.02 \times 10^{-2} \tag{16}$$

Using the divergence relation of equation (2) and the fact that the pion-nucleon coupling implied by expressions (1) and (6) is

$$\frac{1}{2}f\xi \, \overline{N} \, \gamma^{\lambda} \gamma_5 \, \partial_{\lambda} \, (\tau \cdot \varphi_{\pi}) \, N \tag{17}$$

which leads to¹⁵

$$\xi^2 = \frac{1}{2} (m_\pi/m_A)^2 = 8 \times 10^{-3} \tag{18}$$

These give

$$\xi = 0.09$$

$$g = 9.0 \tag{19}$$

The value g = 9.0 is in essential agreement with the phenomenology of strong interactions

Strange Particle Weak Interactions

For the leptonic weak decays of strange particles we extend the scheme by considering the charged strange vector and axial vector fields V_{λ} and A_{λ} on the same footing as the charged non-strange vector and axial vector fields V_{λ} and A_{λ} by the replacement

$$V_{\lambda} \to V_{\lambda} + V_{\lambda}$$

$$A_{\lambda} \to A_{\lambda} + A_{\lambda}$$
(20)

in the primary weak interaction coupling of expression (10). According to equation (1) this implies a coupling of the kaon to the charged lepton currents similar to expression (14). The ratio of kaon and pion transition rates can now be calculated to obtain

$$\frac{\Gamma\left(\mathsf{x}\to\mu\mathsf{v}\right)}{\Gamma\left(\pi\to\mu\mathsf{v}\right)} = \frac{m_\pi}{m_\mathsf{x}} \left[\frac{1-(m_\mu/m_\mathsf{x})^2}{1-(m_\mu/m_\pi)^2}\right]^2 \simeq 1.4$$

Using an experimental value of the pion lifetime and the value of the branching ratio $R=0.65\pm0.02$ for the two-body leptonic mode of the kaon, for the kaon lifetime

$$\tau(\mathbf{x}^+) \; = \; \frac{\Gamma \; (\pi \to \mu \nu)}{\Gamma \; (\mathbf{x} \to \mu \nu)} \; . \; R \; . \; \tau(\pi^+) \; = \; 1 \cdot 17 \times 10^{-8} \; \mathrm{sec}$$

is obtained which is to be compared with the experimental value⁵

$$\tau(\varkappa^+) = 1.22 \times 10^{-8} \sec$$

It is to be emphasized that this rate is predicted without making use of any new smallness parameters (contrast with ref. 18).

For the axial vector decays of hyperons it can be shown that, making use of the divergence relation of equation (2), the effective four-fermion interaction for the leptonic decays of baryons can be computed. It turns out that they are uniformly smaller than the nucleon beta decay coupling by a factor

$$\tan \theta_{\beta} = \frac{m_{\pi}}{m_{\pi}} \left(1 + \frac{M - m}{2m} \right)^{-1} \simeq 0.26$$
 (21)

where M/m is the ratio of the hyperon mass to the nucleon mass. If the requirement that there are no strange scalar particles is used it can be demanded that the strange vector fields be free of divergence

$$\partial^{\lambda} V_{\lambda} = 0$$

This implies, in turn, that (the leading terms in) the vector coupling between the baryons of different mass should vanish. Thus the vector decays of hyperons and the three-body leptonic decays of kaons both proceed by

the smaller momentum dependent couplings; it is not possible to make a simple prediction for the apparent suppression factors. Experimentally, the vector decays have an even smaller ratio to the vector beta decay coupling by a factor of 0.6 as compared with the ratio of the axial vector strengths.

Non-leptonic weak decays of baryons and mesons require a self-coupling of the vector-axial vector strange fields with the corresponding non-strange fields.

Implications for Strong Interactions

It is possible to use the present theory to compute the low energy pion-nucleon interactions (paper in preparation). The dominant contributions come from nucleon and nucleon resonance exchanges and the exchange of the vector meson. A simple calculation for the s-wave scattering lengths yields the values

$$a_1 = +0.160 \ m_{\pi}^{-1}; \ a_3 = -0.080 \ m_{\pi}^{-1}$$

in good agreement with the values obtained from experiment

$$a_1 = +0.183 \ m_{\pi}^{-1}; \ a_3 = -0.109 \ m_{\pi}^{-1}$$

Similarly, for the p-waves it can be predicted that

$$a_{11} = -0.115 \text{ m}_{\pi}^{-1}; \ a_{33} = +0.117 \ m_{\pi}^{-1}$$

 $a_{13} = a_{31} = \frac{1}{4} \ a_{11} = -0.029 \ m_{\pi}^{-1}$

These are in good agreement with the experimental values

$$\begin{array}{lll} a_{11} = -0.101 \ m_{\pi}^{-1}; \ a_{33} = +0.215 \ m_{\pi}^{-1}; \\ a_{13} = -0.029 \ m_{\pi}^{-1}; \ a_{31} = -0.038 \ m_{\pi}^{-1} \end{array}$$

For inelastic pion-nucleon resonance the present theory leads to the prediction that the I = 1/2 amplitude should be $\sqrt{10}$ times the I = 3/2 amplitude¹⁹. The experimental data have been analysed to yield20

$$(A_1/A_3) = + 3.34$$

in excellent agreement.

Mesons were suggested by Yukawa to account for nuclear forces and nuclear beta decay²¹. Within my present scheme there are many mesons, comprising the pseudoscalar, vector and pseudovector mesons. sequently, the nuclear forces which are obtained have three characteristic ranges; the largest range is the result of pion exchange, and it has been known for quite some time that the "tail" of the nuclear force is consistent with this picture. The vector and pseudovector mesons lead to shorter range potentials; but they also lead to spin-orbit forces which are essential to an understanding of the nucleon-nucleon interaction. Both in complex nuclei and for nucleon-nucleon scattering at higher energies

they were phenomenologically introduced with great

We have outlined here a theory of universal primary interactions of particles, including strong, electromagnetic and weak interactions. The most important idea is that the primary interactions of the baryons consist of the strong universal coupling to vector and axial vector fields only. Both electromagnetic and weak interactions of baryons are acquired characteristics. Thus this theory is the logical completion of the idea that the beta decay of the nucleon arose only by virtue of its coupling to the meson. Such diverse items of particle phenomenology such as nuclear magnetic moments, ratio of the Gamow Teller and Fermi coupling constants, weak magnetism, absence of neutral lepton currents, apparent suppression of strange particle leptonic decays, pion-nucleon scattering lengths and the salient features of the nuclear force are quantitatively accounted for by present theory. Such an array of satisfactory predictions leads us to study this theory seriously. A more detailed account of this theory will be published elsewhere.

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Role of Carotenoids in Photosynthesis of Green Plants

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In contrast to other hypotheses, \beta-carotene is responsible for the photic reduction of triphosphopyridine nucleotide during the photosynthetic cycle of electron transfer and energy conversion in green plants.

According to current hypotheses three main systems can be distinguished in the photosynthetic cycle of electron transfer and energy conversion in green plants. illumination, pigment system III, which contains chlorophyll, carotene and possibly accessory pigments, expels electrons to the reducing system (I; pigments-ferredoxin -TPN). The oxidizing system (II; eytochromes f, b and b_3) then returns the electrons to the pigments. Photophosphorylation and oxygen production are probably largely conducted by system II. The electrons lost from cytochrome f are retrieved from the reducing system by way of a flavoprotein1. It can be shown that flavoprotein and cytochromes are operating synchronously and that the amount of reduced pyridine nucleotide (TPNH) decreases on illumination with red light which, however, primarily stimulates system II. In blue-violet light which, on the other hand, stimulates system I, the level of reduction and the amount of TPNH increase. The existence of two pathways, one which photically oxidizes cytochrome f and another which reduces the cytochromes again in the dark, was described in my first communication on the oxidation of cytochrome f by light². Recent experiments have shown that added TPNH can also be oxidized during the restoration of the reduced state of the cytochromes^{3,4} and that the reducing and oxidizing systems are conducted by two separate light reactions. This theory was earlier adopted by others also.

Many authors believe that chlorophyll is directly involved in both reactions, but this hypothesis does not explain the actual difference in their action spectra. Chlorophyll has strong bands of absorption in both the blue-violet and red regions, and experiments with added oxidants, for example, potassium ferricyanide, show a fast rate of oxygen production in both cases. Blue-violet light, however, does increase the production of TPNH more than red light. This fact proves that reactions I

and II are conducted by different pigments.

On the basis of these facts and the results of other experiments on the effect of light of chloroplast carotenoids, I have concluded that reaction I involves excitation of β -carotene and transfer of electrons to ferredoxin-TPN5.6. β -Carotene is oxidized to xanthophyll, as shown by direct determination of the ratio c (carotene)/x (xanthophyll) before and after illumination. With short exposure times of 5–10 min the total amount of carotenoids (c+x) remains fairly constant: the pigment system reduces xanthophyll through system II. This was also shown by Costes? During prolonged illumination, carotenoids may be lost, probably because the electrons coming from system I are trapped in the added oxidant during impaired reduction of the pigments.

Chloroplasts from leaves of spinach were isolated in 0.3 molar sodium chloride + 0.02 molar potassium hydrogen phosphate at pH 8.2. Photosynthetic production of oxygen was determined by means of rapid polarography ('Polarecord', Metrohm, Switzerland). The chloroplast suspension was kept in small plast tubes (about 12×20 mm) which were fitted with rubber stoppers. During illumination the tubes were placed in a cuvette with a quartz window and cooled to 1°-3° C by means of a The tubes were centrifuged before the quantity of oxygen was determined. Illumination was provided by a small 250 W projector, fitted with heat absorbing filters and Schott colour filters or interference filters. Experiments were also performed using a high intensity Bausch and Lomb monochromator and tungsten or xenon lamps.

In vitro experiments were performed using a solution of purified ferredoxin from spinach (by courtesy of Professor D. I. Arnon, Berkeley) containing 1 mg/ml. chlorophyll and a somewhat larger quantity of TPN (Sigma). β -Carotene (7 mg/ml., Sigma, type 4) was added in the form of a fine crystal powder and the mixture was exposed to a stream of bubbling nitrogen. The quartz cuvette (10×40 mm) was illuminated by the projector at room tempera-

ture.

Ferredoxin and TPN reductions were recorded spectrophotometrically. In the oxidized state, ferredoxin from spinach has a high band at about 420 nm and a lower band at about 460 nm. These bands disappear during reduction. Determinations of the band heights are hampered by the fact that the background of the whole spectrum rises from blue—green to ultraviolet. The measurements were therefore made from a straight line drawn between 400 and 500 nm. For measurement of the 340 nm band of reduced TPN, a base line was drawn between 300 and 380 nm.

Measurements of rapid reactions of systems I and II were made by an oscillographically recording scanning

spectrophotometer. Observations were made 20 msec after an electric flash^{1,8} and compared with the values just before the flash. As I have shown³, the photic response is extremely rapid (considerably below 1 msec) but the effect on the enzymes (cytochromes, ferredoxin—TPN) lasts longer because dark reactions proceed more slowly.

Action of oxidants. To interpret accurately experimental results obtained with isolated chloroplasts, it is important to realize that these chloroplasts are cut off from any co-operation with the protoplasm and cannot respire. Direct comparisons with the photosynthesis of whole leaves or whole green cells, such as by determination of the action spectrum, must therefore be made with Chloroplasts are suspended in a limited great care. quantity of medium and are provided with sufficient quantities of chlorophyll, cytochromes, ferredoxin and TPN for intense photosynthesis during 5-10 min without any visible bleaching of the chlorophyll and scarcely any loss of carotenoids. The ratio c/x is decreased but c+xremains nearly constant. For efficient oxygen production, an extraneous oxidant must be added, but, in my own experience, no extra ferredoxin is needed. Addition of ferredoxin or any other reductone of high negative potential, for example, viologen, has no special effect on unwashed chloroplasts. This means that the photolysis of water and oxygen production is coupled to the positive side of the chain, that is, the cytochromes. Hill10 showed that ferricyanide (about +0.4 V) oxidizes cytochrome fand Table 1 and Fig. 1 show that the oxidation of cytochrome f increases with rising concentration of ferricyanide, and oxygen production concomitantly is raised. Simultaneous determination of reduced ferricyanide in red light yields the theoretical value

$$\frac{\text{mole ferricyanide}}{\text{moles } O_2} = 4.$$

These experiments show that reduced ferricyanide is not re-oxidized by chlorophyll: the electrons arriving from system I (or TPNH) are thus trapped in the extraneous oxidant. This means that the chlorophyll cannot deliver new quantities of electrons for reduction of the oxidized β-carotene; a fact that during prolonged illumination (more than 10 min) leads to retarded photosynthesis and probably contributes to the loss of carotenoids by oxidation.

It follows from these observations that the transition,

cytochrome $f^{\underline{e}}$ chlorophyll, is one of the most sensitive points in the cycle. An illustration of this sensitivity is the earlier observed fact that, during increased mechanical or sonic disintegration of the chloroplasts, linkage of the cytochromes with the other components of the stroma is the first to be broken. That electrons nevertheless may pass over from cytochrome f to chlorophyll is shown during very strong illumination, for example, by an electric flash. The experiments also show that, at an extra excitation of system I, for example, by blue light, the ratio ferricyanide/oxygen decreases to less than 4, obviously because some electrons are now bypassing the

Table 1. EFFECT OF POTASSIUM FERRICYANIDE ON THE OXIDATION OF CYTOCHROME f BY AN ELECTRIC FLASH

Red flashes (Sch Drops of potassium ferricyanide	$\begin{array}{c} \text{ott } RG \text{ 2)} \\ \text{Oxidation of} \\ \text{cytochrome } f \end{array}$
None	- 7.8
1	- 9.7
2 5	- 13·0 - 17·3
Blue flashes (filt	
None	+55
1	+ 43
2	+ 5
2 5	0

Differences are between measurements made 100 msec before and 100 msec after the flash. The chlorophyll content was 0-04 mg/ml, and 0-1 molar potassium ferricyanide was added in drops to 3 ml, of suspension.

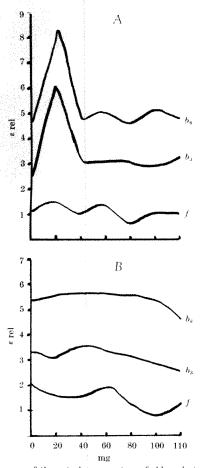


Fig. 1. Response of the cytochrome system of chloroplasts to a blue electric flash (filter BG 12; 0-1 msec). A, Suspension containing 0-3 molar sodium chloride +0-02 molar potassium hydrogen phosphate without extra additions. B. The same with addition of five drops of 0-1 molar ferricyanide in 4 ml.

extraneous oxidant. Weak oxygen production may occasionally also occur without addition of ferricyanide.

According to the circumstances mentioned, photosynthetic production of oxygen by isolated chloroplasts depends not only on the oxidant but also on the ability of system I to deliver electrons. This means that the stream of electrons to system II will be more rapidly exhausted in red than in blue light. A comparison between oxygen production during the first 10 min and during 30 min shows a much more pronounced decline under red light (Table 2). Green light, which is relatively less absorbed by chlorophyll than by β-carotene, supports photosynthesis for nearly as long as blue light.

A suspension of unwashed chloroplasts kept in the dark contains a certain quantity of TPNH which, because of its solubility in water, leaks out into the medium, even if some of it is presumably bound to the structure (or structurally linked enzymes). Table 3 shows that during illumination by red light the quantity decreases, whereas Table 2. RETARDATION OF PHOTOSYNTHETIC OXYGEN PRODUCTION DURING PROJONNED EXPOSURE TO DIFFERENT REGIONS OF THE SPECTRUM

Colour filtar	Ratio 30 min exposure	
Colour filter	10 min exposure	è
RG/2	1.2	
VG 9	2.5	
BG 12	2.6	

Table 3. CONSUMPTION OR PRODUCTION OF REDUCED PYRIDINE NUCLEOTIDE IN CHLOROPLASTS DURING ILLUMINATION USING DIFFERENT COLOUR FILTERS

		Colour filters	
Start (dark)	RG 2	VG 9	BG 12
+22.0 Loss or gain	+ 9.3	+18.3	+ 32.0
Loss or gain	-58 per cent	- 17 per cent	+45 per cent

Suspensions were illuminated in 2.6 ml, plast tubes at 1°-2° C; chloroplasts were removed by centrifugation and the spectrum of the medium was determined between 300 and 380 nm; values expressed in $\epsilon \times 10^{-2}$; exposure was for 20 min. The values are corrected for equal absorption.

it increases in blue light. This is a good illustration of the existence of two separate light reactions; blue light conducting system I and red light conducting the oxidation of TPNH through system II.

Time course of the excitations. An electric flash lasts for only about 0.1 msec, but, for quantitative oscillographic measurements, at least a few msec are needed (the disturbing higher frequencies must be cut off by damping). Continuous recording of the immediate effect and the after effect of a flash shows that the instantaneous oxidation of cytochrome f (at 554 nm) and reduction of TPN (at 340 nm) is followed by an after effect lasting a maximum of up to 100-150 msec before the original 75 per cent reduction of cytochrome f is restored (see Figs. 1A and 2). The time course of the after effect of a flash varies with the conditions of the sample, its age, degree of washing, etc. Figs. 1 and 2 show examples of a rapid and a slower wave of the process. That not only oxidation but also reduction of cytochrome f by one flash can be measured depends on the fact that the cytochromes in the dark are usually only reduced to about 60-70 per cent, not to 100 per cent as erroneously claimed by some authors. As shown in Fig. 2, the time course of cytochrome f oxidation is dependent on the colour of the flash. The optimal effect of red light—that is, exidation of cytochrome f—is attained at about 140 msec, whereas the effect of blue light, namely, reduction of cytochrome f. is maximal at about 200 msec.

The comparatively slow development of the after effect of a flash illustrates the large difference in time course between the photic excitation and the enzyme reactions (compare ref. 9). This gives the impression that the initial photic excitation is temporarily stored by some intermediate factor. The different effects of blue and rod light point either to a slower transference between β-carotene and ferredoxin in system I or to a damping influence of the chain of enzymes which transfers electrons from system I to system II. The chain of oxidation reduction enzymes acts in all circumstances as a pacemaker of the photosynthesis. This, of course, has nothing to do with the fact that, at moderate light intensity, there is a linear relation between light and photosynthesis, because the transference of the initial excitation to the chain of enzymes and the activity of these now run at the same speed.

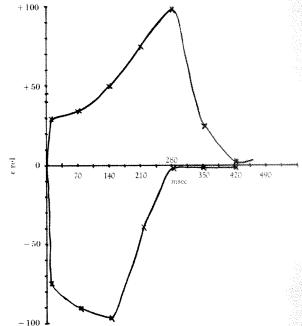


Fig. 2. Response of cytochrome f to an electric flash. Chloroplast suspensions without extra additions. Upper curve, blue flash (filter BG 12). Lower curve, red flash (filter RG 2). Ground light FG 9.

In vitro Reduction of Ferredoxin-TPN by \$\beta\$-Carotene

As shown before, β -carotene in the form of insoluble crystals or dissolved in chloroform acts as a photoreductone for ferredoxin even if the latter is in aqueous solution. Transfer of electrons occurs at the contact surface. The first experiments were carried out during illumination in blue light (BG 12 or 25) and the mixture was exposed to a stream of vigorously bubbling nitrogen^{5,6}. The reduced ferredoxin reduces TPN. This model experiment strongly supports the hypothesis that β -carotene is the photoactive factor of system I. To elucidate the action spectrum of the process different model experiments were carried out in green and red light. The results are shown in Fig. 3.

The new observations corroborate the previous results, namely, strong reduction of ferredoxin and a clear formation of TPNH in blue light. They also show a significant positive effect of green light and an effect of red light (>625 nm) which amounts to about 30-50 per cent of that in blue light. Even in the dark some reduction can be observed, but these results are not yet significant.

Careful examination of the absorption spectrum of β-carotene shows no absorption band in red but a 10–15 per cent absorption was observed in infrared and chloroplasts show a measurable scattering of the light which can also be developed in a suspension of very small crystals. On these grounds, there is no reason for denying the possibility of a limited photic excitation also in the red region. Previous investigations (see Fig. 3) also showed that red light has significant effects on β-carotene in solution, notably a characteristic negative peak at about 510 nm. This peak is considerably more pronounced after illumination with blue light.

Influence of pre-illumination on oxygen production. Direct polarographic measurements of oxygen were made in the cooled chloroplast suspension. As shown in Table 4, blue light (450 nm) with a photosynthetic effect of 21–26 (measured as the deflexion of the polarographic response to oxygen) stimulates the effect of the subsequent exposure to 680 nm (the top of the red absorption band) from 17–26 to 34, whereas the effect of 450 nm following 680 nm is a strong inhibition, from 21–26 to only 7. The effect of pre-illumination with 550 nm (56–57) on 680 nm is less significant (from 17–26 to 17), whereas the effect of 680 nm on 550 nm is a retardation from 56–57 to 41. Still stronger is the retarding effect of 450 nm on 550 nm (decreasing from 56–57 to 23) whereas 550 nm seemed to have no effect on 450 nm.

The flash experiments showed that red light stimulates system Π but retards system I, resulting in an increase of the level of oxidation in the whole cycle and a shortage of TPNH produced. Blue light (450 nm) has the reverse effect, that is, it increases the level of reduction in the cycle and concomitantly facilitates a following activity of system II. Green light is characterized by a high quantum efficiency¹ and stimulates both systems I and II. System I is probably stimulated as a result of absorption by β-carotene in green light and system II is possibly stimulated by means of the cytochromes. The influence of a pre-illumination with green light on the effects of 450 and 680 nm is less significant.

The role of carotenoids in photosynthesis is illustrated by the direct observation of reduction of ferredoxin-TPN

Table 4. EFFECT OF PRE-ILLUMINATION WITH DIFFERENT COLOURS Exposure 1 Exposure 2

450 nm $O_2 = 21$ 680 nm $O_2 = 34$ 680 nm $O_2 = 7$

EXDO	sure i	***************************************		
450 nm 680 nm 550 nm 680 nm 450 nm	$O_{2} = 21$ $O_{2} = 17$ $O_{2} = 57$ $O_{2} = 26$ $O_{2} = 26$ $O_{3} = 56$	680 nm 450 nm 680 nm 550 nm 550 nm 450 nm	$O_{2} = 34$ $O_{2} = 7$ $O_{3} = 17$ $O_{2} = 41$ $O_{2} = 23$ $O_{3} = 19$	
SEA WY				

Figures refer to the deflexion in mm of the polarographic response to oxygen at 10^{-8} amp (mean of deflexions at 0^{-6} and 1^{-2} V). Concentration of chlorophyll was $0^{-0}4^{-0}06$ mg/ml. and 12 drops of 0^{-1} molar potassium ferricyanide were added to 10 ml. of this chloroplast suspension. Exposure time was 10 min at 1^{-2} ° C. Illumination was by monochromator and xenon lamp. Exposure 2 immediately follows exposure 1.

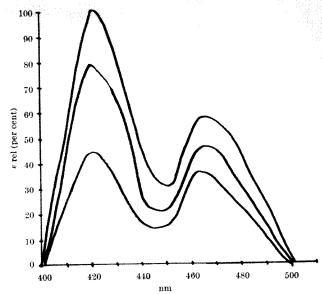


Fig. 3. Model experiment with β -carotene, ferredoxin and TPN (see text). Spectrum of ferredoxin measured from a straight line between 400 and 500 nm. Upper curve, fully oxidized ferredoxin. Middle curve drawn after illumination for 20 min with red light (filter RG 2). Lower curve, illumination with blue light (filter BG 12). See text for description.

in vitro, by the appearance of a negative band at 500–520 nm in chloroplasts and in illuminated β -carotene by the synchronous decrease of the ratio c/x with the rate of photosynthesis, and by the fact that all these reactions are primarily sensitive to blue light. These results support more strongly my hypothesis that, in system I, β -carotene is responsible for the photic reduction of TPN, than the hypothesis that chlorophyll is directly responsible, not only for system II but also for system I. That chlorophyll is reduced by system II is shown by other experimental results and it is likely that reduced chlorophyll when illuminated reduces xanthophyll to β -carotene and thus completes the cycle. Our experiments on changes in the state of oxidation-reduction of the cycle caused by illumination with different colours illustrate the separate activities of systems I and II.

The extreme reactivity of β-carotene^{7,12}, and the fact that isolated chloroplasts are exposed to external influences much more than are those in the intact cell, could cause reactions which lie outside the photosynthetic cycle, for example, oxidative loss of carotene during oxygenation or prolonged illumination⁸.

Loss of total carotenoids during illumination of isolated chloroplasts starts with exposures lasting longer than 10 min in strong light but is observed chiefly in white or red light. Blue light, however, counteracts the losses (Table 3). In addition to this photic consumption of carotene there may be an irreversible oxidation in the dark during oxygenation. It could be experimentally shown that the decrease in the ratio c/x is observed independently of photophosphorylation and photic oxygen production—more facts which support the concept of separate activity of the systems I and II.

This detailed experimental analysis of the conditions and activities in isolated chloroplasts clearly shows the irrelevance of certain objections against the thesis of β -carotene as an active promoter of system I. The fact that during shorter periods strong photosynthetic oxygen production proceeds in red light is satisfactorily explained by an independent activity of system II at the expense of stored TPNH, and a moderate activity of system I also in red light. The rash conclusion that red light does not affect β -carotene is at variance with our direct observations in connexion with the model experiments. Oxygen production of illuminated chloroplasts is furthermore chiefly a property of system II and its supply of electrons from TPNH—a certain quantity of which is present in

the chloroplasts from the start. Its consumption during prolonged illumination with red light is shown both directly and indirectly (Tables 1 and 3).

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Triads in Foetal Skeletal Muscle

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Departments of Physiology and Pathology, University of Louisville School of Medicine In the gastrocnemius muscle of the rat foetus longitudinally oriented triads were often seen near A-I junctions. This suggests that there is a change from longitudinal to transverse orientation during the development of skeletal muscle.

In vertebrate skeletal muscle a triad contains a central element formed from the T system (T) and two lateral elements formed from the terminal segments of the sarcoplasmic reticulum (SR). The T system is continuous with the sarcolemma at the Z level of the line in certain fish1 and frog 2 muscle and at the level of the A-I junction in rat^3 muscle. The triads are located at the Z level of the line in fish and frog muscle and near the A-I junction in rat muscle. Virtually all the triads in vertebrate skeletal muscle of adult animals are oriented transversely, with a few sparsely distributed longitudinal connexions between the transversely oriented \check{T} system⁴. A notable exception is the observation⁵ that triads are frequently oriented longitudinally at the level of the Z line in slow fibres of frog muscle. The space between apposed mem-branes of T and SR in transverse and in longitudinal triads is remarkably constant and is about 100 Å. Revel⁴ described rather regular periodicity of dense bridges traversing this space in bat muscle fibres. Similar dense bridges were reported3 for rat muscle fibres and evidence that these bridges are connexions between T and SR has been presented^{6,7}. The terminal segments of the sarcoplasmic reticulum are frequently called cisternae, but several investigations^{2,4,8-10} have noted electron-opaque material in SR. Structures resembling a membrane within SR have been reported11 for rat muscle. In this article the abbreviation SR applies specifically to the lateral elements of the triad.

In a recent electron microscope study of the morphogenesis of rat skeletal muscle, sarcoplasmic reticulum between the developing fibrils was observed in 16.5-20 day rat foetuses, but no triads were reported12. In lightmicroscope investigations on newborn mice and foetal guinea-pigs, Verattii13 found that the mesh of reticulum is oriented predominantly in the direction of the longitudinal axis of the fibre and rarely in the transverse direction. He observed a regular reticulum, transversely oriented, in fibres from adult mice13. The contrast between the appearance under the light microscope of muscle fibre reticulum of newborn mice and of adult mice suggested that transversely oriented triads typical of adult muscle are rare in foetal muscle. microscopy of skeletal muscle fibres from foetal rats has shown that triads are relatively scarce, with more triads oriented longitudinally than transversely.

The age of rat foetuses was determined by the detection of sperm in the vaginal smear of the mother. The observa-

tions were made on eight 19 day foetuses. Results were compared with those of many previous electron microscope examinations of adult rat muscle fibres. In the case of rat foetuses the entire gastroenemius muscle was fixed, but with adult animals small bundles of fibres were removed from the gastrocnemius muscle of animals anaesthetized with 'Nembutal'. The bundles of fibres were tied to 'Plexiglas' stays and fixed in glutaraldehyde by a slightly modified version of the method of Sabatini et al.14. The tissues were post-fixed in osmium tetroxide by the Palade method, as modified by Caulfield 15. fibres were stained with uranyl acetate during dehydration with alcohol, embedded in 'Maraglas' and sectioned with an LKB microtome. The sections were triple stained with lead, uranyl acetate and lead. A modification of the Reynolds method 16 was used for lead staining. Sections exhibiting grey interference colours were examined with a Siemens 'Elmiskop IA' electron microscope.

Triads are quite easily identified in adult rat muscle fibres because they are almost invariably found transversely oriented between the fibrils near the junctions of the A and I bands. In longitudinal sections, through the centres of two neighbouring fibrils, a cross-section of a triad can be seen. In a longitudinal section of a fibre, with the plane of section passing along the face of a fibril, a longitudinal section of a triad can be seen (Fig. 1). Location and identification of triads in foetal muscle are difficult for several reasons. First, the fibres are small and fragile. Second, the interfibrillar spaces are relatively large and they contain a thick distribution of electronopaque granules. Third, the triads are usually oriented longitudinally. When they are thus oriented they apparently do not encircle the fibrils. Fourth, transverse triads are rare and longitudinal triads are sparsely distributed. There are three structural characteristics of triads in rat muscle fibres that can be seen equally well in transversely and longitudinally oriented triads. These characteristics are: (a) the approximate 100 Å between apposed membranes of T and SR; (b) dense bridges across this space; and (c) electron-opaque material within SR which contrasts rather sharply with the less opaque appearance of material within T. In Fig. 2 are shown the most unusual findings in foetal muscle fibres, that is, a transversely oriented triad. The triad in the upper right side (star) is shown in cross-section. The extent of the transverse orientation around the circumference of the fibril is in doubt because a section of a triad on the other side of the

fibril is not seen. In the lower left side of Fig. 2 an approximate longitudinal section of a transversely oriented triad (asterisk) is seen, and a segment of considerable length is shown in transverse orientation. It is interesting that the transversely oriented triads are always found near the level of the A-I junctions. What is more striking, however, is the observation that the longitudinally oriented triads are also near the A-I junctions (Fig. 3). Triads thus oriented might be expected to be distributed randomly along the interfibrillar space with no specific location in the sarcomere. It is true that the location of a triad in relation to the neighbouring fibril cannot always be definitely established. Spurious appearances may be created by distortion of alignment of fibrils in juxtaposition, and an example of such distortion is shown in Fig. 4. If the position of the triad (star) is judged in relation to the fibril in the left side of the figure, it seems to be near the M line. On the other hand, the triad considered in relation to the fibril near it in the right side of the figure is near the A-I junction. The inset in the upper right side of Fig. 4 shows a high magnification of the triad that reveals three characteristics of triads used for identification. The approximate 100 Å spaces (vertical arrows) between T and SR are visible on both sides of T. Dense bridges across the space on the left side of T can be seen by close inspection. The difficulties encountered in attempting to find several triads in a single section are illustrated in Fig. 5. There are two structures which seem certain to be triads (stars). The structures designated by asterisks are judged to be triads. This judgment is supported by the locations of the questionable structures

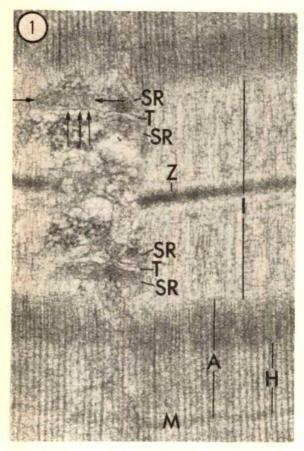
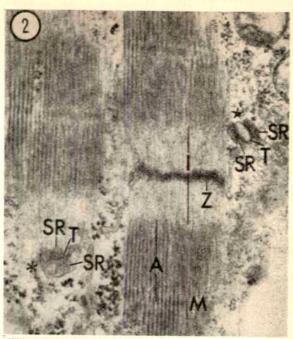


Fig. 1. Electron micrograph of adult rat gastrocnemius muscle fibre showing two transversely oriented triads in a longitudinal section of the fibre. The vertical arrows are directed toward dense bridges across the space between a terminal segment of the sarcoplasmic reticulum (SR) and the T system (T). The horizontal arrows are directed toward membrane-like structures within SR. Z represents the Z line, M the M line, M line, M line, M line, M line, M line M line



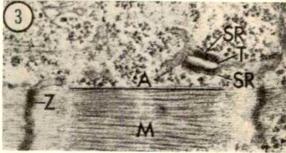


Fig. 2. Longitudinal section of a 19 day foetal rat gastrocnemius muscle fibre showing a cross-section of a triad (star) and a transversely oriented triad (asterisk). Other labels as in Fig. 1. $(\times c.34,000.)$

Fig. 3. Longitudinal section of a foetal muscle fibre showing a triad that appears to be longitudinally oriented. Note the intense staining of SR. (\times c. 32,000.)

near the $A{-}I$ junctions. Fig. 5 also illustrates the complex system of sarcotubules (ST) found in the interfibrillar spaces of rat foetal muscle. In spite of the thick distribution of dense granules the interlaced tubules can be seen in some places. It is clear that the two membranous systems (T system and sarcoplasmic reticulum) cannot be distinguished in the maze of tubules labelled as sarcotubules. In fibres from adult rats (Fig. 1) the T system is confined almost entirely to its transverse orientation in the triads near the $A{-}I$ junctions. It is obvious from examination of extensions of the T system beyond longitudinal triads (arrow in Fig. 5) that such confinement does not exist in rat foetal muscle fibres.

The use of location near the A–I junctions to aid interpretation of electron-opaque structures as triads is illustrated in Fig. 6. All the structures indicated by stars occupy similar positions relative to the A–I junctions of the fibril in the left side of Fig. 6. The second structure from the top shows the central element (T) and one lateral element (SR) as they might be seen in an unlateral element (SR) as they might be seen in an uncated by stars show electron-opaque material comparable with that seen in lateral elements of triads. Whether the absence of one or more components of the triad is peculiar to this unfavourable section or is a result of incomplete development is not known. In Figs. 5 and 6 the findings that longitudinally oriented triads are not

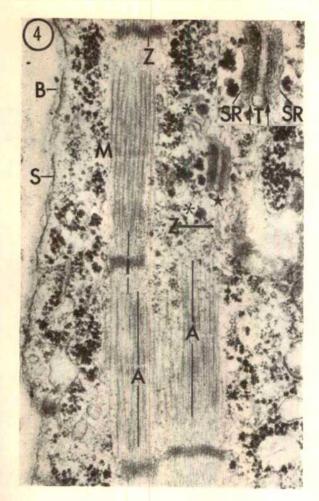


Fig. 4. Longitudinal section of a foetal fibre showing a triad (star) oriented longitudinally. The heavy horizontal line indicates the position of the Z line for the fibril near the triad. Arrows are directed toward spaces between T and SR in the inset of the triad in the upper right side of the figure. Asterisks are placed on the left side of latex spheres about 880 Å in diameter. B. Basement membrane; S, sarcolemma. (\times e, 32,000; inset, \times e, 51,000.)

located at the level of the Z line of the sarcomere are shown. Characteristics of typical fibrils in the 19 day foetal muscle fibre are illustrated in Fig. 6. The average diameter of the largest fibrils in foetal fibres is about equal to the average diameter of fibrils in adult rat muscle fibres. It is interesting that fibrils may attain full size in the 19 day foetus, while the diameter of the fibre is only a fraction of that in the adult. The average fibre diameter is about 10μ in the 19 day foetus, and $40-50\mu$ in the adult. The length of the A band in foetal muscle is equal to that in the adult, even in the thinnest fibrils observed. The M lines are sharply defined and well developed.

We tried to find continuity of the T system with the sarcolemma and extracellular fluid in foetal fibres. Invaginations of the sarcolemma near the level of the A-I junction were found at a few points, but we saw no connexions of these invaginated membranes with lateral elements of triads. In one case, a continuity of the sarcolemma with the membranes of a T system tubule was observed (Fig. 7). Although no opening from T to the extracellular fluid is shown, it seems probable that a more favourable section would have revealed one. Investigations^{3,6,17} of adult rat muscle fibres employing methods of preparation similar to those described here have shown narrow openings through the sarcolemma into enlarged tubules of the T system near the level of the A-I junction. In the adult the subsarcolemmal triads are transversely

oriented. It can be seen in Fig. 7 that the plane of apposition between T and SR is oriented longitudinally Opacity at the site of apposition is very intense and might account for failure to see the characteristic space between apposed membranes of T and SR. On the other hand, an unfavourable plane of section might explain the failure. Nevertheless, the electron-opaque material in the lateral element (SR) and its virtual absence in the central element (T) seem to warrant the conclusion that two of the components of a triad are shown in Fig. 7. It is difficult to make good preparations of membrane-like structures in lateral elements of triads in foetal muscle fibres. The membrane-like structures as shown within SR of adult rat fibres (Fig. 1) have not been clearly demonstrated for foetal rats. The electron-opaque material within SR of fibres from foetal rats, however, might be homologous with the membrane-like structures observed in adult rats11.

These observations pose two questions of particular interest. The first concerns development of the triad; the second excitation–contraction coupling. The prevalence of longitudinally oriented triads and their frequent location near the A–I junctions in skeletal muscle fibres of foetal rats are striking and unexpected findings. So far, observations can only suggest that there is a transition of triad orientation from the longitudinal to the transverse direction during development of rat muscle. This idea is supported by the similarity of sarcolemmal and T system connexions in adult and foetal rats. It is obvious that triad development should be studied in the muscle fibres of newborn and young

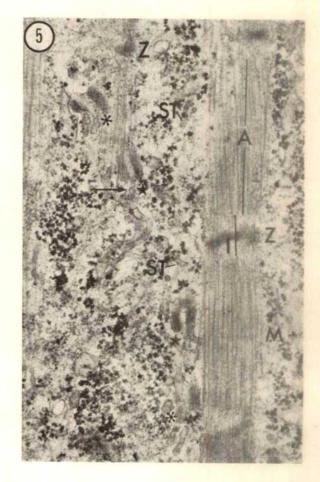


Fig. 5. Longitudinal section of foetal fibre showing triads (stars) and apparent triads (asterisks) oriented longitudinally and located near A-I junctions. The arrow is directed toward an extension of T. ST, Sarcotubules. (x c, 32,000.)

growing rats, and more information about the distribution of the T system would be useful in connexion with these studies. The T system might be traced with ferritin particles2,5, "calcium staining"4 or with potassium pyroantimonate used for localization of sodium18.

The suggestion that the triads are related to inward conduction of excitation from the fibre surface has been made¹⁹ and substantially supported^{20,21}. The T system has been specifically implicated in inward conduction of excitation by virtue of its observed connexions with the sarcolemma¹⁻³. On the other hand, lateral elements of the triads have been suggested to take part in the excitation-contraction coupling aspect of muscle function. Small quantities of calcium ions placed on exposed fibrils are known to induce contraction²². Observations^{23,24} that frog skeletal muscle fibres treated with oxalate show precipitation of calcium ions chiefly in the lateral elements of triads support the idea that these ions are accumulated in these elements. It has been suggested that accumulated calcium ions are released from the lateral elements by inward conduction of excitation along the central element (T system). In the light of various findings and suggestions it seems reasonable to suppose that changes in the structure of triads during the development of muscle fibres might be associated with changes in latent period,

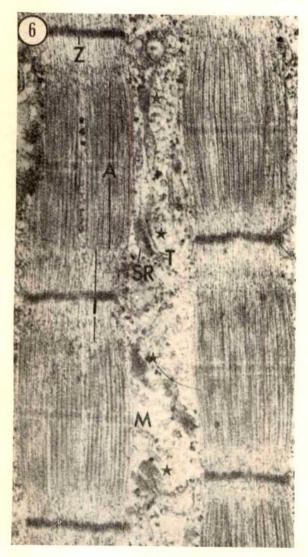


Fig. 6. Longitudinal section of foetal fibre showing dense structures (stars) located near A-I junctions of the fibril in the right side of the figure. One of the dense structures shows what appears to be SR with T on the right side of it. (\times c. 34,000.)

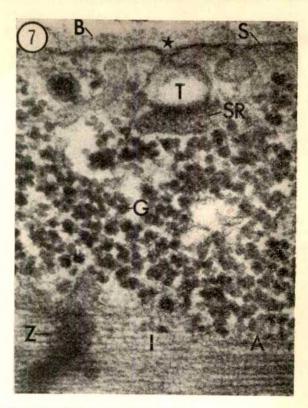


Fig. 7. Longitudinal section of foetal fibre showing an invagination of the sarcolemma (S) that is continuous with the T system (T). The plane of apposition of T and SR is oriented longitudinally. B, Basement membrane; G, dense granule. $(\times c, 90,000.)$

speed of contraction and duration of contraction in response to a single stimulus. Buller, Eccles and Eccles²⁵ have shown that fast muscles, like the flexor digitorum longus of the kitten, show an increase in speed of contraction and a decrease in contraction time of the twitch response during the first 4 weeks after birth. It would be interesting to find out whether growing kittens show changes in triad development which might be correlated with changes in the twitch response.

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LETTERS TO THE EDITOR

PLANETARY SCIENCE

Intensity of the Earth's Magnetic Field in the Geological Past

RECENTLY, Krs1,2 has obtained two estimates of the Permo-Carboniferous geomagnetic field intensity from cassiterite, and one estimate of the Permian field intensity from ignimbrite, in central Europe, using the method developed by Thellier and Thellier³. Both the Permo-Carboniferous values (0.50 oersteds and 0.60 oersteds at lat. $50\cdot12^{\circ}$ N., long. $12\cdot80^{\circ}$ E.; maximum error ± 10 per cent) and the Permian value (0.50 ± 0.03) oersteds at lat. 50.63° N., long. 16.30° E.) were similar to the present field intensity in the same region (0.482 oersteds), the implication being that the Earth's field intensity has not changed appreciably since the Permo-Carboniferous. Furthermore, because the cassiterite was obtained from a greisen body, Krs considered that its thermoremanent magnetization was acquired over a period long enough to average out secular variations in intensity. The quoted Permo-Carboniferous field intensity estimates were thus thought to represent the mean geomagnetic field rather than the instantaneous field corresponding to a certain point on the secular variation curve.

Although the Permo-Carboniferous, Permian and present field intensities in central Europe may be similar, this does not indicate that the geomagnetic dipole moment has remained approximately constant over the past 270×10^{6} yr or so, because no account has been taken of the site latitude change during this period. The palaeomagnetic directions for the cassiterites4 (declination 207°, inclination +11°) and for the ignimbrite² (declination 192°, inclination -3°) indicate more southerly site latitudes at the time of acquisition of the respective thermoremanent magnetizations. Because the field intensity produced at the Earth's surface by the geomagnetic dipole varies with geomagnetic latitude, the field intensity values obtained by Krs indicate ancient dipole moments higher than the present $(8.0 \times 10^{25} \text{ gauss cm}^3)$. Virtual dipole moments⁵ calculated using these ancient field intensities and palaeomagnetic inclinations are 12.2×10^{25} gauss cm³ and 14·6×10²⁵ gauss cm³ for the Permo-Carboniferous and 12.8×10^{25} gauss cm² for the Permian, all of which are over 50 per cent higher than the present dipole moment.

A second point arises in comparing the new virtual dipole moments from cassiterite and ignimbrite with those previously published. Fig. 1a shows all virtual dipole moments covering the geological time scale (defined here as greater than 10^4 yr) available up to June 1967 (ref. 6). The new virtual dipole moments from cassiterite (open circles) and ignimbrite (cross) are differentiated symbolically from the rest of the data. Fig. 1b shows the trend curves based on data in Fig. 1a. Curve (1) is the previously published curve⁵ based on the mean virtual dipole moments from sets of rocks containing ten or more separate rock units; curve (2) is the mean curve for all data, obtained by averaging virtual dipole moments and ages in 100×10^6 yr intervals; curve (3) is the least squares regression curve for all data assuming a second order variation.

The equation determined for curve (3) is

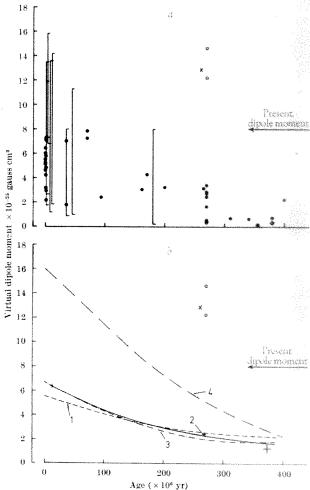
$$P(10^{-25}) = 6.76 - 2.86 (10^{-2}) t + 4.05 (10^{-5}) t^{2}$$

where P is the virtual dipole moment (gauss cm³) at time t (million years) from the present. Curve (4) is the

upper boundary of data in Fig. 1a excluding the virtual dipole moments from cassiterite and ignimbrite.

Curves (2) and (3) agree closely. Curve (1) agrees less well with these, but is not based on all the data. All three curves indicate a gradual increase of mean geomagnetic dipole moment with time by at least a factor of two over the last 400×10^6 yr. The upper boundary, curve (4), suggests further that the spread of virtual dipole moments decreases with age. Back to at least 300×10^6 yr, curve (4) lies $(150\pm20$ per cent) higher than the mean curves (2) and (3). If the spread of virtual dipole moments is chiefly caused by fluctuation of the main dipole the results suggest that the maximum amplitude of this fluctuation is approximately proportional to the mean dipole moment.

The new virtual dipole moments from cassiterite and ignimbrite seem, however, to be inconsistent with previously published data. They are considerably higher than the present dipole moment, whereas the mean curves over the whole range, and all other individual virtual dipole moments obtained so far from rocks older than 80×10^6 yr, lie below the present moment. Accepting curve (4) as a rough guide to the maximum dipole fluctua-



Age (× 10^4 yr) Fig. 1. a. Virtual dipole moments for geological time (greater than 10^4 yr) from the literature. Solid circles represent virtual dipole moments from separate rock units in cases where less than ten such units have been measured; vertical lines represent the range of virtual dipole moments in cases where ten or more units have been measured. Open circles represent virtual dipole moments from cassiterites'; the cross represents virtual dipole moments from ignimbrite'. Ages according to Holmes' revised time scale', except in a few cases where radioactive ages are available. b. Trend curves based on data in a. (1) Previously published curve' based only on virtual dipole moments from sets of ten or more rock units; (2) mean curve for all data (except cassiferite and ignimbrite) obtained by averaging virtual dipole moments and ages in 100×10^4 yr intervals and constructed by eye (error bars represent standard errors); (3) regression curve for all data used for curve (2) assuming second order variation; (4) upper boundary of all data except cassiterite and ignimbrite.

tion amplitude would mean that ultimately all virtual dipole moments obtained from rocks older than about 200 × 10° yr should be smaller than the present dipole moment. If the virtual dipole moments from cassiterite represent mean values2, the discrepancy between them and curves (2) and (3) amounts to a factor of about 7. Krs's suggestion, however, that secular variation had been averaged out in the cassiterite thermoremanent magnetization presumably referred to secular variation of the nondipole field rather than of the dipole field, in which case the virtual dipole moments from cassiterite are not mean values in the same sense that curves (2) and (3) represent the variation of mean virtual dipole moments. But, even if the eassiterite virtual dipole moments are "instantaneous" values with respect to dipole fluctuations, they still seem to be inconsistent with the pattern of such fluctuation as defined by the boundary curve (4).

In view of the general paucity of data from rocks older than 50×10^6 yr, it is perhaps too soon to say that this inconsistency necessarily implies validity of all virtual dipole moments except those from cassiterite and ignimbrite. Krs^{1,2} has shown that both the cassiterite and ignimbrite used by him possessed exceptionally high palaeomagnetic stability. Furthermore, there is still some doubt about whether the apparent increase of mean geomagnetic dipole moment with time represents a real increase or whether it arises merely through gradual decay of rock magnetization over geological time. It is also possible that the natural magnetization in some of the older rocks analysed may have been chemical rather than thermal, in which case the experimental method is not valid7.

The tentative conclusions that the mean geomagnetic dipole moment has been increasing gradually over the past 400×10^6 yr and that the amplitude of dipole fluctuations has also been increasing are based on the bulk of data currently available. When more data have been obtained these conclusions may have to be revised.

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Effect of the Tunguska Meteor and Sunspots on Radiocarbon in Tree Rings

SEVERAL hypotheses have been advanced to explain the peculiar circumstances associated with the fall of the Tunguska Meteor on June 30, 1908, in Siberia (lat. 60° 55′ N., long. 101° 57′ E.). An exhaustive description of the phenomena is given by Krinov¹. Especially puzzling is the apparent absence of a meteoric crater, but Krinov² does report some recent analyses of soil in the region which show nickeliferous iron and silicate globules.

Cowan et al.3 have discussed the possibility of the phenomena being produced by the annihilation of antimatter in the atmosphere. They have tried to measure the increase in atmospheric carbon-14 which should have been produced by the neutrons generated in the process and consequently recorded in tree rings. Venkatavaradan4 has discussed the measurements and has suggested that they correlated with the solar activity cycle.

Table 1

Analysis No.	Tree ring date	δ Carbon-13* (per mil)	△ Carbon-14† (per mil)
GrN 4886	1894	- 26.01	$+0.6 \pm 1.6$
GrN 4904	1898	-25.60	-2.3 + 1.6
GrN 4887	1901-2	-25.03	-4.2 ± 1.8
GrN 4756	1904	-24.03	-1.5 ± 1.6
GrN 4747	1907	-25.07	-3.7 ± 1.7
GrN 4710	1908	-25.15	-3.8 ± 1.7
GrN 4701	1909	- 25.44	-5.6 ± 0.9
GrN 4702	1911	-24.07	-6.9 ± 1.6
GrN 4790	1912	-24.83	-8.7 ± 1.2
Grn 4942	1914	-25.09	-4.0 ± 1.5
Grn 5025	1915-7	~ 24.83	-7.3 ± 1.6

^{*} Relative deviation in the carbon-13/earbon-12 ratio from the PDB standard sample

This communication describes new measurements of radiocarbon in tree rings, with higher accuracy. results show no significant deviations which could be correlated either with the Tunguska meteor or with the sunspot cycle.

Eleven samples have been analysed. Nine were individual tree rings, and the other two contained two and three rings, respectively. All of them were taken from a section of the same tree, a poplar, Populus balsamifera (supplied by S. Westin, of the Norges Tekniske Høgskole, Trondheim, Norway). The tree grew in a windy place outside Trondheim (lat. 63° 25′ N., long. 10° 26′ E.). It was felled during the spring of 1957. For each analysis 30 g of wood was treated successively with diluted acid, alkali and acid. It was then burned to form carbon dioxide, which was purified and used as the counting gas in a proportional counter with a volume of about The carbon-13/carbon-12 ratio of the gas was measured and used to correct for variations in isotopic fractionation5.

The reference activity used was 0.95 of the National Bureau of Standards oxalic acid standard, which was known for the counter with a σ (standard error) of $\pm\,2\cdot2$ per mil. The activity of each sample was corrected for age to 1950 using a radiocarbon half life of 5,730 yr. The results are given in the last column of Table 1 and plotted in Fig. 1.

The sloping line in Fig. 1 is the least squares fit to the individual measurements. It shows a decrease in the carbon-14 activity of 0.35 per mil/year, during the time_{\odot} span covered. This is about twice as large as Fergusson's value⁶. The difference may be caused by a local effect which is at present being investigated.

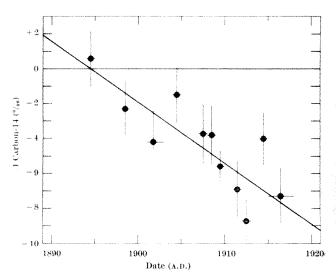


Fig. 1. Deviation of the carbon-14 activity in tree rings. The sloping line is the least squares fit to the points, the slope being caused by industrial dilution and secular variation effects.

[†] Deviation of carbon-14 activity corrected for isotopic fractionation: $^{13}\text{C} = \delta^{14}\text{C} - 2(\delta^{13}\text{C} + 25)(1 + \delta^{13}\text{C} \times 10^{-3})$ where $\delta^{14}\text{C}$ is the deviation of the age corrected activity of the sample.

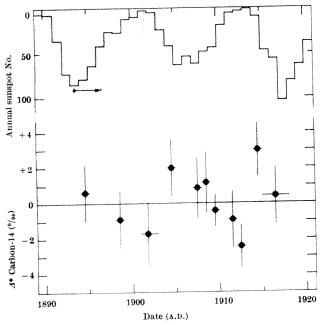


Fig. 2. Deviation of the carbon-14 activity of the tree ring samples relative to the sloping line in Fig. 1; and the annual sunspot number (solar activity). The atmospheric radiocarbon concentration should be proportional to the upper curve, with a maximum allowable phase shift as indicated by the arrow.

In Fig. 2, the same measurements are plotted with reference to the sloping line. Thus the points represent the carbon-14 deviations referred to the average activity of the atmosphere. In the same figure the annual sunspot number' is plotted with an inverted ordinate axis, to allow a direct comparison of the carbon-14 deviations with the fluctuations in the production rate of the isotope⁸.

From theoretical considerations Cowan et al.3 estimate an increase of 7 per cent for the global atmospheric content of carbon-14 as a consequence of the interaction of a mass of anti-matter large enough to produce the energy release of the Tunguska meteor. Marshall⁹ agrees with them, but Gentry¹⁰ calculates only a 2.4 per mil increase. Cowan et al. report having measured an increase of about 1 per cent for the carbon-14 concentration in the atmosphere for 1909*, the accuracy of each measurement being ±5 per mil. We do not understand their corrections for isotopic fractionation so it is impossible to give the exact magnitude of the deviation measured by them. According to Gentry, their result does not contradict his estimation, and consequently his hypothesis that the Tunguska meteor consisted of anti-matter.

From our results shown in Figs. 1 and 2, it is concluded that no deviations larger than 3 per mil have been measured, and that any possible deviation around 1909 must be smaller than about 3 per mil (for a degree of certainty of 30). Thus if the explosion was caused by annihilation of anti-matter, the first mentioned calculation has not been experimentally verified.

Lingenfelter⁸ has calculated the amplitude of the variations in the rate of production of carbon-14 by cosmic ray neutrons, assuming that the galactic component of the cosmic radiation is modulated by the solar activity. The amplitude of the atmospheric carbon-14 variations can be obtained by dividing that amplitude with the attenuation coefficient deduced from Houtermans¹¹. A maximum value of about 2 per mil results for the cycles represented in Fig. 2. The maximum phase shift which is permissible is one-quarter of a period, that is, about 3 yr. This shift

is indicated by an arrow in Fig. 2. Although there is some suggestion of periodicity in the results, the small amplitude of about 4 per mil does not allow any definite conclusion to be drawn except that the effect must be smaller than this. A much higher accuracy would be needed to detect the expected variations within an individual sunspot cycle with any certainty.

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Topography and Heat Flow of the Fiii Plateau

THE Fiji Plateau is an extensive region at a depth intermediate between continent and ocean basins. Above it rise the islands of Fiji where acidic plutonic rocks occur within an ocean basin in a tectonic setting which can only be compared with the granitic Seychelles Islands in the deep western Indian Ocean. The results of the few seismic refraction lines run on the plateau indicate a variable crust of intermediate thickness¹. Thus the Fiji Plateau is an unusually interesting and promising region for tectonic studies. In the spring and summer of 1967, the Scripps Institution of Oceanography expedition Nova studied this region. In this preliminary communication we discuss results in topography and heat flow and suggest a relationship between them.

Twenty-five measurements of heat flow were made from R.V. Horizon and three from R.V. Argo during July Nineteen previous measurements taken and August. during the Scripps Institution of Oceanography expeditions Capricorn and Proa are also considereds, present measurements were all taken with a long, thin, Bullard-type probe, 2.0 cm in diameter and 2.3 m long, which penetrates the top few metres of the sediment. Three elements, which were sensitive to temperature, spaced I m apart enabled us to measure two different temperature gradients in the sediment. A fourth thermistor above the instrument package measured the absolute temperature of the superjacent sea water. Conductivities of the sediment penetrated were determined by the transient needle probe method4 on cores taken nearby. When such a core was not available, the weighted mean of nearby conductivity values was used. measured conductivities for the plateau differ very little, so it is unlikely that the assumed values introduce a significant error.

The Fiji Plateau ranges in platform depth from about 1,000 to 1,700 fathoms and west of Fiji the general trend of contours is north to south. The surface is quite irregular

^{*} Suess measured 0 ± 6 per mil for a tree ring dated 1908 (ref. 12). We think that the peak of the carbon-14 increase should be sought in the tree ring of 1909. Although the Tunguska event happened in 1908, it was already late in the season 19 and it is necessary to add some months delay for atmospheric transport 14.

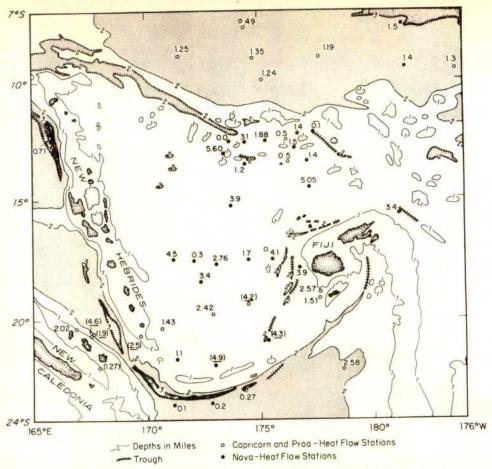


Fig. 1. Heat flow and bathymetry of the Fiji Plateau. Stations with a measured conductivity have two figures after the decimal point while those with an estimated conductivity have only one. Stations showing a partial penetration are underlined and those showing a tilt of greater than 30° are in parentheses.

on a small scale, and sub-bottom profile records show a thin but apparently uniform blanket of sediment. A few volcanic seamounts rise above the central region of the plateau and a few fault troughs cut into it. In general, it is rather subdued in aspect, seems old and is only slightly active seismically. A zone of en echelon troughs and ridges, resembling the crest of the Mid-Atlantic or Gorda Ridges lies just west and north of Viti Levu Island. This zone is seismically active and the north-south section seems to be the result of active rifting of the sea floor. Although of intense topographic relief, the zone lacks corresponding magnetic anomalies while the subdued plateau elsewhere has anomalies of several hundred gammas and an apparent

wavelength of tens of miles. It is not yet known whether the anomalies on the plateau are linear like those associated with the mid-ocean ridge system.

The great relief of the margins of the plateau contrasts markedly with the subdued centre. To the west and east are the active island arcs of the New Hebrides and Tonga. To the north is a zone of north-westerly fault troughs and large volcanoes, many of which are drowned atolls6 of Tertiary age, and the island of Rotuma which has youthful ash cones and virtually uncroded lava flows. To the south is the Hunter Fracture Zone identified by Hess and Maxwell7 and first surveyed by Horizon during the Nova Expedition. The western part of the fracture zone curves in a smooth are into the southern section of the New Hebrides Trench. Farther east, a regional difference in depth of more than a mile is marked by en echelon troughs as much as 3,800 fathoms deep. The fracture zone continues east to Vanua Levu Island and includes Tertiary volcanoes as well as the active volcanoes of Matthew and Hunter Islands. Despite the great relief, neither the northern nor the southern zones of boundary faulting is seismically active.

The heat flow measurements range from 0.0 to 5.60 µcal/cm²/sec (Fig. 1). The central subdued topographic region of the Fiji Plateau has a uniformly high heat flow; discounting partial penetrations, it has a mean value of 2.43 with a standard deviation of 1.56 µcal/cm²/sec. In contrast, the mountainous northern and southern bound-

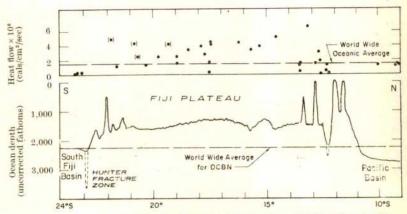


Fig. 2. A generalized profile of heat flow and bathymetry along 174° E. Stations showing a tilt of greater than 30° are in parentheses.

aries have extremely variable heat flow, both high and low, while, further to the north, the deep Pacific Basin has relatively constant normal values. The values shown on Fig. 1 in the latter area have a mean of 1.22 and a standard deviation of 0.29 µcal/cm²/sec. Fig. 2, a generalized profile of the heat flow and bathymetry along 174° E. and between 11° S. and 24° S., illustrates the striking

differences between the three regions.

The high mean heat flow through the plateau indicates unusually high temperatures at quite shallow depths. Although this high average value is similar to that through the Japan Sea, the Sea of Okhotsk and the Shikoku Basin⁸⁻¹⁰, the greater spread of values found on the Fiji Plateau may indicate a different origin. The Japan Sea has a uniformly high heat flow with almost all values lying between 1.7 and 2.5 µcal/cm²/sec. This high mean flow and low variance about the mean indicate a widespread region of anomalously high temperatures at shallow depths. On the other hand, the values found on the Fiji Plateau range widely. While the very high values surrounding Viti and Vanua Levu suggest a region of anomalously high temperatures beneath the islands, the high and low values found on the stations to the west indicate isolated igneous intrusions of recent origin.

Six out of twenty-five stations from R.V. Horizon yielded values of less than 0.3 μcal/cm²/sec. Such a high percentage of very low values is difficult to explain. Three of the values were found in the mountainous region north of the Plateau. The station at 12° 14' S. and 176° 11' E. on Fig. 1 with a bar over the value of 0.1 consisted of three separate penetrations of the probe. All three measurements gave the same overall temperature gradient. These stations were taken in a sediment trough adjacent to the island of Rotuma, so it is possible that the temperature gradient was disturbed by a recent sediment slump. The other two low values were also found in sediment troughs at the base of regions of high relief and consequently may have the same explanation. It seems a little unreasonable, however, to attribute so many low values to rapid sedimentation in a region which has been quiescent since the Miocene. The three low values just south of the Hunter Fracture Zone present a similar problem. The equivalent of a pebbly mudstone in the upper 30 cm of a gravity core, taken near the most easterly station, indicates a recent sediment slump which may account for the unusually low temperature gradient. It is possible that the low values found on the other two stations have a similar explanation. But the large distances (300 miles) between them and their position in the subdued topography, south of the sharp relief of the Hunter Fracture Zone, make such an explanation unlikely. On the other hand, Fig. 2 indicates that the regions of variable heat flow may be associated with the topographically rough northern and southern boundaries of the Plateau. This association of rough topography and mixed heat flow values is also found over the crest of the mid-ocean ridges and off the west coast of central America¹¹. It is possible that this association may be connected with the tectonic development of these regions.

The high mean heat flow of the North Fiji Plateau contrasts strongly with the uniform normal flow of the western Pacific basin. Three other regions which border the western flanks of the Pacific, the Sea of Okhotsk, the Japan Sea and the Shikoku Basin have a high average heat flow, though the values of the Shikoku Basin are more variable than those found in the other two seas (M. Langseth, private communication). The heat flow of these three marginal seas is also in strong contrast with the uniform normal flow of the western Pacific Basin. But the Japan Sea, the Sea of Okhotsk and the Shikoku Basin are all inland seas at a lower elevation than their surroundings, while the Fiji Plateau is a plateau which, although at the same depth as the marginal seas, is high in relation to the surrounding topography. This suggests



Relation, in the western Pacific, of high and normal heat flow ndesite line. ———, Andesite line (after MacDonald, 1949); hatched areas, known regions of high mean heat flow.

that on the western flanks of the Pacific there is a correlation between a given depth and high heat flow. On the other hand, this relationship may be purely coincidental. Fig. 3, which shows the distribution of known regions of high heat flow in the western Pacific, clearly illustrates that the island arcs, roughly traced by the andesite line, separate these regions from the Pacific Basin. A deep sea trench, large gravity anomalies, volcanoes and deep focus earthquakes are the features usually associated with island arcs. The distribution of the regions of high heat flow suggests that a high average heat flow in the marginal seas is another principal feature of the western Pacific island arcs.

The Nova Expedition is supported by the US National Science Foundation and the US Office of Naval Research. We would like to thank the captains, crew and scientists aboard R.V. Argo and R.V. Horizon for their co-operation during expedition Nova, and Mr F. Dixon and Mr J. Greenhouse for taking many of the heat flow measurements.

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Pentameral Symmetry in **Echinoderms**

NICHOLS, in his recent restatements1,2 of the suture-line theory, has made out a strong case for the adaptive significance of pentameral symmetry in the newly metamorphosed echinoderm. Its admittedly transient advantages at this stage, however, do not seem to account for a pentamerous form persisting to determine the symmetry of the adult body. There are exceptions-Nichols1 quotes Promachocrinus—but why are they not the rule?

Nichols has looked for a reason for pentamerism in the life of modern echinoderms, but the evolution of echinoderms is a continuous process which has gone on since Pre-Cambrian times³. Once the pentameral condition had been established at some time in the past, in many members of the phylum it was likely to persist tenaciously in a variety of ways of life because any disadvantages of pentamerism are likely to be less than the disadvantages of changing it. Conversion of pentameral symmetry to a higher symmetry could not be done gradually because there is no intermediate between five-fold and six-fold radial symmetry. If it occurred as one mutation, the whole body-plan of the animal would be altered. groups in which parts of the skeleton fit together to form a rigid box, such as the theca of blastoids or the corona of echinoids, the disruption caused by this mutation would certainly be disadvantageous. In echinoids, with a corona of elaborate architecture and, in most groups, a complex jaw assembly also, the angular relationships and so the shapes of most parts of the skeleton would have to be altered to fit. This is presumably why the only echinoids without five rays are occasional teratological individuals. Where the constraint of a rigid test has been reduced, pentameral symmetry has been less persistent. In the asteroids, the basic skeletal structure is in the flexible arms. In consequence, the asteroids have both species (for example, Oreaster reticulatus4) and genera (for example, Luidia⁵) with pentameral and other symmetries. adaptability of the asteroid skeleton to this sort of disruption is illustrated in the growth of Pycnopodia helianthoides where the extra arms "wedge apart" the adambulacral and interbrachial ossicles as they develop.

Bather's theory' is an account of how the pentamerous condition may have arisen, and it is still favoured today. It may be possible to explain how the original trimerous condition arose in terms of the demands of bilateral symmetry, but, as Nichols^{1,2} points out, it is not clear why only the lateral rays divided, and those only once. Unless there is some constant, but unknown, ecological or anatomical factor involved, the failure of the anterior ray to divide must be explained by applying the suture-line theory to this stage in evolution. This would eliminate forms with an even number of rays produced by division of the anterior ray or only one lateral ray. Seven or nine rays, produced by a further division of the lateral rays, seem to have been unnecessary. It may be that five ambulacra carried sufficient podia for the early echinoderms, which tend to be smaller than their later relations. (This is better demonstrated from a collection of specimens than a collection of references.) Also, under the suture-line theory, seven or nine rays would make the animal more vulnerable. An echinoderm, which had evolved pentameral symmetry like this, might be capable of changing gradually in such ways as to give rise to all the major groups of echinoderms, without being able to attain a higher symmetry for the reason given in the second paragraph. Nevertheless, there seems to have been a need in many of the later groups for an increased number of podia, which has been met without losing the pentameral In Palaeozoic echinoids, the number of symmetry. ambulacral columns has been increased; in later echinoids, compound ambulacral plates have been developed10; in edrioasteroids, the ambulacra have become longer and

curved11; in crinoids the arms have frequently branched extensively12.

It would seem that a complete theory of echinoderm pentamerism should consider the history of these animals, especially in Cambrian and Pre-Cambrian times. Such a theory can be more firmly based as recently discovered fossils2,3 are further interpreted.

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PHYSICS

Drag of Spheres in Dilute High **Polymer Solutions**

White showed that the drag on spheres in water, at Reynolds numbers exceeding the critical value (vd/v> about 2×10^5), could be increased by dissolving a very small quantity of polyethylene oxide (Union Carbide 'Polyox WSR301') in the water, and suggested that this effect resulted from suppression of turbulence in the boundary layer by the additive, which caused earlier separation and a corresponding increase in the wake size.

Experiments carried out below the critical Reynolds number, where the boundary layer is laminar, have shown that the drag on a sphere is considerably reduced by adding 'Polyox'²⁻⁴, although drag reduction in pipe flow occurs only in the turbulent regime. Flow visualization studies3 showed delayed boundary layer separation and a smaller wake size with the polymer solutions, which is consistent with the reduction of drag.

Tests have now been carried out for a wider range of Reynolds number to span the critical region; the technique of measurement was similar to that of the previous investigators. Steel spheres were dropped down through cylinders containing the test liquid; the spheres were

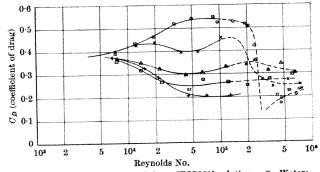


Fig. 1. Drag of spheres in 'Polyox WSR301' solution. ○, Water; ×, 10 p.p.m. 'Polyox' solution; △, 30 p.p.m. 'Polyox' solution; □, 60 p.p.m. 'Polyox' solution; +, 120 p.p.m. 'Polyox' solution.

released from a point just below the surface of the liquid by an electro-magnet. The apparatus was kept in a darkened room and for each test the falling sphere was illuminated by a strobo-flash unit operating at mains frequency (50 c/s). Measurements from multiple image photographs on high speed 'Polaroid' film enabled the terminal velocities to be determined, from which the corresponding drag coefficients were calculated.

Two rigs were used: spheres between 0.25 in. and 1.5 in. in diameter were dropped into a 'Perspex' tube 10 ft. high by 6 in. in diameter, and the larger spheres ranging in diameter from 1.5 in. to 4.5 in. were dropped into a steel vessel 22 ft. high by 2 ft. in diameter which had 'Perspex' windows near the bottom for observation purposes. The spheres frequently deviated from a vertical path during their descent and struck the sides of the containers, and in all such cases the results were discarded.

The results obtained with water and dilute 'Polyox' solutions are shown in Fig. 1. The drag coefficients at low values of Reynolds number agree to a large extent with the results of previous investigators. It can be seen that at high Reynolds numbers adding polymer increases the drag, as the preliminary experiments suggested. Small increases in viscosity have been taken into account in evaluating the Reynolds numbers because these very dilute solutions seem to be Newtonian under steady laminar capillary flow. Results obtained in the large rig are shown connected by dashed lines. Tests have also been carried out with equimolar solutions of cetyltrimethylammonium bromide (CTAB) and 1-naphthol. This complex soap system was previously found to exhibit very large drag reductions in turbulent flow in pipes, although the effect terminated when the shear stress of the wall exceeded a certain value, probably because of changes in or disruption of the micelle structure. This could account for the reduced effectiveness in drag reduction at higher values of Reynolds number as seen in Fig. 2. The highest concentration was found to be slightly pseudoplastic at low shear rates with a flow index, n, of about 0.9, and because possible structure changes at high shear rates make viscosity difficult to interpret, the Reynolds numbers of Fig. 2 have been based on the viscosity of water.

Sphere drop tests in a 500 p.p.m. solution of guar gum exhibited no significant reduction in drag although similar solutions in pipe flow produce a large decrease in flow resistance6

Bizzell and Slattery' predicted that for a power law fluid the separation point would move towards the rear of the sphere as the flow index, n, decreased, but all the solutions used in this investigation were Newtonian under steady laminar capillary flow (n=1), except for the highest concentration of the soap system. Gadd's has shown that freshly mixed solutions of 'Polyox' exhibit normal stress differences in shear flow, while this effect is not found with dilute guar gum solutions. Normal stress measurements have not yet been carried out with the

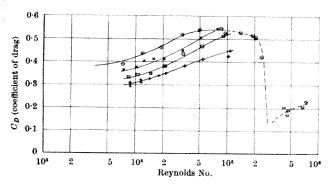


Fig. 2. Drag of spheres in equimolar solutions of cetyltrimethylammon-lum bromide (CTAB) and 1-naphthol. \bigcirc , Water; \times , 264 p.p.m. total concentration; \square , 508 p.p.m. total concentration; +, 1,016 p.p.m. total concentration.

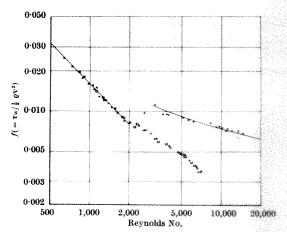


Fig. 3. Comparison of fresh and aged 'Polyox' solutions in pipe flow. (Pipe diameter, 0-090 in.) ♠, Water; ×, 30 p.p.m. 'Polyox' solution (fresh); ○, 30 p.p.m. 'Polyox' solution (aged for 6 days).

soap systems, which are certainly visco-elastic as the erratic rise of air bubbles through the solutions shows.

Brennen and Gadd' have found that the elastic effects with a dilute 'Polyox' solution disappear after agoing for several days, although the reduction of skin friction remains unchanged. The results shown in Figs. 1 and 2 were obtained with freshly mixed solutions; the effect of ageing on the drag of a sphere in 'Polyox' solution is illustrated in Table 1.

Table 1. SPHERE DIAMETER 0.5 IN.

Age (days)	C_{B}
Fresh	0.324
1	0.346
2	0.362
3	0.390
6	0.443

Cp in water was 0.475, and 30 p.p.m. of 'Polyox WSR301' was used.

After 6 days the sphere drag reduction virtually disappeared. Pipe friction measurements were then carried out with this same aged solution and also with a freshly mixed solution at the same concentration. Fig. 3 shows that the increasing drag on a sphere with age cannot be ascribed to degradation, because practically identical pipe friction results were obtained with the fresh and aged solutions.

These results then seem to indicate that the delayed laminar separation is caused by visco-elastic effects. because the phenomenon seems to occur only with solutions which exhibit normal stress difference behaviour. It is possible that the stress system set up in the shear flow within the boundary layer tends to strangulate the fluid onto the sphere in a way somewhat similar to the Weissenberg effect with flow in an annulus with an outer rotating cylinder.

I thank the Hoffmann Manufacturing Company Ltd., Chelmsford, for providing a wide range of precision steel balls used in this investigation.

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Absolute Experimental and Theoretical Determination of the Copper-63(n,2n)Copper-62 Reaction Cross-section

The copper-63(n,2n) copper-62 reaction cross-section has been accepted1 as a standard for measuring neutron flux in the 14 MeV region. Unfortunately, this crosssection varies rapidly with neutron energy, and thus it is important that the cross-section should be predictable on a theoretical basis in order to relate the various experimental determinations. Usually 2,3 (n,2n) cross-sections are calculated on the statistical model using an experimental value for the relevant (n,n) cross-section. In this investigation the necessity of the measured (n,n) crosssection has been eliminated.

After Vandenbosch and Huizenga⁴, the cross-section $\sigma(J_c)$ for forming any angular momentum state J_c in the compound nucleus is given by

$$\sigma(J_c) = \pi \left(\frac{\lambda}{2\pi}\right)^2 \frac{\sum\limits_{S=|I-s|}^{I+s} \frac{J_c+S}{\sum\limits_{I=|J_c-S|} (2I+1) (2s+1)} \frac{(2J_c+1)}{(2s+1)} T_l(E) (1)$$

where I is the spin of the target nucleus, s is the spin and the angular momentum of the incident particle, and $T_l(E)$ is the barrier penetration coefficient for a given l and incident neutron energy, E. The total cross-section σ_{end} for the compound nucleus formation is obtained by summing over the available J_c values. The compound nucleus can then decay by the various particles evaporating into different final nuclei.

The (n,2n) cross-section, $\sigma(n,2n)$, is given by

$$\sigma(n,2n) = \sigma_{cpd} \frac{P(2n)}{\Sigma P(x)}$$
 (2)

where P(2n) is the probability of evaporating two neutrons, P(x) is the probability of evaporating any particle x, and $\Sigma P(x)$ is the total probability of decay of the compound nucleus.

Vandenbosch and Huizenga⁴ give the cross-section for forming a spin state J_F in the final nucleus, $P(J_F)$, as

$$P(J_F) \propto (J_c) \
ho(E) \ rac{(2J_F+1)}{2\sigma^2}$$

$$\exp\left(-(J_F + \frac{1}{2})^2/2\sigma^2\right) \sum_{S=|J_C - S|}^{|J_F|S|} \sum_{l=|J_F - S|}^{|J_F|S|} T_l(\varepsilon)$$
 (3)

Where $T_l(\varepsilon)$ is now the barrier penetration coefficient for a particle X leaving the compound nucleus with excitation energy ε . Here s is the spin of the emitted particle X. The probability, P(x), of any given particle x being emitted is assumed to be proportional to the sum of the $P(J_F)$ values over the available J_F states.

Expression (3) refers to the emission of a single particle from the compound nucleus and is not immediately useful for the emission of two neutrons. Okumura⁵ approaches the problem of the second emitted neutron by neglecting the angular momentum changes caused by this emission. In this investigation the calculation is performed by first using expression (3) to calculate angular momentum distribution caused by the emission of one neutron and then repeating the calculation for the second neutron.

The value of σ , the spin cut-off parameter, was taken to be 4.0, in accordance with the large amount of experimental evidence published on this parameter in work on isomeric cross-section ratios for nuclei of mass number near 60 (refs. 6-9). It should be pointed out, however, that the effect of changes of ± 1 in this parameter will cause little change in the final value for the (n,2n) crosssection because its function is to prevent the distribution of angular momentum in the final nucleus reaching high values. This is the case for both P(2n) and $\Sigma P(x)$.

The value of $\rho(E)$, the level density in the final nucleus for zero spin, is taken to be

$$ho(E) \propto \mathrm{e}^{2\sqrt{Ea}}$$

where E is the excitation of the final nucleus, and a is the level density parameter, taken to be 2 MeV-1 after Blatt and Weisskopf¹⁰ and also in accordance with the method used by Chatterjee¹¹ using the Weisskopf-Ewing

For an incident neutron energy of 14.7 MeV, the copper-63(n,2n)copper-62 cross-section is given by this calculation to be 582 millibarns which is in good agreement with the experimental measurements made in this laboratory; namely, 594 ± 26 millibarns for incident neutrons of energy 14.7 ± 0.2 MeV. Pasquerelli¹² reports a lower experimental value of 511±15 millibarns for a neutron energy of 14.7 ± 0.1 MeV, and Grimeland obtained values of 568 ± 16 millibarns and 548 ± 10 millibarns for 14.8 ± 0.1 MeV neutrons.

In this laboratory the neutrons were obtained from the T(dn)He4 reaction using an incident deuteron energy of 140 keV. The neutron yield was measured by the associated particle technique, detecting alpha particles at 150° to the deuteron beam using a silicon surface barrier detector. The variation in yield with time was recorded and integrated both numerically and by an electrical analogue method. In order to relate the alpha and neutron yield an isotropy correction appropriate to thick target neutron production was applied.

Copper disk samples were positively located close to the back of the tritium titanium target and received neutrons emitted over a cone of solid angle 0.66π steradians. The effect of the finite source size and non-uniformity was determined theoretically¹⁴. This gave an uncertainty in the estimation of the neutron flux at the copper sample

of ± 2.9 per cent.

The induced activity of the samples was determined from the photopeak of the annihilation radiation, detected using a 3 in. x3 in. NaI (Tl) scintillation spectrometer; the annihilation radiation source being localized by placing the copper samples in an aluminium converter positioned 10 cm from the crystal. The efficiency of the crystal was taken from the theoretical works of Heath¹⁵ and Grosjean¹⁶, and was cross-checked with a standard sodium-22 source using the 1-28 MeV photopeak. The peak to total ratio of the crystal was determined experimentally and the accuracy of the counting equipment was estimated to be 3.1 per cent.

The overall accuracy of the measurement, which included the statistical errors and uncertainties caused by the source and sample positions, was obtained by combining the errors quadratically, and was estimated to be $4 \cdot 3(4)$ per cent.

The theoretical variation of the cross-section with neutron energy is also in close agreement with preliminary experimental results over the range 14 MeV to 15 MeV. This substantiates the value of a as 2 MeV⁻¹ as being the correct one. In addition, the calculation has proved capable of enabling a prediction of the value of the copper-65(n,2n)copper-64 cross-section which was computed to be 890 millibarns at 14.7 MeV in close agreement with the value obtained experimentally in this laboratory of 968 ± 68 millibarns at 14.7 ± 0.2 MeV.

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MOLECULAR STRUCTURE

Solid "Liquid Crystal" Films of Poly-Y-benzyl-L-glutamate

WE present here evidence for a planar layered structure in solid films of poly- γ -benzyl-L-glutamate (PBG) made by casting from solution. The (idealized) structure is composed of successive layers of planes parallel to the film surface. The planes themselves consist of "domain' regions of parallel helical molecules. In a given domain the molecules in adjacent planes show a slight angle of twist relative to one another, characteristic of cholesteric liquid crystal structures. An interesting manifestation of this suggested structure is the unusually anisotropic swelling of these films. Films plasticized with a nonvolatile solvent also maintain this layered cholesteric structure with a uniform separation of the PBG planes as shown by birefringence studies.

The structure of reasonably concentrated solutions of PBG has already been extensively characterized¹⁻⁵ and these solutions, which were in a fluid condition in contrast to our solid films, were shown to have a cholesteric liquid crystal structure. Close to the container surface, the molecular planes were parallel to the surface. Within the fluid, however, the molecular planes of the cybotactic regions were isotropically arranged.

The structure of the solid films (unplasticized and plasticized) which are discussed here was inferred from (a) swelling studies, (b) birefringence studies, and (c) X-ray observations6.

Films of PBG of high molecular weight (Pilot Chemicals; molecular weight, 105) 0.1 cm thick were prepared by slow evaporation of chloroform solutions. Solutions with initial concentrations of approximately 10 per cent PBG were contained in cylindrical polyethylene rings placed on a mercury surface. Homogeneous films plasticized with 'Aroclor' (chlorinated polyphenyls) were prepared by similar casting of predetermined ratios of 'Aroclor' and PBG dissolved in chloroform, then evaporating the chloroform.

The mechanical properties of these films are dependent on the concentration of plasticizer. The room temperature torsional modulus, G, measured after 10 sec, is shown in Table 1.

The PBG molecule has been shown to retain its α -helical conformation in the solid state, and oriented plasticized films (20 per cent PBG) also exhibit the characteristic infrared dichroism associated with a helical molecular conformation.

When the completely dried films (100 per cent PBG) were introduced into benzene immediate anisotropic swelling of the films was observed. The film shape was retained

T	able 1
Per cent PBG	3G (dynes/cm ³
100	5 × 10°
60	2.3×10^{s}
50	1.2×10^{9}
30	2×10^{8}
20	3.5×10^7

and dimensions of apparent equilibrium were attained within several minutes. There was a 400 per cent increase in the thickness of the film in a direction N normal to the surface of the film while the other dimensions (parallel to the film surface) increased about 20 per cent. The film remained in a solid condition and could be handled without change of shape.

Plasticized films also showed the same anisotropic swelling in benzene. The swelling was reversible, and the film approached its original dimensions as the solvent

evaporated.

The structure of the swollen film was studied in further A thin section detail with a polarizing microscope. $(2-3\mu)$ was cut perpendicular to the surface of a 100 per cent PBG film and swollen in benzene as described. Between crossed polars, the direction of viewing was in the plane of the original film. In the swollen condition, regularly spaced retardation lines were seen parallel to the surface of the film (and perpendicular to N_L maximum swelling the spacing S between retardation lines was about 2u

In concentrated fluid solutions of PBG, the existence of retardation lines was regarded as very strong evidence for the liquid crystalline (cholesteric) structure 1-5. Robinson also gave a relation between the spacing S and the concentration of PBG in solution². We find the relation to be approximately valid for our swollen (but solid) film.

It is possible that retardation lines on the 100 per cent PBG film (unswellen) cannot be resolved under a microscope because the value of S is smaller than the wavelength of visible light, and this may be why they were not observed by Elliott and Ambrose¹. The retardation lines can, however, be observed in films sufficiently plasticized with 'Aroclor', just as they can in films swollen in

The structure of solid films of PBG east from chloroform solutions has also been examined by X-ray measurements in this laboratory. This structure was tentatively interpreted in terms of co-existing paracrystalline and mesomorphic regions with uniplanar orientation of the molecules in the plane of the film⁶. The new results reported here support this interpretation.

Films (100 per cent PBG) moderately crosslinked in the solid state by gamma irradiation also showed anisotropic swelling, which indicates the preservation of anisotropic order in the crosslinked solid. Perhaps the crosslinks form preferably in the parallel planes. The plasticized films can be readily handled in their original form but may also be crosslinked to lock in permanently the cholesteric structure.

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Denaturation Kinetics of Biopolymers by Differential Thermal Analysis

Several workers have described equations to calculate the activation energy of a reaction from the exothermic or endothermic peaks which occur during a differential thermal analysis (DTA) experiment¹⁻². There is a second type of differential thermal analysis curve, one not

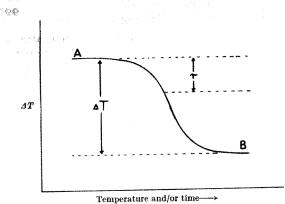


Fig. 1. Idealized sigmoid type thermogram; explanation in text.

characterized by a maximum or a minimum but by a sigmoid shape caused by a change in the heat capacity of the system during the experiment. Fig. 1 shows an idealized sigmoid type isotherm. Such curves are well known in polymer chemistry, where they indicate the occurrence of a so-called second order phase transition in the solid polymer. We report here the observation of such thermograms for aqueous solutions of several biopolymers including proteins and nucleic acid, and direct attention to the possibility of obtaining kinetic data from them.

It can easily be shown that if A represents the state of the system along the upper baseline of Fig. 1 and B represents a second state along the lower baseline, then the degree of conversion of A to B at any time is given by

$$\alpha = \frac{\tau}{\Delta T} \tag{1}$$

where ΔT is the total displacement of A from B and τ the displacement at time t. The extent of the reaction at any time t after the start may be expressed by

$$\int_{\frac{1}{\sqrt{1-\alpha}}}^{a} \frac{\mathrm{d}\alpha}{(1-\alpha)^n} = At \exp\left(-\frac{E}{RT}\right)$$
 (2)

where E is the activation energy for the process, A is a constant, R the gas constant, T the temperature and nthe order of the reaction. If the reaction involves only a change in heat capacity, then its activation energy can be obtained from equation (2) by a plot of

$$\ln \left[\frac{1}{t} \int \frac{\mathrm{d}\alpha}{(1-\alpha)^n} \right] \text{ against } 1/T$$

Our data for the thermal denaturation of pepsin and ovalbumin gave linear plots for n=1 and yielded values for the activation energies slightly lower than those published. Thus we find 39 kcal/mole for 0.5 per cent pepsin for which Casey and Laidler4 report 56 kcal/mole, and 79 kcal/mole for 0·1 per cent ovalbumin where Lewis⁵

reports 132 kcal/mol.

There are several possible reasons for this discrepancy. Undoubtedly one factor involves our need, at present, to work with unbuffered systems. This is necessitated by the effect of temperature on the ionization of the buffer; the resulting enthalpy changes swamp out the changes in heat capacity caused by the denaturation process. It is also possible that the biochemical criterion for heat denaturation measures a slighty different process from that detectable by differential thermal analysis. Furthermore, Casey and Laidler4 report an activation energy for the initial rate of reaction while the differential thermal analysis experiment gives an average for the temperature range of the transition. The same workers also reported that the activation energy increases as the concentration of the solution decreases, a factor which could account for

the discrepancy between our value for ovalbumin and that of Lewis.

Similar experiments performed on gels of calf thymus DNA also produced sigmoid type thermograms in the temperature range of 60°-80° C. A wide variety of experiments by other workers have shown that DNA undergoes a helix-coil transition in this temperature range. Calculation of the activation energy for the process measured by the differential thermal analysis experiment gave a linear plot for n=1, corresponding to E=30.0 kcal/mole.

It is apparent that the differential thermal analysis experiment provides a valuable insight into structural changes occurring in solutions of biopolymers such as proteins and nucleic acids.

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CHEMISTRY

Formation of Metastable Atoms of Phosphorus, Arsenic and Antimony by Flash Photolysis

ELECTRONICALLY excited atoms (${}^{2}D^{0}, {}^{2}P^{0}$) of phosphorus, arsenic and antimony have been observed in absorption after isothermal flash photolysis of phosphine, arsine and stibine. These metastable atoms were also observed with phosphorus trichloride and tribromide, arsenic trichloride and tribromide, and antimony trichloride. So far, nine atomic lines of phosphorus, eight of arsenic and forty-four of antimony have been identified, arising from the excited states, and two transitions involving the ground state (${}^4S^6$) of antimony have also been observed. The states involved and the transitions observed are listed in Table 1. Several of the transitions were also observed in emission and these are indicated by an asterisk in Table 1.

Vibrationally excited P2, As2 and Sb2 were also observed with the exception that no Sb₂ was produced from

antimony trichloride.

Arsine and stibine were prepared according to Gunn et al.1. Phosphine (Matheson), phosphorus trichloride, phosphorus bromide and arsenic trichloride (Baker and Adamson), arsenic tribromide (K and K) and antimony trichloride (May and Baker) were purified by distillation Pressures used for these compounds were usually in the range 0.5-0.1 mm of mercury, although as low as 10-4 mm of mercury was used for antimony. The compound, with a large excess (about 250 mm of mercury) of nitrogen added, was flash photolysed at an energy of about 1,000 Joules in a 50 cm long reaction vessel. The spectra were recorded on Ilford HP3 plate using a Hilger quartz spectrograph, model E742.

The production of PH2 and PH and of vibrationally excited P2 in the flash photolysis of phosphine has been explained by the mechanism²

$$PH_3 + h\nu \rightarrow PH_2 + H \tag{1}$$

$$H + PH_3 \rightarrow H_2 + PH_2$$
 (2)

$$2PH_2 \rightarrow PH_3 + PH \tag{3}$$

$$2PH \rightarrow P_2 * + H_2 \tag{4}$$

Table 1. SUMMARY OF ATOMIC TRANSITIONS OBSERVED IN ABSORPTION

Atom	State Designation	J	Level ⁸ (cm ⁻¹)	Transitions ⁵
	3p3 2D0	3	11,362	48 ² P ← ² D°
Phosphorus		ĝ	11,377	4s ² P ← ² P**
•	3p3 * P*	ÿ	18,722	48′ ½D ← 2P0
		3	18,748	
	4p3 2De	3	10,593	5s ² P ←— ² D ^{6*} †
Arsenic	4p D	5	10,915	58 *P ← *P**
Aibeine	4p3 2P0	<u>3</u>	18,186	58' ¹ D ← ¹ P*
	491-	3 2	18,648	36 -1) 1
	5p3 4S0	3	0	68 ⁴P ←—⁴S*
		4	A F10	6s ² P ← ² D ⁰ *
	5p2 *D*	ã	8,512	68 ⁴ P ² D**
		ğ	9,854	68′ 2D ←—2D°†
Antimony				(a) ← 2D°
		1	16,396	68 ² P ² P**
	$5p^{3-2}P^{6}$	3		68 ⁴P ←—²Pe
		3	18,465	68′ ³D ←—³P°
				78 ¹ P ←— ¹ P ⁹
				78 ⁴P ← ²P*
				(a)2Pa

- * Transitions also observed in emission.
- † Transitions involving ${}^{a}D_{3}^{0}$ not observed.
- (a) Transitions to unspecified upper states also observed.

The reaction

$$H + PH_2 \rightarrow H_2 + PH$$
 (5

was considered less likely. The same mechanism can be applied to the flash photolysis of NH₂ (ref. 3) and it seems likely that similar reactions occur for all the compounds studied. The observation of the spectra of AsH2 and AsH (refs. 4 and 5), SbH₂ and SbH (ref. 5), NCl₂ and NCl (ref. 6) and of PCl (ref. 7), AsCl (unpublished work) and SbCl (ref. 5) in the flash photolysis of the appropriate hydride or halide is consistent with this The vibrationally excited P₂ which we observe with phosphorus trichloride and phosphorus tribromide as well as with phosphine could arise from reactions similar to equation (4) and the same applies to the production of vibrationally excited As, from arsine and the arsenic halides. That excited Sb₂ is produced from stibine, while no Sb₂ was observed with SbCl₃, is in accord with the probable endothermicity of the corresponding reaction for SbCl.

The production of ground state (4S0) atoms can readily be explained by a logical extension of the mechanism. Representing the phosphorus, arsenic or antimony atoms by A and the chlorine or bromine atom by X, the nineteen reactions possible for the compounds studied are summarized by the equations

$$AH + H(AH,AH_2) \rightarrow A + H_2(AH_2,AH_3)$$
 (6)

$$AX + AX(AX_2) \rightarrow A + AX_2(AX_3) \tag{7}$$

The three reactions involving hydrogen atoms are sufficiently exothermic to produce $A(^{2}D^{0})$, but to account for the presence of excited atoms in all systems, the AX or AX_2 radicals (and perhaps AH or AH_2 also) must be vibrationally or electronically excited.

Calculation shows that a sufficient concentration of the vibrationally excited radical could probably be produced by termolecular recombination. Reactions (6) and (7) then follow with the additional possibility, in some cases, of atom excitation by energy transfer from these (or other) excited species.

Alternatively, excited AX(AH) and $AX_2(AH_2)$ radicals could be produced by primary or secondary photolysis:

$$AX_3(AH_3) + hy \rightarrow AX_2^*(AH_2^*) + X(H)$$
 (8)

$$AX_3(AH_3) + h\nu \rightarrow AX^*(AH^*) + X_2(H_2)$$
 (9)

$$AX_2(AH_2) + hv \rightarrow AX^*(AH^*) + X(H)$$
 (10)

A logical extension of this approach is the direct production of excited atoms by secondary photolysis:

$$AX_2(AH_2) + hy \rightarrow A^* + X_2(H_2)$$
 (11)

$$AX(AH) + hv \rightarrow A* + X(H)$$
 (12)

Whichever mechanism is adopted, it seems probable that the initial production of atoms in only one of the two excited states observed need be accounted for, because interconversion by secondary absorption is possible.

$$A(^{2}D^{0}) \xrightarrow{h_{\nu}} A(^{2}P) \xrightarrow{M,-h_{\nu}} A(^{2}P^{0})$$
 (13)

Evidence that these processes occur is provided by the fluorescence we observe from the 2P state to both metastable states.

Until the mechanism of production of excited atoms is understood more fully and the concentrations of atoms in all three electronic states are measured, the extent to which the mechanisms for the flash photolysis of the compounds AH_3 and AX_3 need to be modified remains uncertain. This work is being continued and will be extended into the vacuum ultraviolet for studies on ammonia and on ground state phosphorus and arsenic atoms.

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Lewis Base Adducts of Cobalt and Iron Bis-1,2-dithiolenes

TREATMENT of the dimeric dianionic iron and cobalt dithiolenes, $[MS_4C_4R_4]_1^2$, $[Fe(S_2C_6H_3Me)_3]_1^2$ and $[Fe(S_2C_6C_4)_2]_1^2$, where R is CN, or CF₃, with Lewis bases such as pyridines, phosphines, stibines, CN- or N₃-, results in the dissociation of the dimers and formation of five co-ordinate adducts, $[M(L)-S_4]^{-1}$ or $[M(L')-S_4]^{-k}$, where L is a neutral and L' is a monoanionic Lewis base. With bidentate bases such as a, a'-dipyridyl and o-phenanthroline, six-co-ordinate complexes, $[M(N-N)-S_4]^{-1}$, were formed, and similar adducts of the cobalt dithiolenes with ethylenediamine and dimethylglyoxime were also prepared.

Similar dissociative behaviour and adduct formation had been observed1,2 in the reactions of nitric oxide with these dimeric iron and cobalt dithiolenes, and the fiveco-ordinate nitrosyls had been observed to undergo facile one-electron transfer reactions. A voltammetric study of these new Lewis base adducts in dichloromethane established that they, too, could undergo one-electron transfer reactions. Thus almost all the complexes studied (some species were labile in solution, thus precluding an investigation of their voltammetric behaviour) displayed reversible voltammetric waves corresponding to the generation of neutral and dianionic species, and many also exhibited waves corresponding to monocations being formed (Table 1). It seems therefore that, in these adducts of two- and four-electron donors, a four-membered electron-transfer series can be envisaged, that is

$$\begin{array}{l} [M(L)-\mathbf{S}_4]^{-2} {\longleftrightarrow} [M(L)-\mathbf{S}_4]^{-1} {\longleftrightarrow} [M(L)-\mathbf{S}_4]^{0} {\longleftrightarrow} M(L)-\mathbf{S}_4]^{+1}, \\ [M(\mathbf{N}-\mathbf{N})-\mathbf{S}_4]^{-2} {\longleftrightarrow} [M(\mathbf{N}-\mathbf{N})-\mathbf{S}_4]^{-1} {\longleftrightarrow} [M(\mathbf{N}-\mathbf{N})-\mathbf{S}_4]^{0} {\longleftrightarrow} \\ [M(\mathbf{N}-\mathbf{N})-\mathbf{S}_4]^{+1} \end{array}$$

The limited amount of voltammetric data available suggests that the half-wave potentials for these redox reactions depend on four factors: the central metal atom; the nature of the sulphur ligand (that is, its substituents); the nature of the adduct donor atom; and the donor atom substituents. The dependence of half-wave potentials on the sulphur ligand substituents and metal atom has already been observed in other dithiolene systems1-5 and it is recognized that the electron-releasing ligands tend to stabilize the more oxidized members of an electron transfer system. A similar effect seems to be exerted by the adduct donor atoms and their substituents, but a more extensive study is necessary before the precise effects of the donors can be assessed.

The five-co-ordinate iron monoanions, $[Fe(L)-S_4]^{-1}$, and [Fe(N₃)-S₄]-2, containing weak-field ligands, were highspin $(\tilde{S}=3/2)$ and had magnetic moments at room temperature consistent with three unpaired electrons. This observation indicates that the so-called anomalous magnetic behaviour of the simple iron dithiolenes in donor solvents6 is probably caused by the dissociation of the low-spin dimers (S=1/2) into high-spin monomers which may be complexed by one molecule of solvent. The fiveco-ordinate iron monoanions, and [Fe(CN)-S₄]-2, which contain strong-field ligands, are low-spin (S=1/2), as are the six-co-ordinate iron complexes. All of the cobalt adducts are diamagnetic.

The adducts with spin-doublet ground states exhibited electron spin resonance signals in solution at room temperature (Table 1). Thus the monoanionic phosphine

Table 1. Magnetic, electron spin resonance and voltammetric data from lewis base adducts of iron and cobalt Bis-1,2-disubstituted dithiolenes

	1/1	THIOMBI	1993		
Complex	Spin ground state	$g_{\mathrm{av},\mathrm{a}}$	Aav.	$\frac{E_{1/2}}{(\mathrm{V})}$	Electrode process [†]
$\begin{array}{l} [\mathrm{Fe}(\mathrm{pyr})\mathrm{S_4C_4(CN)_4}]^{-1} \\ [\mathrm{Fe}(\mathrm{P}Bu_3)\mathrm{S_4C_4(CN)_4}]^{-1} \end{array}$	$\frac{3}{2}$ $\frac{1}{2}$	2.046	28-11	-0.51^{e} -1.60^{e} $+0.35$	$ \begin{array}{ccc} -1 & \rightarrow & -2 \\ -1 & \rightarrow & -2 \\ -1 & \rightarrow & 0 \end{array} $
$\begin{array}{l} [\mathrm{Fe}(\mathrm{CN})\mathrm{S_4C_4}(\mathrm{CN})_4]^{-2} \\ [\mathrm{Fe}(\mathrm{dipyr})\mathrm{S_4C_4}(\mathrm{CN})_4]^{-1} \end{array}$	$\frac{1/2}{1/2}$	$2.054 \\ 2.085$	*******	-0.64 +0.41	$-1 \rightarrow -2$
$[\mathbf{Fe}(\mathbf{P}Et_3)\mathbf{S_4C_4}(\mathbf{CF_2})_s]^{-1}$	1/2	2.044	27-9f	$-1.22 \\ +0.02$	$\begin{array}{c} -1 \rightarrow 0 \\ -1 \rightarrow -2 \\ -1 \rightarrow 0 \end{array}$
$[\mathbf{Fe}(o\text{-phen})\mathbf{S_4C_4}(\mathbf{CF_3})_4]^{-1}$	1/2	2.084	Water Control	-1.06 -0.08	$\begin{array}{c} -1 \rightarrow -2 \\ -1 \rightarrow 0 \end{array}$
$[\mathrm{Fe}(\mathrm{P}Bu_3)\mathrm{S}_4\mathrm{C}_4Ph_4]^0$	dia.	White parts.	None,	+ 1·21 - 0·72 + 0·55	$0 \rightarrow +1$ $0 \rightarrow -1$ $0 \rightarrow +1$
$[\mathrm{Co}(\mathrm{P}Et_2)\mathrm{S_4C_4}(\mathrm{CN})_4]^{-1}$	dia.		********	-0.88 +0.49 +1.58	$\begin{array}{ccc} -1 &\rightarrow & -2 \\ -1 &\rightarrow & 0 \end{array}$
$[\mathrm{Co(dipyr)S_4C_4(CN)_4}]^{-1}$	dia.	******	Morros	-1.00 + 0.60	$0 \rightarrow +1$ $-1 \rightarrow -2$ $-1 \rightarrow 0$
$[\mathrm{Co}(\mathrm{P}\textit{Et}_3)\mathrm{S_4C_4}(\mathrm{CF_3})_4]^{-1}$	dia.	******		+ 1·25 - 1·27e + 0·13	$0 \rightarrow +1$ $-1 \rightarrow -2$ $-1 \rightarrow 0$
$\begin{array}{l} [\operatorname{Co}(\operatorname{P}Et_4)(\operatorname{S}_2\operatorname{C}_4\operatorname{H}_3\operatorname{M}e)_2]^{-1} \\ [\operatorname{Co}(\operatorname{P}Ph_3)\operatorname{S}_4\operatorname{C}_4Ph_4]^{\circ} \end{array}$	dia. 1/2	2.013	26.41	+ 1·25 b 1·04 0·41	$0 \rightarrow +1$ $-1 \rightarrow -2$ $0 \rightarrow -1$
$[\mathrm{Co}(\mathrm{P}Bu_3)\mathrm{S_4C_4}Ph_4]^o$	1/2	2.020	23·4i	$^{+0.30}_{-0.58}$ $^{+0.28}$	$0 \rightarrow +1$ $0 \rightarrow -1$ $0 \rightarrow +1$
$[\operatorname{Co}(\operatorname{P}(\operatorname{O}Ph)_3)\operatorname{S_4C_4}Ph_4]^a$	1/2	2.014	24-1i	g g	0 7 7 1

^{*} Measured in acetone at, or near, room temperature.

complexes, [Fe(PR₃)-S₄]-1, displayed doublet signals due to phosphorus-31 nuclear hyperfine coupling, and had g-values and hyperfine splittings averaging 2.045 and 28.0 gauss, respectively. These data are in direct contrast to those obtained from the formally isoelectronic cobalt complexes $[Co(PR_3)S_4C_4Ph_4]^6$ (ref. 7) where cobalt-59, but not phosphorus-31, hyperfine splittings were detected; an exception to this occurred in [Co(P(OPh)₃)S₄C₄Ph₄]⁰ where a small phosphorus-31 coupling (about 8.5 gauss) was resolved. The g-values and metal hyperfine splittings in these neutral cobalt complexes averaged 2.017 and 24.6 gauss, respectively. The dipyridyl and o-phenanthroline adducts $[Fe(N-N)-S_4]^{-1}$, and $[Fe(CN)-S_4]^{-2}$, displayed broad electron spin resonance signals and no nitrogen-14 or carbon-13 hyperfine structure was detected. The width and resolution of some of the resonance signals detected in solutions containing the phosphine adducts and CNwere found to be temperature dependent. A detailed study of the magnetic properties of these adducts and of their electronic structure is in progress.

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Preliminary Study of Variations of Oxygen and Sulphur Isotope in Natural Sulphates

WE have recently developed a method for studying variations in oxygen-18 and sulphur-34 in sulphates using the same specimen for the preparation of carbon dioxide and sulphur dioxide for mass spectrometry.

One of us¹ described in detail a method for the extraction of oxygen from sulphates for studying their oxygen isotope ratio, and showed that some normal chemical procedures for the recovery of sulphates could bring about changes in the original oxygen isotope ratio. We² studied these changes in greater detail, and developed a satisfactory method for the recovery of sulphates from a variety of natural sources. Sulphates can be recovered, purified and reduced by graphite for conversion of the oxygen to carbon dioxide for mass spectrometer measurement of the ratio of the oxygen-18 to oxygen-16. The procedures described cause no changes in the original ratio. The recovery of the oxygen from the sulphate is 98-99 per cent and reproducibility of the method is ± 0.18 p.p.t. The sulphur is recovered for measurements of the ratio of sulphur-34 to sulphur-32 as described earlier³.

Longinelli and Craig4 described a method similar to ours for the study of oxygen-18 variations in sea water and saline lakes. They also reported δ oxygen-18 values for sulphates and water with respect to standard mean ocean water (SMOW). The conventional method for reporting measurements of oxygen isotope abundance in carbon dioxide is as parts per thousand with respect to carbon dioxide liberated from PDB Chicago Limestone Standard

^b Cobalt-59 or phosphorus-31 isotropic hyperfine splittings.

c Measured in dichloromethane using a rotating platinum electrode and results quote against a standard calomel electrode.

d For the reaction $\{M(L)-S_4\}^2 + e \rightleftharpoons [M(L)-S_4]^2 \pm 1$, in dichloromethane. Estimated error in $E_{1/2}$ values \pm 10 mV, and all results corrected for iR drop.

e Irreversible waves.

f Phosphorus-31 hyperfine splitting.

g Not measured.

h Not measured because of lability of complex in solution.

i Cobalt-59 hyperfine splitting.

by reaction with ortho-phosphoric acid and then expressing these results with respect to SMOW, using the relationship

$$\delta^{18} O_{sample} = 1.0409$$
 $\delta^{18} O_{sample} + 40.92 \text{ p.p.t.}$
with respect with respect to SMOW to PDB

 δ is the deviation in parts per thousand of the oxygen-18 to oxygen-16 ratio from that in the standard, where

$$\delta \ {\rm oxygen\text{-}18 \ p.p.t.} = \frac{{}^{18}{\rm O}/{}^{16}{\rm O}_{\rm sample} - {}^{18}{\rm O}/{}^{16}{\rm O}_{\rm standard}}{{}^{16}{\rm O}/{}^{16}{\rm O}_{\rm standard}} \times \frac{1000}{1}$$

An immediate comparison between the results of Longinelli and Craig and the results from this laboratory was possible. Longinelli and Craig showed that sulphate ions in sea water and saline lakes were enriched in oxygen-18 by between 7 and 23 p.p.t. relative to SMOW. We have studied oxygen-18 enrichment in sulphates from several sources and attempted to show correlations between sulphate oxygen and the water in which the sulphate existed and between the oxygen and sulphur isotope variations in the sulphate ions themselves. Results are shown in Figs. 1 and 2.

Longinelli and Craig found that the sulphate ions in the ocean were enriched in oxygen-18 by 9·5 p.p.t. relative to SMOW. We have measured the δ oxygen-18 values in six coastal sea waters and eleven samples from an ocean profile to a depth of 2,500 m collected around New Zealand. These seventeen samples gave an average of 9·9 p.p.t., so there is little difference between δ oxygen-18 values in sulphates from sea water measured in our laboratory and those of Longinelli and Craig. There was no isotopic temperature correlation between the sulphate and water oxygens in sea water, which supports the known stability of the sulphate oxygens which have been shown to exchange extremely slowly⁵.

An interesting series of sulphate and water samples from Lake Vanda, Antarctica, were available. It was known that these samples came from layers of strongly density-stratified non-connective saline waters in the temperature range 19·4°-25·5° C (ref. 6).

As we reported, between depths of 180 and 215 ft. the δ sulphur-34 values varied progressively from +20 to +39.4 p.p.t. with respect to meteoritic sulphur. The δ oxygen-18 values in these sulphates gradually increased from -9.4 to -3.6 p.p.t. with respect to SMOW, while the saline water oxygen increased from -31.2 to -28.9 p.p.t. with respect to SMOW. Antarctic snow water in this area has a δ oxygen-18 value of -30 p.p.t. with respect to SMOW. Assuming equilibrium conditions to have been established, we calculated the isotopic equi-

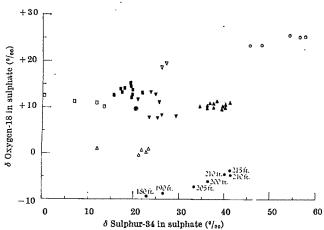


Fig. 1. Correlation of \$\delta\$ oxygen-18 and \$\delta\$ sulphur-34 values in a variety of sulphates. O, Sulphate ion in sea water; \$\triangle\$, sulphate ion in geothermal water, Wairakei, New Zealand; \$\triangle\$, sulphate ion in Lake Vanda, Antarctica; \$\triangle\$, plante; no rain water, New Zealand; \$\triangle\$, barite, \$\triangle\$, sechery, Tasmania; \$\triangle\$, barite, Pennines, England; \$\triangle\$, secondary barite, Pennines, England; \$\triangle\$, diagenetic barite, Kaipara, New Zealand.

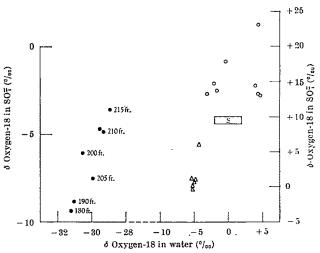


Fig. 2. Correlation of δ oxygen-18 values in sulphate ion and the associated water. $[\overline{S}]$, Sea waters^{2,4}; \triangle , geothermal water, Wairakei, New Zealand; \bigcirc , saline water⁴; \bullet , Lake Vanda, Antarctica.

librium temperatures of around -20° C from the oxygen isotopic data. This temperature does not correlate with the present water temperatures but with the mean air annual temperature (-20° C) for this region. As shown in Fig. 1, the δ oxygen-18 and δ sulphur-34 values of the sulphate steadily increase with depth. We can speculate that the oxygen-18 variation has taken place at the same time as the sulphur-34 variation, although we cannot explain at present how such oxygen-18 and sulphur-34 variations have occurred. These samples also show a clear correlation between oxygen-18 variations of sulphate ions and associated water (Fig. 2). This may represent some interaction of sulphate ions with the water, but we cannot at present explain this because we do not know the origin of the sulphates. The δ oxygen-18 values in the water and sulphate above the non-convective layers will be studied when Lake Vanda can be sampled again.

Because the sulphur isotope variations in the sulphate and hydrogen sulphide, from geothermal bore waters at Wairakei, New Zealand, had already been studied, we decided to examine the oxygen-18 variations in the sulphates and water from these bores. It was observed that while the δ sulphur-34 values for the sulphates were very similar to those in sea water sulphates (approximately +20 p.p.t. with respect to meteoritic sulphur), the δ oxygen-18 values for the sulphate oxygen were very close to those of SMOW (not approximately 10 p.p.t. heavier as is the 8 oxygen-18 value for sea water sulphate). The δ oxygen-18 value for geothermal bore-water was found to be approximately -5 p.p.t. with respect to SMOW. From these results, assuming equilibrium conditions, we calculated the isotopic equilibrium temperature in the bores to be about 140° C, while the measured temperature is around 270° C. It seems likely that the high temperature of this geothermal water produces isotopic equilibrium between the sulphate ions and the water, and so we think it possible that the isotopic equilibrium temperature obtained is too low because of uncertainties in the theoretical isotopic equilibrium constants calculated by Urey⁸.

The ratio of oxygen-18 to oxygen-16 has also been measured in a number of rain water sulphates. It was found that their δ oxygen-18 values (approximately +11 p.p.t.) were 1-3 p.p.t. heavier than sea water sulphate oxygen.

We know from our investigation of sulphur isotope that the sulphate in rain water does not come principally from sea water spray. Rain water in this area has a δ oxygen-18 value of -6 p.p.t. with respect to SMOW and in the evaporation procedure for recovery of the sulphate this increases to +25 p.p.t. If the sulphate oxygens are as

stable as reported, this evaporation procedure should not change the δ oxygen-18 value. This is now being tested using sulphates of known oxygen-18 to oxygen-16 ratio. Atmospheric oxygen has a δ oxygen-18 value of +22.73p.p.t. (ref. 10). Tests are being run to determine the isotopic fractionation which may take place if rain water sulphate is formed by the atmospheric oxidation of hydrogen sulphide, to explain our δ oxygen-18 values for rain water sulphates.

To evaluate further the spread in δ oxygen-18 values in nature, thirty-seven barite specimens were examined. Their oxygen-18 values covered the range 7.8 to 25.5 p.p.t. relative to SMOW. Small variations within different barite deposits were observed, but distinct variations existed between different mining areas. Secondary barites were clearly different from primary barites in δ oxygen-18 values. Remarkable enrichments in oxygen-18 and sulphur-34 as large as +25.5 p.p.t. with respect to SMOW and +57.9 p.p.t. with respect to meteoritic sulphur, respectively, were found in diagenetic barite (from upper cretaceous concretions, Kaipara, New Zealand, collected by W. H. Hodgson, University of Otago) associated with calcite. These enrichments may represent effects of bacterial reduction of either sulphates or interaction of the barite with the calcite or both. Good specimens of co-existing sulphate-oxide minerals have yet to be examined to test the usefulness of this method in palaeotemperature studies.

In this paper we have found some clear correlations between oxygen-18 and sulphur-34 variations both within and between each different type of sulphate, as shown in Fig. 1, and between oxygen-18 in sulphate and its associated water, as shown in Fig. 2. The extent to which biological, chemical and physical processes are involved in such distribution requires further detailed studies of all the factors involved.

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Parachor and Critical Co-ordinates

In 1923, Macleod discovered an empirical formula¹ which was valid near the critical point of a pure substance

$$\sigma = \text{constant} \cdot (d_i - d_g)^4 \tag{1}$$

where d_l and d_g are the densities of the liquid and gas, respectively, and σ is the surface tension of the interface separating the liquid and gas. This formula was expressed by Sugden as2

$$P = \sigma^{1/4} M/(d_i - d_g) \tag{2}$$

where M is the molecular weight of the substance, and Pis a constant (characteristic of the substance) called the parachor. As is well known3, equation (1) is only approximately true; that is, the parachor varies slightly with temperature near the critical point. In recent years the

. COMPARISON OF EXPERIMENTAL AND THEORETICAL VALUES OF THE CRITICAL VOLUMES FOR A FEW REPRESENTATIVE SUBSTANCES

Substance	α	\boldsymbol{P}	p_c	T_c	v_c exp.	V_c formula
Equilibrium hydrogen	0·310	35·2	12·77	33	65·5	65·3
Methane	0·290	73·2	45·8	191·1	99	98
Methyl acetate	$0.255 \\ 0.231$	177·0	46·3	506·9	228	229
Water		52·4	218·3	647·4	56	55

parachor has fallen into desuetude, undoubtedly because it was linked2 to an empirical view of the structure of atoms and molecules, which is no longer acceptable. Nevertheless, equation (2), considered only as an approximate experimental law, can still be useful as will be seen

Recent numerical results4,5 show that the threedimensional Ising model, which is believed to describe correctly many real substances near the critical point, satisfies equation (1) within the precision of the numerical method. Thus, at least for such a statistical model, the existence of the parachor may have some theoretical justification*. An Ising model is defined by three parameters, for example, p_c , V_c , T_c , pressure, molecular volume and temperature at the critical point.

Dimension considerations show at once that for such a model

$$P = f(\alpha) \ p_c^{1/4} \ V_c^{13/12} \tag{3}$$

where $f(\alpha)$ is an unknown function of α , and α is the dimensionless parameter

$$\alpha \equiv p_c \ V_c/R \ T_c \tag{4}$$

where R is the universal gas constant. It happens that α varies only slightly from substance to substance. (If a were a constant the law of corresponding states would apply exactly.) Then an excellent representation for $f(\alpha)$ should be

$$f(\alpha) \simeq A\alpha^B \tag{5}$$

assuming that $f(\alpha)$ has no pathological behaviour. A and B are two universal but unknown constants. Comparison with experiments gives $A = 1.01 \times 10^{-2}$, B = 5/12 and

$$P = 1.01 \times 10^{-2} \alpha^{5/12} p_c^{1/4} V_c^{13/12}$$
 (6)

Equation (6) is a relation between the four parameters $(P, p_c, T_c \text{ and } V_c)$. V_c is the most difficult to obtain experimentally. Equation (6) is useful to obtain V_c , when P, p_c, T_c are known, or in practical units

$$V_c \text{ (cm}^3) = 7.10 \ P(\text{c.g.s.}) \ T_c \ {}^{4/18}_{\circ} \ p_c \ (\text{Atm})$$
 (7)

WE used equation (7) for all substances listed by Kobe and Lynn¹⁰ for which the critical co-ordinates are known. The parachor is always known either experimentally or using precise rules^{2,11}. The agreement between the experimental values and those given by equation (7) for V_c was better than 5 per cent. For most substances (75 per cent of cases) the agreement was even better than 3 per cent. This agreement is excellent considering the experimental uncertainty of V_c . There are about fifty substances for which P, p_c , T_c are known and V_c unknown. Equation (7) may be used with confidence to obtain a reliable value for V_c . Numerous empirical relations have already been proposed, linking the parachor and the critical co-ordinates^{2,7,10,11}. Most are a special case of equation (7) for a certain class of substances, for example, for a given α or a given p_c . Contrary to equation (7) none of them applies to all substances. In particular,none can be used at the same time for equilibrium hydrogen (largest α) and for water (one of the smallest α). In

 $\sigma \simeq \text{constant}$, $(d_1 - d_0)^3$

instead of equation (1).

^{*} Fowler's derivation of the parachor for a "classical" (van der Waals type) substance is incorrect. He assumed a discontinuous interface between bulk liquid and bulk gas, while it is well known. Interface between the interface becomes infinitely thick as the temperature approaches its critical value. Actually "classical" substances satisfy an equation of the form.

Table I we give a sample of four substances which cover the whole range of a. (Experimental results are from refs. 2 and 10.)

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BIOPHYSICS

Electron Paramagnetic Resonance in Frozen and Dried Biological Materials

Kent and Mallard¹ and Truby and Goldzieher² have recently indicated that free radicals formed in organic material are artefacts, apparently resulting from lyophil-Our findings are not in agreement with this concept. We believe that lyophilization of living biological materials, of and by itself, produces few if any free radicals. Our investigations indicate that the electron paramagnetic resonance signals observed by others3-5 were the result of a reaction between oxygen and an unidentified component in dried specimens which formed free radicals during the interval between lyophilization and observation in the electron paramagnetic resonance spectrometer.

For several years we have been observing easily detectable free radical production in dried bacterial preparations^{6,7}. As long as oxygen was excluded, no free radicals were detected in dried bacteria, even at the highest sensitivity of our instrument. In the presence of dry air, however, we had to attenuate sensitivity to measure the signals formed.

In some experiments material was dried in a device which enabled the sample to be transferred into electron paramagnetic resonance tubes without exposure to air. Later, the samples were formed into pellets which could be dried in 4.4 mm internal diameter thin-wall 'Pyrex' sample tubes. This latter method of drying the samples is more convenient and more certain of excluding oxygen than is the method of packing in vacuum.

Fig. 1 presents results of an experiment in which rat liver was homogenized, frozen in small spheres and dried in the electron paramagnetic resonance cavity. The liver was removed from the rat, rapidly cooled by washing in cold saline, and homogenized with a 'Pyrex' tissue grinder. Large particles were removed by centrifugation to ensure the uniformity of the suspension. Drops of this homogenate were frozen in a mixture of 'Freen 112' and heptane to form spheres as described previously8. Pellets were transferred to the electron paramagnetic resonance tubes at -70° C and attached to a suitably trapped high vacuum system. Sublimation of water kept the pellets frozen after the tubes were inserted into the electron paramagnetic resonance cavity, so the strength of the electron paramagnetic resonance signal in the frozen material was easily determined before drying as well

as at intervals thereafter—all without interrupting the drying process. As shown in Fig. 1, the concentration of free radicals in either the frozen or dried sample was too low to be detected. Within five minutes after admitting dry air, an appreciable signal appeared at about g = 2.0045. It is evident that measurements made at any time after exposure to oxygen do not represent the concentration in the tissue before drying. Because Dettmer et al.³ stored their samples at room temperature for "lesthan 3 days" before examining them in the spectrometer, the conclusion they drew regarding the effect of time between killing the animal and the appearance of a free radical in the tissue at the time of freezing may not be They implied that free radicals observed in tenable. lyophilized tissue were present before the tissue was frozen. We recognize that there are limited amounts of free radicals in normal tissue. The bulk of the electron paramagnetic resonance signal Dettmer et al.3 observed. however, probably arose from subsequent reactions with oxygen because these workers apparently made no attempt to exclude air from the samples.

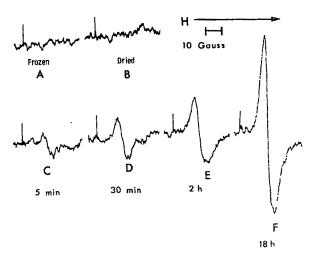


Fig. 1. Effect of drying (A and B) and exposure to air (C–F) on electron paramagnetic resonance spectra of rat liver homogenate. The short vertical line on each trace is b magnetic field marker. These spectra were obtained with a dual-cavity X-band Varian spectrometer, model 4500, with a 9 in. electromagnet and 100 kc/s field modulation. The modulation amplitude was approximately 3 gauss with a microwave power of about 30 mW.

With regard to the stability of the radical, Dettmer et al.3 stated that there was a severe loss of signal amplitude during 2.5 weeks and that moisture suppressed the electron paramagnetic resonance signal. In our studies we found that the stability of the radical was related to humidity and at any relative humidity above 30 per cent the signal intensity decreased after reaching a peak in less than 5 days. Fig. 2 shows the effect of humidity on both the increase and subsequent reduction in electron paramagnetic resonance signal intensity. We have observed that in dry air (<10 per cent relative humidity) free radical concentration remained unchanged for years.

Miyagawa et al.9 indicated that the paramagnetic resonance in dried biological substances was caused by sorbed oxygen because the signal decreased when oxygen was removed. We have found, however, that the signal intensity, developed in air, was not reduced by evacuation at 10-4 to 10-5 torr for 2-3 days. Morozova and Bluymen. fel'd4 also found that high vacuum did not reduce the signal in dried rat liver or spleen and they concluded that the reversibility mentioned by Miyagawa et al.9 was an artefact. In view of the relatively weak signals reported by Miyagawa et al. and the fact that the resonance curves seem to have a shape different from those in Fig. 1. we believe that the free radicals we find in dried tissue and bacteria are the result of a reaction between oxygen and some organic material in the sample-not just a result of

physical sorption of oxygen.

Dettmer et al.3 indicated that the largest signal was obtained from liver and the smallest from spleen. We and others4,8 found, however, that the spleen consistently produced a higher concentration of free radicals than the liver did. The most reasonable explanation of Dettmer's finding is that moisture in his sample may have caused rapid loss of free radicals because we have observed that dry spleen is more hygroscopic than dry liver.

It is difficult to identify or measure the material (free radical precursor) which reacts with oxygen to produce free radicals because this precursor is present in limited amounts and accumulation of the free radicals is influenced by many factors. A number of substances such as ethylenediamine tetraacetic acid (EDTA), sucrose, glucose, ribose or even sodium chloride inhibit or prevent subsequent free radical production when added to the tissues before they are frozen. In bacterial preparations the extent of free radical production seems to be a function of viability, and if cells were killed before lyophilization no free radicals were produced when samples were exposed to air6.

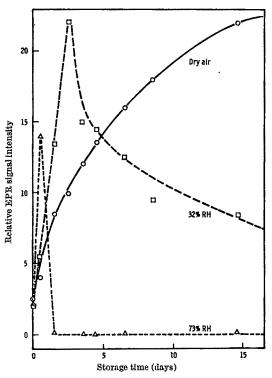


Fig. 2. Effect of relative humidity on electron paramagnetic resonance signal intensity of lyophilized rabbit liver. Saturated salt solutions were used to maintain relative humidity in air above the samples.

We have tried to determine the nature of the free radical Preliminary evidence indicates that two components are involved: one with a molecular weight between 50,000 and 100,000 and the other with a molecular weight less than 25,000. These react to form the precursor. Propyl gallate or adrenaline can react with the larger molecule to form a precursor which seems to be the same as that in whole tissue. The high molecular weight material seems to be heat stable. Although these studies on identification of the system in micro-organisms and tissues are incomplete, we believe that the act of lyophilization, per se, produces no free radicals in biological systems.

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PHYSIOLOGY

Position of Onset of Fast Phase in Optokinetic Nystagmus

OPTOKINETIC nystagmus is the cycle of slow forward and fast return phases of the eye when it follows a continuous movement in the whole visual field. Although optokinetic nystagmus has been frequently studied in a variety of animals, there is no information as to how the point of onset of the fast return phase is determined. In the crab Carcinus both eyes respond to the movement of a striped drum, although one eye moves towards the midline while the other moves away from it. For both eyes to respond in either direction it is sufficient for one eye to see the drum1. J. Barnes, of this laboratory, has recently shown that the eye which makes the slow forward phase towards the midline "governs" the onset of the fast phase and leads by 30-50 msec (unpublished results).

The onset of the fast phase is not directly controlled by proprioceptors or mechanoreceptors of the eye socket. Impulses to muscles which bring about the fast phase can be recorded in the oculomotor nerve of a blind eye which is driven by the other eye, and their pattern is unchanged by clamping the responding eye or even by The same result is obtained when the removing it1. oculomotor nerve of the driving eye is cut so that there is no movement or muscle tension in the driving eye, and no visual information from the blind moving eye2. Furthermore, the position reached by either eye or the tension in muscles does not control onset of the fast phase because a blinded eye, driven by a clamped seeing eye, can itself be clamped or set in different positions with no effect on the frequency of nystagmus, as recorded in the nerves.

The onset of the fast phase is not controlled directly by the visual input, as optokinetic memory experiments showed3. A small movement of a striped drum around the crab is made during a dark period; when the light is restored the eyes follow the drum. Because the eyes respond while the drum remains stationary, the stripes will seem to move in the opposite direction to the response. When a clamped, seeing eye drives a blind, moving eye in this experiment, both the seeing eye and the drum remain stationary when the light comes on. The blind eye can be induced to undergo a nystagmus at any time during its response by first moving it under the control of the illuminated drum. Experiments such as these show that the onset of the fast phase cannot be controlled directly by what the crab sees at that moment. There is no distinction between the mechanism of onset of the fast phase and that which determines the impulse frequency to the muscles which move the eye. The stimulus for each is the total movement seen since the previous fast phase. Each fast phase wipes clean the mechanism for a fresh start on the efferent but not on the afferent side.

The question thus arises as to how the brain of the crab acquires the information to make the fast return phase at the appropriate position of the eye without visual or mechanoreceptor clues. In any one crab the position of the onset of the fast phase is constant to about 1°, but crabs differ greatly in this respect. We have shown that at the onset of the fast phase the slow motor impulse frequencies to the appropriate eye muscles which cause the slow forward phase vary considerably between crabs, but each crab has eye movements which are suited to the anatomy of its own exoskeleton. To test whether learning is involved, we tried the following experiment. With both eyes free to move and the left eye blinded, movement of the right eye was recorded with the slow phase towards the midline, that is, an eye leading in the fast phase. When the right eye reached a point about 1° before the normal onset of the fast phase, the crab received a shock to the carapace in the area innervated by the antennular or tegumentary nerve. If this did not cause a withdrawal reflex, further shocks were received at about 10/sec until the eye was withdrawn inside the limit beyond which shocks were given. Meanwhile, the movement of the drum continued to drive the eye towards the point of shock. At first the shocks cause retraction before the eye makes a normal fast phase, but as long as only a small change is demanded of the system, the onset of the fast phase soon appears after a shorter traverse so that the shock is avoided. The adaptive change lasts for many minutes before the excursions in nystagmus recover their former size. We have found that records of the instantaneous frequency of muscle twenty-one, which is responsible for the slow forward phase towards the midline, show the same phenomenon. These results are now in preparation. The onset of the fast phase occurs at a lower motor output frequency to muscle twenty-one after treatment with shocks which would cause eye with-This shows that the effect is not caused by a sustained partial withdrawal which would merely hold the eye away from the midline. A full description of the interaction between the eye withdrawal reflex and the optokinetic response, in terms of motor impulse frequencies, is in preparation. This adaptive change, however, can scarcely be classed as conditioning because shocks delivered without reference to eye position also cause the fast phase to occur after a shorter than normal traverse of the eye, or after a less than normal frequency of slow motor impulses to muscle twenty-one. Shocks which cause withdrawal of the eye have no lasting effect on the eye position or on the frequency to the relevant muscle, but subsequent fast phases occur at a lower frequency and therefore further from the midline.

The significance of these results is perhaps as follows. The brain seems to make no use of information on the position of the eye except that which is equivalent to the motor output to the eye muscles. When the slow phase is towards the midline, contact of the eye with the edge of the socket will excite mechanoreceptors of either the eye or the socket which can in fact induce eye withdrawal. Whether or not there is a complete withdrawal, repeated excitation in these nerves shortens the traverse of subsequent nystagmus movements by influence on the onset of subsequent fast phases. The withdrawal reflex is a movement away from the midline, and the fact that the eye, making a slow phase towards the midline, governs the onset of the fast phase for both eyes agrees with the idea that the withdrawal reflex provides the reinforcament. By this means the animal could learn the fine control of the position at which it must make a fast phase to avoid running its eye into the edge of the socket, which changes at each ecdysis. Such a mechanism, compatible with the observed independence of proprioceptive

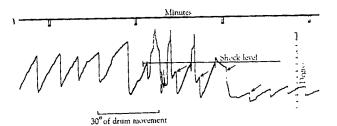


Fig. 1. Effect of shocks to the socket region (in this case to the right antennule) on the initiation of the fast phase by the right eye, with the striped drum moving towards the midline for this eye. Horizontal line marks the point beyond which further movement in the slow phase caused shocks to be applied at about 10 c/s. Note the fast phase occurring at successively lower points (arrows) after each period of shocks and the new level of much earlier fast phases which no longer reach the position where shocks are delivered.

control, would provide the animal with a means of assessing the detail in its own anatomy and would explain the differences between individuals. I do not believe that the whole efferent pattern is learned, but only the modification of some aspects of the fine detail. Accuracy of following in the slow forward phase is achieved by the feedback loop which is the consequence of the movement of the eye. Although the tests with indiscriminate shocks on the onset of the fast phase show that the shortening of the eye traverse is not caused by the association of the shock with the point at which it is received, they are irrelevant to the situation in the crab because the direction of the effect is that required to be adaptive.

Comparable experiments have shown that the postural control of the legs in insects4 is also achieved without proprioceptive feedback by a mechanism which seems to use the appropriate efferent motor frequency as its only means of knowing the position of the leg⁵. In the insect system, however, there proved to be an element of conditioning and the efferent frequency can be modified so that it is either greater or less than the initial spontaneous rate. The crab eye preparation is the second which illustrates an adaptive mechanism in which a non-specific reinforcement (stimulation of a mixed sensory nerve of the relevant anatomical area) influences a subsequent part of a central programme which is not directly under proprioceptive control. It is intriguing to suppose that this type of mechanism of plasticity is the one most suited to animals in which many of the activities are controlled by central programmes of motor impulse sequences which are not instantaneously monitored by mechanoreceptors.

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Reduction of Strontium Absorption in Man by the Addition of Alginate to the Diet

CONSIDERABLE effort has been expended in the search for a non-toxic substance which will reduce the intestinal absorption of radioactive strontium without interfering with calcium absorption. A compound which approaches these requirements is an extract from brown seaweed known commercially as sodium alginate. It consists of a mixed polymer of L-guluronic and D-mannuronic acids. of which the ratio, referred to as the G/M ratio, depends on the botanical source and the method of extraction.

The effectiveness of sodium alginate in reducing strontium absorption in both man and laboratory animals has often been investigated¹⁻¹⁰, but, because of the variability in the composition of the alginates used, comparison of results has been difficult. When, however, various commercial forms of sodium alginate (from Alginate Industries Ltd., 22 Henrietta Street, London, WC2) with measured G/M ratios were added to the normal diet of the rat, the alginate, 'Manucol SS/LD', with the highest G/M ratio was the most effective in reducing the skeletal uptake of strontium without affecting the calcium uptake.

An alginate derivative, referred to as O.G.1, and with a G/M ratio of 97 per cent, has now been prepared in this laboratory¹¹. When O.G.1 was compared in rats with 'Manucol SS/LD' with a G/M ratio of 71 per cent, a correlation was again observed between the guluronic acid content of the salt and the degree of reduction of strontium absorption. Calcium uptake was not affected.

In view of these results an experiment was designed to test the relative efficacy of 'Manucol SS/LD' and O.G.1 in suppressing strontium absorption in man, and the results of the experiment are presented in this communication.

The two alginates tested, 'Manucol SS/LD' with a G/M ratio of 71 per cent and O.G.1 with a G/M ratio of 97 per cent, were dissolved separately in homogenized milk in the ratio 10 g to 1 l. of milk. The strontium-87m, as a neutral solution of the chloride, was added to the mixtures of milk and alginate immediately before ingestion. In the control experiments, the milk contained only the radioactive marker.

All volunteers adopted the following regimen. Subjects fasted overnight and at about 09.15 h each drank 250 ml. of milk containing 10 μc . of strontium-87m (control), or milk containing the same radioactive marker and either 'Manucol SS/LD' or O.G.1. The same quantity of milk, or milk with alginate but unlabelled, was ingested at 12.30 h. No other liquids or solids were taken during the period of experiment (09.15–16.15 h). Three subjects repeated the tests in control and the two experimental conditions with an interval of 1 or 2 days between each test to allow for the decay of the strontium-87m and excretion of the alginate additives.

Two hours after the radioactive dose had been given, 25 ml. of blood was taken by venipuncture into a heparinized centrifuge tube and the plasma separated for counting. Urine was collected for 7 h from the time of ingestion of the radioactive dose and the count rate in 10 ml. samples of plasma or urine was compared with that of a standard source of strontium-87m in a well type scintillation counter. The percentage of the dose in 1 l. of plasma and in the 7 h urine was derived from these observations. The calcium contents of the milk and urine were measured by flame spectrophotometry.

In Table 1 the concentrations of radioactive strontium in plasma, expressed as the percentage of the dose/l., are shown for all four subjects in each experimental situation. Individual results usually differ from their means by less than 5 per cent and the lowest plasma concentration of strontium-87m occurred in all subjects after the ingestion of O.G.1.

Table 1. CONTENT OF STRONTIUM-87m IN THE PLASMA OF FOUR SUBJECTS, 2 H AFTER AN ORAL DOSE

			Per cent Subj		
Diet		E. H.	G. H.	A. S.	J. L.
Milk (control)	Mean	0·530 0·638 0·584	0·555 0·590 0·578	0·475 0·485 0·480	0.718
Milk + SS/LD	Mean	0·245 0·151 0·198	0·305 0·325 0·315	0·107 0·105 0·106	0.203
Milk + O.G.1	Mean	0-083 0-092 0-088	0·078 0·109 0·094	0·079 0·079 0·079	0.130

Table 2. CONTENT OF STRONTIUM-87m IN THE URINE AFTER AN ORAL DOSE

		,	Subj		
Diet		E. H.	G. H.	A. S.	J. L.
Milk (control)	Mean	1·06 1·36 1·21	1·12 1·58 1·35	1·0 0·75 0·88	3.37
Milk + SS/LD	Mean	0·348 0·198 0·273	0-575 0-520 0-548	0·099 0·137 0·118	0.748
Milk + O.G.1	Mean	0·146 0·10 0·123	0·153 0·227 0·190	0·096 0·142 0·119	0.462

Amounts of strontium-87m excreted in the urine during the 7 h following the dose are given in Table 2. Individual results and means are shown for each subject as in Table 1, but, unlike the results for plasma, the individual values differ from their means by 10-20 per cent. Concentrations of strontium-87m in the urine follow the same pattern as the plasma, with the lowest output occurring after ingestion of O.G.1. The high calciuria and strontiuria for subject J. L. has been observed before 12.

The ratios, experimental to control, of strontium-87m in plasma and urine are given in Table 3. Results for O.G.1 show that the marker concentration in the 2 h plasma samples was reduced about six-fold and in the 7 h urine samples about eight-fold, compared with the controls. Treatment with SS/LD produced a three-fold decrease of strontium-87m in the plasma and a four-fold decrease in the urine compared with the controls.

Table 3. ratios of experimental : control contents of strontium-87m in plasma and urine

	Milk + SS	LD: milk	Milk + O.G.1 : milk		
Subject	Plasma	Urine	Plasma	Urine	
E. H.	0-340	0.226	0.151	0.10	
G. H.	0.550	0.406	0.164	0.141	
A. S.	0.221	0.134	0.164	0.135	
J. L.	0.282	0-220	0.181	0.137	
Mean	0.348	0.246	0.165	0.128	
S.D.	+0.189	+ 0-098	+ 0.015	+0.016	

Table 4. MEAN CONTENT OF CALCIUM IN URINE FROM EACH OF THE FOUR SUBJECTS IN THE THREE EXPERIMENTAL SITUATIONS

	Calciu	n (mg/7 h)	
Subjects	Milk	Condition Milk + SS/LD	Milk + O.G.1
E. H.	47.5	31.5	30.9
G. H.	68.5	61.9	58-9
A. S.	44.2	28.9	32.1
J. L.	151	162	117

Concentrations of calcium in the urine of the four subjects are given in Table 4. In general, there is a reduction in urinary calcium after milk supplemented with either SS/LD or O.G.1 had been ingested.

Some of the advantages of using strontium-87m to estimate intestinal absorption have been discussed previously¹³. The short half life (2·8 h) was particularly useful in these studies because the whole series of tests could be performed in 3 weeks.

Milk was considered to be a satisfactory form of diet, because it has been shown that strontium in milk is fully available for absorption¹⁴ and it was desirable to start with a basic material which was free from substances which might inhibit uptake of strontium. Errors resulting from non-uniform mixing of the alginates with the diet were avoided, for the two alginates were dissolved in the milk. Variation in composition between different batches of milk was small and the constancy of the calcium content, 1·0–1·1 g/l., was an advantage as it was an approximate equivalent to the 10 g of alginate added to each litre of milk. This 10:1 ratio of alginate to calcium was also used in the rat diet^{5,8}.

Concentrations of radioactive strontium in plasma (0.48-0.72 per cent dose/l.) observed during the control experiments were in agreement with values found earlier^{13,15,16}. Following the ingestion of 'Manucol SS/LD' there was a mean reduction of 64 and 75 per cent in the concentration of radioactive strontium in the plasma and urine, respectively. These values compare well with the reduction of 66 and 71 per cent in the radioactive marker

content of plasma and urine of rats after feeding with SS/LD⁵. Absorption in these rats, estimated from the sum of the radioactive strontium skeleton and urine, was reduced by 73 per cent.

Concentrations of strontium-87m in plasma and urine were even lower after O.G.1 had been ingested. The urine indicated a reduction in absorption of 87 per cent and the plasma indicated a reduction of 83 per cent. These results again correspond well with a reduction of 78 per cent found in the urine of rats fed on a diet containing $O.G.1^{s}$. Hesp⁴ also found that urine showed a greater reduction than did the plasma, but both values were in reasonably good agreement with the figures for true absorption as measured by means of a whole body counter.

Results for the calcium content of the urine are incon-There is a slight indication in three subjects that the calcium content in the urine falls after the ingestion of 'Manucol SS/LD' or O.G.1, but further experiments would be necessary to verify this point. When rats were fed for 1 yr on a diet containing a 10 per cent supplement of 'Manucol SS/LD', however, absorption and excretion of calcium were found to be normal (unpublished work of T. E. F. Carr).

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Coupling Resistance of Double **Barrelled Microelectrodes**

THE double barrelled microelectrode is a convenient way of introducing two electrode tips into the same cell for simultaneously passing a current and recording. The chief source of artefact is the coupling resistance (Rc) (ref. 2), which is manifest when a current passed through the stimulating barrel causes a voltage to appear at the recording barrel. It presumably represents the resistance of electrolyte immediately adjacent to and shared by both tips, for most of it disappears if the very tip of the electrode is broken off. When current is passed from an intracellular double barrelled electrode, the coupling resistance with the electrode intracellular (Re_i) is in series with the cell membrane resistance viewed by the recording barrel. Rc_i must therefore be subtracted from the observed input resistance to obtain the true value of the input resistance and of voltage displacements. The problem is to know the value of Rc_i , because Rc can only be measured directly with the electrode extracellular. The simplest method² is to assume that Rc does not change on impalement, which is to assume that Rc_i is the same as Rc_{e^-} the value obtained on withdrawal from the same cell.

These experiments were designed to test this assumption. The voltage record from the recording barrel of a double barrelled electrode was monitored by a second, nearby intracellular electrode to give an independent measure of the input resistance, Rm. Rc_i was then determined by subtracting Rm from the value for $(Rm + Rc_i)$ found with the double barrelled electrode. The cells were fibres of the sartorius muscles of the frog Rana temporaria (or, in the case of electrodes 9 and 10, the toad Bufo bufo). They were bathed, at room temperature, in frog Ringer solution, to which 1 mg/ml. cocaine had been added to prevent electrical and mechanical responses. Double barrelled micropipettes were made and filled with 3 molar potassium chloride as described by Wardell³. The resistance of each barrel in the bathing solution was accepted in the range 17-90 M Ω , and the coupling resistances (Rc_e) ranged from 40–650 k Ω . No further selecting was done, hence the series represents the first ten electrodes to satisfy these criteria. Conventional methods of stimulating and recording were used with a beam-splitting device to display the three records (current and voltage from separate barrels of the double electrode, and voltage from the separate recording electrode) on a Tektronix type 502 oscilloscope, where they were photographed. Single fibres were impaled simultaneously with both the double and the single barrelled electrodes, the two being less than 50µ apart along the fibre. Five current pulses of 200 msec duration, ranging in strength from 1 to 10×10^{-8} amp, were passed in each direction through the lower-resistance barrel of the double barrelled electrode. The latter was then withdrawn and the current pulses repeated to obtain Rce. For each current pulse the voltage recorded by the single electrode was subtracted from that recorded by the double electrode. This difference was plotted against the strength of the applied current, and Rci was taken as the slope of a straight line fitted to these points by eye. A fluctuating voltage record (representing instability of Rc_i) was sometimes obtained from the double barrelled electrodes, particularly with the highest current pulses. Such records were discarded. No correction was considered necessary for the separation of the electrodes along the fibre, because the distance of 50µ is much smaller than the space constant of 2 mm found by Fatt and Katz4. Any error caused by this assumption would tend to overestimate Rci. On an average, three randomly selected fibres were studied with each of the ten double barrelled electrodes.

The results are summarized in Table 1 and Fig. 1. The chief finding was that the coupling resistance increased in twenty-six out of the thirty impalements. Over the series the mean value of Rc_e was 322 k Ω , while Rc_i was larger by a mean value of 316 k Ω . In threequarters of the impalements the increase was less than 100 per cent of Rc_e , ranging in value from $-100 \text{ k}\Omega$ to $+360~k\Omega.$ The remaining quarter included four very large increases, the worst being 1,380 $k\Omega$ (= +430per cent of Rce). In addition, marked rectification sometimes appeared, as shown in Table 1.

The large increases only occurred with electrodes of higher Rce. Of the eight impalements with Rce less than 250 k Ω (mean = 156 k Ω), all increases were smaller than 200 k Ω (mean=112 k Ω =95 per cent of Rc_e). Of the twenty-two impalements with Rc_e greater than 250 k Ω (mean = 383 k Ω) the mean increase was 392 k Ω (= 112 per cent of Rce) and it was this group which contained all the large increases. As seen from Fig. 1, one cannot predict that any given electrode of high Rce would necessarily give more error than one of lower Rce, but, in general, electrodes of Rc_e less than 250 k Ω have a smaller and

Table 1. CHANGE OF Rc ON IMPALEMENT

Electrode number	Bar resistan Record		Out	In fibre 1	Out	Coupl In fibre 2	ling resis (kΩ) Out	tance In fibre 3	Out	In fibre 4	Out	Mean increase during impalement $(k\Omega)$
1 2 3 4 5	23 40 60 30 63	17 20 50 23 45	400 285	201 142 590 525 520	137 40 640 295 315	163 95 550 751 455	167 40 590 280 295	370 1,600 310	435 300 310			30 79 -52 667 122
6 7	35 30	28 23	260 300	390 1,200	200 360	395 (1,450) (*780)	210 300	380 (2,160) (*1,240)	215 320	600	300	180 835
8 9 10	45 75 90	40 65 50	360	520 600 380	410 410 240	470 730 (840) (*360)	370 370 380	700 700 400	350 480 360	2,200	540	120 608 133

Mean Rc change for ten electrodes, thirty impalements = $\pm 316 \text{ k}\Omega$.

* Impalements in which Rci showed marked rectification. Upper figure is resistance to depolarizing currents, and lower figure resistance to hyperpolarizing currents. The mean of the two has been taken in subsequent calculations and in plotting Fig. 1.

more consistent increase on impalement than those of larger Rc_e . There was no correlation between barrel resistance and the increase of Rc on impalement.

Thus, over the whole series, Rc nearly doubled on It is not surprising that the increase impalement. occurred, for two reasons. First, there is evidence that the specific resistance of myoplasm is three times that of the external solution⁵. This would cause part of Rc to rise by 200 per cent, but would probably not affect any component of Rc arising within the tip of the pipette as a result of, for example, the interbarrel septum finishing short of the tip. The resultant increase in Rc would thus be expected to be less than 200 per cent. Second, the area in and around the very tip of the pipette, which would be expected to contribute most of Rc, is also the part most liable to be blocked by cell organelles or membranes which could cause Rc to rise manyfold. It is tempting to speculate that such a blockage occurred in those four impalements (Fig. 1) in which Rc increased by exceptionally large amounts. If this is true, it is surprising that it did not happen more often, because the cell is full of membranes and organelles which could cause blockage. Over the whole series, allowance for a

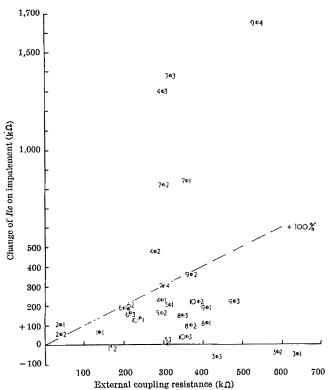


Fig. 1. Relationship of the change of coupling resistance on impalement to the external coupling resistance. Each point is denoted by the number of the electrode (1 to 10) followed by the number of the fibre impaled by it (1 to 4).

doubling in Rc on impalement would have given a good correction of the error, but where Rc_c was greater than 250 k Ω , individual records could not be relied on because of the occasional very large increases. For individual records to be meaningful, Rc_c should be as small as possible and in any case smaller than 250 k Ω , while the cell input resistance should be relatively large compared with Rc_c . Even then there is no guarantee that an individual electrode is giving an accurate record; results using several electrodes should thus be compared.

The exact numerical values obtained here should not be assumed to apply directly to other series of electrodes because of likely differences in their physical properties, but the trend might be expected to be followed.

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Relation between Ganglion Cell Activity and the Local Electroretinogram of Cat Retina

DELAY in the decay of the late receptor potential (late RP) of monkey retina has been demonstrated accompanying a progressive increase in the intensity of a 20 This delayed decay of the late receptor potential is accompanied, in the same retina, by a delayed decay of the d.c. component of the local electroretinogram². The d.c. component is thought to originate in the inner nuclear layer, and the late receptor potential in the receptor layer^{1,3}, and so the authors suggest that "the late receptor potential of the receptors is a crucial event which controls the activity of cells of the inner nuclear They indicate further than other off-responses of the visual system might be similarly related to the late receptor potential if they could be delayed by increases of stimulus intensity. With this in mind, I observed a relevant effect of stimulus intensity on the discharge pattern of cat ganglion cells. The on-response, at high intensities, continued into the off-period instead of terminating at the "off" of the flash. In addition, this ganglion cell effect seemed to be related to the behaviour of the d.c. component of the local electroretinogram.

Ganglion cell discharges and intraretinal slow potentials were recorded in unanaesthetized cats, decerebrated and paralysed with gallamine triethiodide. A closed eye recording and stimulating technique, almost identical with that described earlier⁴, was used. All potentials

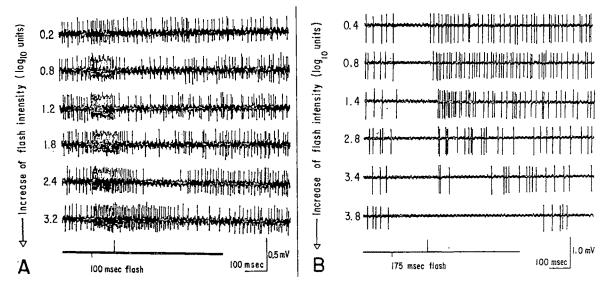


Fig. 1. The ganglion cell on-response as a function of flash intensity. A, On-excitation of a ganglion cell as a function of flash intensity. A 0.33 mm stimulus spot, 100 msec in duration, was centred on the microelectrode in the area centralis, and repeated once every five seconds. A 2.0 mm adapting spot was also centred on the electrode. The stimulus spot was optimal in size for stimulation of the receptive field centre of this ganglion cell as determined by an area-threshold technique. The intensity of the adapting spot was 10²⁻⁶ trolands while the threshold intensity of the stimulating spot was 10²⁻⁶ trolands. Threshold intensity was defined as the intensity that produced a stimulus-locked change in spontaneous activity that was both audible and visible to the experimenter with every flash. Amplification was condenser-coupled with the bandpass set from 30 c/s to 30 kc/s. Negative responses are displayed upward. B, On-inhibition of a ganglion cell as a function of flash intensity. The intensity of the adapting spot was 10¹⁻⁶ trolands, and the threshold intensity of the stimulating spot was also 10¹⁻⁶ trolands. Otherwise, the conditions of stimulation and recording are the same as in A.

were recorded with 3 molar potassium chloride glass micropipettes referred to a vitreal chlorided silver wire. The cornea was protected by a contact lens and the pupil was dilated by instillation of 10 per cent phenylephrine hydrochloride.

A dual-beam ophthalmoscope⁵ was used for stimulating and viewing the retina with tungsten filament lamps in both beams. A shutter in the stimulus beam produced step functions adjustable in duration and rate with equal rise and fall time of 1 ± 0.05 msec. The stimuli were in the form of spots (diameter on the retina: 0.06 mm-2.0 mm), and annuli focused on the retina after the refractive error of the eye had been corrected. The maximum available retinal illuminance in the stimulus beam was 105,9 trolands. The receptive field centre was first located by centring the flash on the tip of the microelectrode, and a spot was chosen with a diameter which stimulated optimally only the centre mechanism (area-threshold technique)6,7. A background spot, larger in diameter than the receptive field, was also centred on the micro-

Fig. 1 shows the responses of ganglion cells to flashes of increasing intensity. At moderate intensities (0.5-1.5 log₁₀ units above threshold), the on-response abruptly terminated at the "off" of the flash and was followed by an off-response of opposite sign, for example, in Fig. 1Bon-inhibition was followed by off-excitation 8-14. Actually, the on-response always continued past the off-transient of the flash for a brief period of time (usually 10-25With additional increases in flash intensity, however, a threshold was reached at which the onresponse extended farther into the off period. Additional increases in intensity enhanced this extension of the onresponse by increasing its strength and duration. was observed in both on-excited and on-inhibited receptive field centres, as an extension of excitation, in the former (Fig. 1A, 1.8-3.2), and of inhibition (Fig. 1B, 2.8-3.8), in the latter. This phenomenon occurred only when the flash intensity was well above threshold and was most clearly defined at intensities greater than 2.0 log10 units above threshold. Lowering the intensity of the background beam lowered both the threshold of the onresponse and the threshold for extension of the onresponse. Extension was observed at all the flash durations tested between 5 msec and 1 sec. It was still observed with flashes smaller than the receptive field centre; with very small flashes (area $< 0.01 \text{ mm}^2$), the threshold was raised.

This effect occurred at high intensities relative to threshold ($>2.0\log_{10}$ units), and so stray light may also have been a significant additional stimulus. It could be postulated that stray light stimulated the receptive field surround and that extension was actually an off-response of the surround appended to the on-response from the centre. At the intensities necessary to produce extension, however, the response of the unit was almost always dominated by the centre mechanism. Even, with stimulation of the surround alone, using annular flashes, stray light often triggered the centre mechanism making it difficult to study the surround on-response at high flash intensities.

The off-response was also altered at high flash intensities. In some cells the off-response, although weakened, could still be identified after the termination of the extended on-response (for example, the inhibitory period after the on-excitation in Fig. 1A), while in others it could not be identified easily⁸⁻¹⁰. The persistence of the off-response at high intensities was also related to the duration of the flash. For example, with flashes of 250 msec and longer, in on-excited cells, a brief period of inhibition sometimes remained at a short latency from the "off".

The appearance of the local electroretinogram in the light-adapted area centralis is shown in Fig. 2. potential was presumably recorded just distal to the outer margin of the inner nuclear layer, because the microelectrode had been adjusted to record a maximum offresponse¹⁵. It consisted principally of a negative b-wave and a negative d.c. component, together forming PII of Granit¹⁶. Following the off-transient of the flash, the d.c. component formed an off-response by decaying rapidly toward the baseline. At moderate intensities (<1.5 log₁₀ units above threshold), the off-response always followed the off-transient of the flash at a short latency, for the d.c. component continued into the off-period for only 15-25 msec. Intensities which were much greater than threshold (>2.0 \log_{10} units) always altered this response. The principal effect of high intensity illumination was to increase the duration of the d.c. component. In Fig. 2,

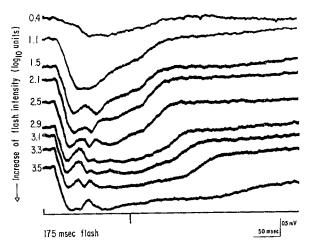


Fig. 2. The local electroretinogram as a function of flash intensity. A 1-0 stimulus spot, 175 msec in duration, was centred on the microelectrode in the area centralis and repeated once every five seconds. A 2-0 mm adapting spot was also centred on the electrode. The stimulus spot was optimal in size for the production of a local electroretinogram at the lowest stimulus intensity as determined by an area-threshold technique. The intensity of the adapting spot was 10¹⁻² trolands while the threshold intensity for the stimulus spot was 10¹⁻⁴ trolands. Threshold intensity was defined as the intensity that produced a local electroretinogram, 0-1 mV in amplitude, with every flash. Intensities are specified in log₁₀ units above threshold. Amplification was direct-coupled. Positive responses are displayed upward.

3.3, a continuation of the d.c. component into the offperiod and a delayed off-response are both observed. At maximum intensity, the d.c. component continued into the off-period for several hundred msec, the slope of decay was markedly reduced (Fig. 2, 3·5) and the off-response became difficult to identify¹. The effects on the decay of the d.c. component were present at all flash durations tested between 5 msec and 1 sec, but were most prominent at flash durations less than 500 msec.

The local electroretinogram was usually recorded by centring a 1.0 mm diameter spot of light on the microelectrode. Larger spots did not alter the threshold for this response 18. With spot diameters of less than 0.33 mm, the threshold of the local electroretinogram was increased along with the threshold for modifying the offresponse. With very small spots (0.01 mm²), a decrease in the slope of decay could still be observed.

The bipolar cells probably give rise to the d.c. component1 and also are the principal source of ganglion cell input19, so that these results imply that the d.c. component of the local electroretinogram contributes to the formation of ganglion cell responses.

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Ventral Root Afferent Fibres and the Recurrent Inhibitory Pathway

RENSHAW1 showed that stimulation of ventral roots of the cat spinal cord may inhibit motoneurones. This also occurs when muscle nerves are stimulated in preparations with cut dorsal roots. Renshaw proposed several alternative hypotheses to account for this. One was that antidromic action potentials in motor axons propagate along collaterals which terminate within the ventral horn2 and inhibit motoneurones either directly or through an inter-The discovery by Renshaw of ventral horn interneurones which were discharged repetitively by ventral root stimulation gave support to this idea. Eccles, Fatt and Koketsu³ reported evidence for synaptic links between cholinergic terminals of ventral root axons and the interneurones of Renshaw and between the interneurones and motoneurones. They also provided evidence against a second hypothesis of Renshaw that recurrent inhibition may be produced by interaction between the dendrites of motoneurones. There is as yet little direct evidence against the third hypothesis of Renshaw that afferent fibres within the ventral roots might inhibit motoneurones. Such afferents would have to arise, at least in part, from muscle to account for recurrent inhibition produced by muscle nerve stimulation. There is evidence for the presence of afferents in ventral roots. Sherrington4 found as many as forty degenerating axons in chronically sectioned ventral roots. He confirmed previous observations that there are often a few ganglion cells within the ventral root, although he failed to demonstrate a relationship between the "recurrent" afferent fibres and the ganglion cells. Ventral root afferents have recently been described in the cat⁵ and the rat⁶; at least some of them appear to arise from muscle6.

Because of the great theoretical interest in the recurrent inhibitory pathway, it seemed advisable to investigate the role of ventral root afferents in this pathway. One or more ventral roots were sectioned intradurally under pentobarbital anaesthesia in each of five cats. Aseptic procedures were used and broad spectrum antibiotics were given following the surgery. The animals were kept for 7-18 days. The dissection during the terminal acute experiment involved re-exposure of the spinal cord and preparation of roots, including the chronically sectioned ones, for stimulation or recording. Glass microelectrodes with initial resistances of about 10 M Ω were employed for recording from single units within the spinal cord. They were filled with I molar potassium acetate saturated with methyl blue. Details of equipment and procedures can be found in a recent paper from this laboratory?. The dye was used for marking the positions of neuroness. Records were made from Renshaw cells and from moto-The appropriate spinal cord segments and neurones. roots were preserved in neutral formalin for histological controls. Serial sections of the central ends of the chronically cut ventral roots were stained and examined for the presence of ganglion cells. Although three of the ventral roots contained an occasional ganglion cell (1, 2 and 4, respectively), there were none in three other roots (including the ones employed for Fig. 1A, B and D). It was possible to demonstrate the presence of a recurrent inhibitory mechanism in all the animals, and the range over which the recurrent inhibition could be graded exceeded the largest number of ganglion cells observed. Inhibitory postsynaptic potentials were recorded from fifteen of seventeen motoneurones. The motoneurones were identified by antidromic invasion of an action potential when a chronically sectioned ventral root was stimulated10. Recurrent inhibitory potentials were observed when the stimulus strength was at or below threshold for the motor axon3. In three experiments, records were made from a total of five Renshaw cells, all activated by stimulation of the central stumps of chronically sectioned ventral roots. It was, in addition, possible to demonstrate recurrent inhibition of the monosynaptic reflex.

Fig. 1 illustrates the results of these experiments. A and B are intracellular records from a motoneurone. Stimulation of a chronically sectioned ventral root evoked the antidromic action potential in A. When the stimulus strength was lowered below threshold for the motor axon of this cell, a recurrent inhibitory postsynaptic potential was observed (B). The extracellular field potential shown in C was a response to stimulation of a chronically sectioned ventral root in another experiment. Similar field

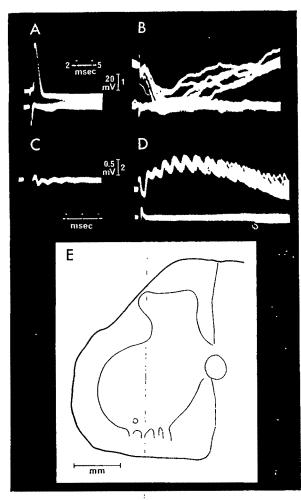


Fig. 1. The electrical records in A-D were made from the lumbosacral spinal cord in cats with chronically sectioned ventral roots. The upper traces in A and B were recorded intracellularly from a motoneurone, identified by antidromically conducted action potential (A) which resulted from stimulation of the chronically sectioned LT ventral root. When the stimulus strength was below threshold for the motor axon, a recurrent inhibitory postsynaptic potential was observed (B). The lower traces were recorded from the dorsal root entry zone by a ball-tipped platinum electrode. The trace in C is the field potential recorded extracellularly from a population of Renshaw cells by a microelectrode placed in the ventral horn. The stimulus was applied to the chronically sectioned SI ventral root. The action potentials shown in D were recorded intracellularly from a Renshaw cell damaged by the microelectrode impalement. The position of this neurone was marked and found to be at the site shown in the drawing in E. The upper time scale is 2 msec for A and 5 msec for B. The lower time scale is for C and D. The upper potential scale is 20 mV for A and 1 mV for B. The other is 0.5 mV for C and 2 mV for D.

potentials have been associated with the discharge of a population of Renshaw cells³. The burst discharge in Dwas recorded from a Renshaw cell following stimulation of a chronically sectioned ventral root. The microelectrode probably damaged the neurone, because the recording was intracellular and yet the action potentials were very small. The position of this Renshaw cell was marked by the electrophoretic ejection of methyl blue from the electrode. The cell was in the ventral part of Rexed's lamina VII¹¹, just medial to the motor nucleus (Fig. 1E). This position is characteristic of Renshaw cells^{7,8}.

There is at least one report consistent with these findings. Eccles, Libet and Young12 in their study of chromatolytic motoneurones observed recurrent inhibitory postsynaptic potentials in response to stimulation of chronically sectioned ventral roots. They made no histological sections, nor did they report finding Renshaw cells activated by stimulation of the same ventral roots.

It can be concluded that, although there are afferent fibres in ventral roots, these are not essential for the production of recurrent inhibition.

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Calcification of Cell Nuclei in Experimental Necrosis in vivo

WE have reported previously1-3 that calcium, phosphorus. magnesium, lipids and polysaccharides can be deposited in the nuclei of dead cells in vitro. In such a nucleus a highly refractile granule is formed which grows in size. and, in suitable conditions, structures many times the size of a cell may grow out from the granule3. This may be pertinent to the study of certain processes in the organism, such as the calcification of necroses. arteriosclerosis and other cases of pathological calcification3 and so it seemed important to ascertain whether the phenomenon also occurs in the nuclei of dead cells in vivo. We therefore examined kidney tissue with ischaemic necroses caused by ligature of blood vessels4,5. Such necroses were known to calcify rapidly4-7 but the nature of the calcification was unknown.

In four male Wistar rats each weighing 230 g the left kidney was ligatured to cut off completely its blood supply. In two of the rats the kidney was removed for examination after 6 days and in the other two after 30 days. calcium content was assessed in each case as follows. The kidney was weighed after extirpation and dried overnight at 105° C. The dry matter was triturated and extracted with 2 ml. of 0.1 normal nitric acid for 5 days.

Calcium was assessed chelatometrically in the tissue extract and was calculated in g/100 g wet weight. Samples for histological examination were fixed in 10 per cent formalin and embedded in paraffin wax. Sections were stained with haematoxylin and eosin. Calcium was detected by the Kóssa reaction.

The normal kidney of a 230 g rat weighed 0.8 g; it was 1.4 cm long, brownish-red in colour and contained about 0.09 per cent calcium. Six days after ligature the kidney was a normal size but it was greyish white and the calcium content had risen 0.2 per cent. Thirty days after ligature the kidney was much smaller than that of a control animal. It was furrowed, yellowish grey in colour and contained 3.7 per cent calcium—a forty-fold rise in calcium content. Sections from kidneys 6 days after ligature showed nuclei with and without deposited calcium (Figs. 1 and 2). Calcification of the nuclei varied in intensity. In some nuclei there were only a few granules giving a positive reaction for calcium, while in others this reaction was so strongly positive that only phase contrast microscopy could show that the calcified masses were cellular nuclei (Fig. 2). In sections made 30 days after ligature, haematoxylin and eosin staining revealed many basophilic granules varying in size in a wide area beneath the surface of the kidney. Some basophilic granules were suggestive of nuclei in size and shape, and in all of them the reaction

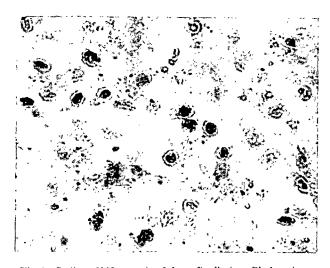


Fig. 1. Section of kidney cortex 6 days after ligature. Black spots are nuclei showing a calcium-positive reaction. Nuclei without calcium are also present. (×200.)

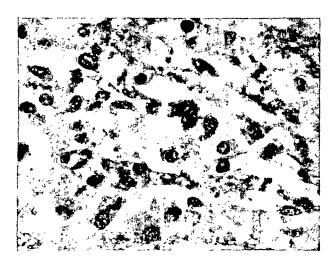


Fig. 2. Phase contrast microscopy of the same site as in Fig. 1 showing that calcium is deposited in the nuclei. (×200.)

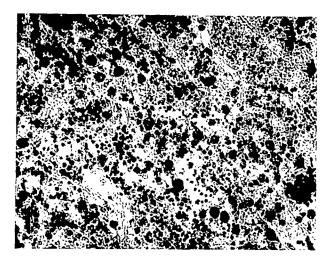


Fig. 3. Section of kidney cortex 30 days after ligature. Basophilic calcium-positive granules. Some structures resemble nuclei. (×100,)

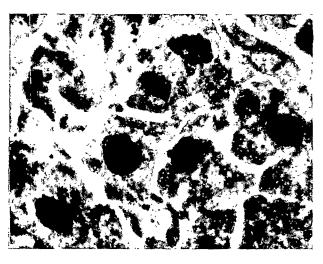


Fig. 4. Phase contrast illumination of same site as in Fig. 3. According to the position of the calcium-positive fragments it is evident that they were nuclei originally. (\times 450.)

for calcium was positive (Fig. 3). In preparations stained by the method of Kóssa, phase contrast microscopy in some cases revealed that the structures suggestive of nuclei occupied the centre of partially disintegrated cells and that really they were nuclei in which large amounts of calcium had been deposited (Fig. 4).

Our findings show that the nuclei of dead cells can calcify in vivo. It is, however, to be expected that processes occurring in vivo differ somewhat from those in vitro. We presume that there is a "nucleating material" in the nuclei of cells and that this material asserts itself only after the death of the cell. Here the term nucleating material is understood in the broadest possible sense. Our pilot experiments showed that DNA is one such substance. This nucleating material is the determining factor in the calcification of necrotic tissue. In such cases the material can be liberated not only from the nucleus into the cytoplasm but it can leave the cell after its disintegration and pass into the surrounding tissue with its nucleating properties intact. This may explain the numerous calciumpositive granules of basophilic material found in necrotic tissue, which did not in any way resemble nuclei. believe that nucleating compounds from cell nuclei, active in vitro and in vivo, may be very important not only

for the deposition of calcium in necrosis but also in other cases of calcification.

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Mobilization of Lymphocytes from Lymph Nodes and Spleen by Polysaccharide Polysulphate

One of us has reported the production of lymphocytosis by polysaccharide polysulphates in experimental animals1. Maximal lymphocytosis was observed 3 h after administration of sulphated polysaccharides, and so lymphocytosis seems to be caused by the mobilization of lymphocytes from storage. The investigation described here was carried out to find the place or places from which the lymphocytes are mobilized into the circulating blood.



Fig. 1. a, Lymph node from a rat given dextran sulphate ($\times 22.5$), b, Lymph node from control rat ($\times 22.5$). Note the depletion of lymphocytes in a in contrast with the appearance of "fullness" in b.





Fig. 2. a, Spleen from rat given dextran sulphate (×6). b, Spleen from control rat (×6). Note the lightly stained periarterial areas in a in contrast with the darkness of the same areas in b. An increase in dark nuclei in the red pulp can also be recognized.

Adult female rats weighing 150-200 g were kept in stress-free conditions for 5 days and starved overnight before the experiments, which were all carried out at about the same time of day to avoid diurnal variation in the leucocyte count. Dextran sulphate with a molecular weight of about 5,000 and a 19 per cent sulphur content was dissolved in sterile physiological saline to give a concentration of 25 mg/ml. and it was injected intraperitoneally into the rats in a dose of 0.2 ml./100 g body weight. Control rats were injected intraperitoneally with the same volume of physiological saline. Preliminary experiments showed that maximal lymphocytosis was obtained 3 h after intraperitoneal injection of dextran sulphate, and so the rats were anaesthetized 3 h after administration by an intraperitoneal injection of sodium pentobarbital (7 mg/100 g body weight) and blood samples were collected by cardiac puncture for leucocyte count and differential count. At the same time tissue specimens were collected. One part was subjected to ordinary histological examinations, and the other part was analysed for DNA content, by a modification of the Schmidt-Thannhauser-Schneider method to compare the number of cells contained in the normal and treated tissues. In all rats injected with dextran sulphate a marked lymphocytosis between two and six times the normal lymphocyte count was observed in circulating blood.

The results of the DNA analysis are shown in Table 1. A marked decrease of DNA after the administration of dextran sulphate was observed only in lymph nodes, while there was a marked increase in circulating blood and a slight increase in lung and spleen.

As shown in Figs. 1 and 2, marked reduction of lymphocytes was noted histologically in lymph nodes and white pulp of spleen after the dextran sulphate had been given. In the spleen (Fig. 2) while lymphocytes were depleted from the periarterial areas of white pulp, a considerable increase in number of lymphocytes was seen in the red pulp in a rather blotchy pattern. This increase seemed to be more than just a reflexion of lymphocytosis in circulating blood. This finding may account for the fact that the DNA content in the spleen of animals injected with dextran sulphate was not lowered as in the lymph nodes. The thymus showed no recognizable change after adminis-

Table 1. Analysis of rat tissues for dna after injection of dextran sulphate

	DNA-P mg/10	00 g fresh tissue	
Tissue	Control	3 h after injection	P
Lung Liver Kidney Spleen Thymus Intestine Blood Bone marrow Lymph nodes	$\begin{array}{c} 62.8 \pm 6.1 \\ 26.6 \pm 3.4 \\ 35.4 \pm 2.9 \\ 105.4 \pm 14.4 \\ 234.2 \pm 20.6 \\ 54.0 \pm 6.7 \\ 24.8 \pm 1.9 \\ 145.8 \pm 28.6 \\ 193.1 \pm 13.9 \end{array}$	$\begin{array}{c} 69.8 \pm 5.0 \\ 26.5 \pm 3.3 \\ 36.0 \pm 2.9 \\ 122.8 \pm 7.8 \\ 240.1 \pm 24.2 \\ 54.9 \pm 6.5 \\ 35.5 \pm 3.0 \\ 138.9 \pm 23.4 \\ 159.4 \pm 13.0 \end{array}$	<0.02 Not significant Not significant <0.01 Not significant Not significant <0.001 Not significant <0.001 Not significant

Each value represents a mean $\pm S.D.$ for between seven and thirteen rats.

tration of dextran sulphate, which coincides with the result of DNA measurement. The observation supports the view that the thymic lymphocytes differ from lymphocytes in the other lymphoid tissues².

These results show that the mobilization of lymphocytes from lymph nodes and spleen is the chief mechanism of the lymphocytosis in peripheral blood after the administration of dextran sulphate.

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pP_y : a Measure of Potentiating Activity

THE absence of a generally applicable pharmacological method for estimating the potency of potentiating compounds such as anticholinesterases and antiamine oxidases is perhaps partly a result of the accuracy with which these compounds can be assayed by manometric techniques on isolated enzymes. Inhibitory potency determined in this way, however, is not necessarily indicative of the potentiating activity to be found in the whole animal or even in isolated tissues.

This communication describes the determination of pP_y values. pP_y , analogous to pA_x (ref. 1) is defined as the negative logarithm of the molar concentration of a potentiating drug which increases the response of a fractional dose (y = 1/x) of agonist to that of an initial submaximal dose. The term pSx has been used previously^{2,3} in a similar context.

The test preparation was the isolated rectus abdominis muscle of the frog; the agonist was acetylcholine placed the muscle bath, which contained oxygenated frog Ringer solution, for 60 sec in each 5 min cycle. The potentiating drug (an anticholinesterase) added to the bath 2 min before the first fractional of acetylcholine and immediately usually for twelve cycles (57 min contact), after each double wash. To obtain pP_y values, different concentrations of anticholinesterase were applied to the right and left muscle, respectively, and then, after a suitable recovery period, each muscle received the alternative concentration. Ideally this enabled four estimations of pP_y to be made on one pair of muscles (Fig. 1), but because of its irreversible action only the first procedure was possible with dyflos.

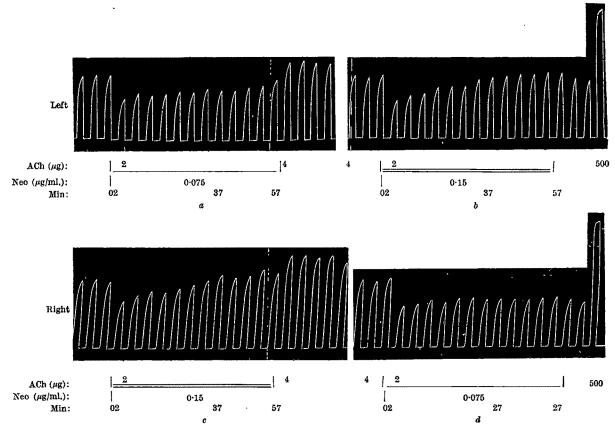


Fig. 1. Frog rectus abdominis muscle in 4 ml. bath. Acetylcholine chloride (ACh) contractures induced every 5 min. ACh doses shown only when changed. Top, left muscle; bottom, right muscle. Between (a) and (b), and (c) and (d), 25 min (five cycles). At _____, 0.075 \(mu g/ml.\), and at \(\begin{array}{c} \begin{array}{c} \begin

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Because the initial control response to acetylcholine varied about the 50 per cent maximum response in different experiments, dose-response experiments were performed with edrophonium (four experiments) and eserine (two experiments) to test for linearity and parallelism over the dose-response curve. Three-dose latin square experiments were performed before, during and, in the edrophonium experiments, after the presence of the anticholinesterase. The acetylcholine dose ratio was 4. In only one out of six experiments was there a significant deviation (P < 0.01) from parallelism although in two other experiments there was a significant difference (P < 0.05 and < 0.01, respectively) in quadratic curvature. Inspection of the data and graphs suggested that the significance of these factors would have lessened if a smaller dose-response range had been selected.

Table 1. pP_2 values of anticholinesterases on contractures of the frog rectus abdominis muscle induced by acetylcholine

Compound	Contact time (min)	pP ₂ ± standard deviation (No. of experiments in brackets)	Limits of error $(P=0.05)$ as percentage of mean pP_z	Previously quoted pSx values ^{2,3}
Escrine	17 27 37 57	$5 \cdot 29 \pm 0 \cdot 06$ (3) $5 \cdot 46 \pm 0 \cdot 09$ (8) $5 \cdot 55 \pm 0 \cdot 14$ (18) $5 \cdot 56 \pm 0 \cdot 11$ (12)	± 4·3	6.3
Neostigmine	17 27 37 57	6.34 ± 0.02 (2) 6.49 ± 0.04 (4) 6.49 ± 0.12 (8) 6.52 ± 0.11 (10)	± 3·7	6.28
Edrophonium	2 7 17 57	5.84 ± 0.28 (8) 5.56 ± 0.61 (7) 5.45 ± 0.70 (8) 5.76 ± 0.17 (9)	± 6·6	5-44
Dyflos	117 177	1·35±0·18 (5) 4·43±0·15 (5)	± 8.8	0 44

 pP_2 values of four commonly used anticholinesterases are shown in Table 1. The standard deviations of equilibrium pP_2 values ranged from 0.11 to 0.17 and the limits of error ($\tilde{P}=0.05$) from 3.7 to 8.8 per cent, results which are similar to those found for pA_2 estimations^{1,4}. Table 1 shows both differences in potency and differences in the time required to reach equilibrium conditions. Neostigmine, the most potent compound in this series $(pP_2 = 6.5)$, attained its maximum effect after 27 min, whereas eserine required contact for 37 min and was about nine times less active ($pP_2 = 5.55$). Edrophonium ($pP_2 = 5.8$) showed its maximum effect after contact for only 2 min, the pP_2 values after 2 and 57 min not differing significantly. At intermediate times there was greater variability in the

values. The pP2 values and their equilibrium times for neostigmine and edrophonium agree closely with previously quoted pSx values although my value for eserine seems relatively less active2. These authors also found a close correlation between acetylcholine potentiating activity and IC_{50} values for neostigmine $(0.5 \times 10^{-5}$ molar) and eserine $(1.4 \times 10^{-5} \text{ molar})$ on frog rectus acetylcholinesterase. Dyflos required 2-3 h to exhibit its maximum effect, insufficient potentiation being obtained after contact for 57 min with the concentrations used to obtain pP_2 values. This compound was about thirteen times less active than eserine. Because the molecular weight of dyflos, the slowest acting and least potent compound tested. is similar to that of edrophonium, which achieved its maximum effect very rapidly, it is obvious that diffusion to the site of action is not an important factor in the action/time relationship of these compounds in this preparation. Three estimates of pP_8 were also obtained with eserine $(pP_8 = 5.07 \pm 0.17)$ although the concentrations necessary were such that in other

experiments reduction of the response followed an initial period of potentiation. This accords with previous findings. $pP_2-pP_8=0.49$ for this compound shows that a four-fold decrease in agonist concentration is compensated by a 3·1-fold increase in eserine concentration.

The method is currently being used in a systematic study of the possible acetylcholine potentiating activity of anti-adrenaline compounds.

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Tea and Atherosclerosis

MANY people have pointed out a relationship between atherosclerosis and serum lipids¹⁻⁴. Little *et al.*⁵ showed that the concentration of serum lipids is positively correlated with coffee drinking, but slightly negatively correlated with tea drinking. Pauls reported that people who developed coronaries drank more cups of coffee than those without coronaries. Snapper' stated in his book that there was little atherosclerosis in the Chinese, who drink tea almost exclusively.

The absence of atherosclerosis in the Chinese, the Bantus of South Africa, the Negroes in Central Africa and the Yemenites in Asia Minor has been attributed to low contents of serum lipids. Similarly, it should be noted that the cholesterol of the Chinese was lower than that in Europeans or Americans⁹. The work reported here was undertaken to explore the relationship between tea drinking and atherosclerosis.

During the past decade we have developed a quantita tive method of grading the degree of atherosclerosis10. and by this system we can determine the degree of

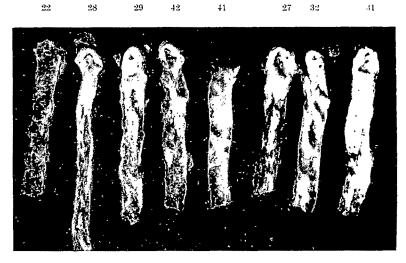


Fig. 1. Aortae from rabbits fed with different ingredient as indicated in each case below: No. 22, control, rabbit chow and water; Nos. 27, 28 and 29, 3 per cent Wesson oil, 0.5 per cent cholesterol and water; Nos. 41 and 42, 3 per cent Wesson oil, 0 per cent cholesterol and tea; Nos. 31 and 32, 3 per cent Wesson oil, 0.0 per cent cholesterol and tea; Nos. 31 and 32, 3 per cent Wesson oil, 0.5 per cent work of the control of the cholesterol and water for 3 months and then water was replaced by tea for another 2 months. Tea was made by boiling 1 g of tea leaf in 2 l. of water and filtered through a filter paper.

atherosclerosis at different segments and even the same segment of the different sectors. Table 1 shows that the overt coffee drinking population has a higher degree of atherosclerosis than the overt tea drinking population.

When rabbits were maintained for 3 months on a diet consisting of rabbit chow augmented with 3 per cent Wesson oil and 0.25 per cent cholesterol, they readily developed atherosclerosis¹¹. Fig. 1 compares aortae from rabbits drinking different beverages. The degree of atherosclerosis is much lower in the rabbits drinking tea than in those drinking water. It is evident that tea seems to protect the aorta from forming atheroma.

Table 2 shows the relationship between atherosclerosis and serum lipid concentration of the experimental rabbits. The concentration of lipid seems to correspond well to the degree of atherosclerosis. The control group had a smaller concentration of serum lipoproteins and there was virtually no atherosclerosis. Rabbits which were given a diet with a high fat content and water to drink showed sclerosis. Rabbits given a diet with a high fat content and water containing the ophylline at a concentration of 10^{-5} g/ml. showed slightly less sclerosis.

Table 1. Degree of atherosclerosis in habitual coffee drinkers and habitual tea drinkers

Population	Coronary artery $(I/E)c^*$	Cerebral arter: $(I/E)\iota^*$
Coffee drinkers	58 (155)†	33 (150)
Tea drinkers	37 (50)	12 (40)

These data were accumulated during the past 14 years of investigation of human coronary and atherosclerosis. The coffee drinkers were Americans (Caucasian) and the tea drinkers were Chinese. • $(IE)_c$, coronary atherosclerosis; $(IE)_c$, atherosclerosis of the brain, where I is intima material area, E is total arterial cross-section, c is coronary artery, and h is brain.

artery, and b is brain.
† Numbers in parentheses are numbers of cases studied.

Table 2. RELATIONSHIP BETWEEN ATHEROSCLEROSIS AND THE CONCENTRATION OF SERUM LIPOPROTEINS IN RABBITS

	Sf 0-12	Lipoproteins 12-20	20-100	Degree of atherosclerosis
Control (6) Diet alone (5) Diet and theophylline (10)	50 309	16 251	0 623	0 7·2 6·5
Diet and tea (4) Diet*early and tea later (10)	$\frac{214}{374}$	79 1 69	437 389	2·0 6·0

* Numbers in parenthesis represent the number of rabbits investigated. † Diet started 3 months before tea was supplied in the drinking water.

On the other hand, rabbits which were given a diet with high fat and tea to drink had much less sclerosis than either of the other groups. The group given only the diet with high fat content for 3 months, and then maintained on the diet and tea regimen for another 2 months, showed less sclerosis than those rabbits given the high fat control diet. These data show that tea decreases concentration of lipids in the serum as was reported earlier^{5,12}. They further suggest that if the atherosclerosis proceeds beyond a reversible stage, tea cannot reverse it. Probably tea must be supplied simultaneously with fat or immediately after a meal of fat. Theophylline and theobromine are far less effective, if at all, than tea in protecting the aorta from forming atheroma. Thus it seems that some active principle other than theophylline in the tea extract must be responsible for preventing the formation of atheroma.

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CYTOLOGY

Growth of the Pre-erythrocytic Tissue Stages of Plasmodium berghei in Isolated, Perfused Tree Rat Liver

RECENT improvements in the maintenance of isolated organs in perfusion systems1-4 suggested that these techniques could be used to study certain pathogens which need a specific cellular environment and an organ of choice. The malaria parasites of man and all other mammalian plasmodia undergo a primary development cycle in the parenchyma cells of the liver of their hosts before invasion of the blood takes place. We thought therefore that successful growth of the tissue phase of the malaria parasites (commonly known as pre-erythrocytic schizogony) in isolated, perfused liver could shed some light on the nutritional requirements of plasmodia during their initial growth in the body and be of use in studies of chemotherapy and immunity. We now report the successful growth of the pre-erythrocytic tissue stages of Plasmodium berghei in isolated, perfused tree rat liver.

We used the liver perfusion-aeration apparatus designed by Professor Leon H. Miller (Department of Biochemistry, University of Rochester) and produced commercially by Metaloglass, Inc., which consists of a thermoregulated plastic chamber in which the perfusion fluid is moved through the glass and plastic tubing by a peristaltic action pump. Oxygenation of the perfusion fluid is regulated by manometric valves in the reservoir cylinder (Fig. 1). The apparatus was sterilized by filling the entire assembled system with an antiseptic solution of 'Ioclide' (Clay

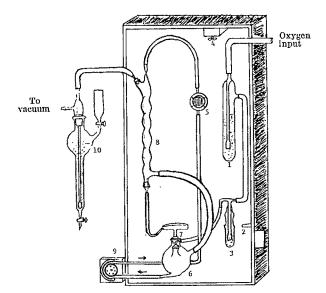
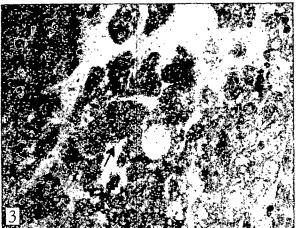


Fig. 1. Liver perfusion-aeration apparatus (drawn from Bulletin M81-B, Metalogiass, Inc.). 1, Humidifier; 2, thermoswitch; 3, gaseous manometer; 4, fan; 5, filter; 6, blood reservoir; 7, liver platform; 8, gaseous exchange tube; 9, pump; 10, carbon dioxide trap.

Adams Co., New York) for 18 h and rinsing several times with sterile water, sterile saline and finally with sterile Ringer's solution. The removable liver platform was sterilized separately in the autoclave. An adult male tree rat (Thamnomys surdaster) from our laboratory colony⁵ was injected intravenously with 60,000 sporozoites of P. berghei. Twenty-three hours after sporozoite inoculation the tree rat was anaesthetized and 100 U.S.P. units of heparin was administered. Laparotomy was immediately performed and plastic tubing (P.E.10, Clay Adams Co.) inserted into the portal vein and into the superior vena cava. All other adjacent vessels were ligated and the inflow and outflow of fluid ascertained by slow flushing with warm heparin-Ringer solution (37° C). Hepatectomy was then performed and the isolated liver attached to the perfusion-aeration system. The perfusion medium was similar to that employed by Folkman et al.6. Its constituents were as follows: Eagle medium ($\times 10$ conc.), 100 ml./l.; calf serum, 250 ml./l.; haemoglobin (human), 1.0 g per cent; amino-acids (×50 conc.), 20 ml./l.; L-glutamine, 2 mmoles/ml.; glucose, 250 mg per cent; aqueous insulin, 0.25 units/ml.; ampicillin, 0.4 mg/ml.; polymyxin B, 100 units/ml., HCl to pH 7.3; sterile distilled water to 11. The liver was perfused for 26 h to permit maturation of the tissue schizonts (a cycle of 48-51 h). It was then removed, cut and fixed in Carnoy's solution, sectioned and stained in Giemsa colophonium. Microscopic examination revealed a number of mature and bursting pre-erythrocytic schizonts in the parenchyma cells. The tissue schizonts showed all the characteristics observed and described in infections in tree rats induced by sporozoites7-8 (see Figs. 2 and 3).





Mature pre-erythrocytic tissue schizonts of Plasmodium berghei showing merozoites. Stained sections from liver of tree rat TR164 maintained in perfusion-aeration apparatus for 26 h. Polaroid land camera, \times c. 285.

We are continuing work on the growth of the tissue stages in the isolated perfused liver of natural and experimental rodent hosts of P. berghei.

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Incomplete Inhibition of Synchronized Cell Division by Hydroxyurea and its Relevance to the Normal Cellular Life Cycle

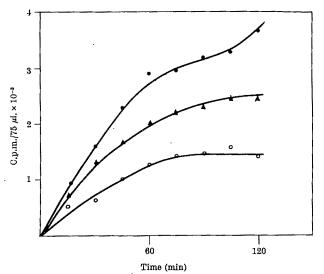
ONE of the more puzzling aspects of synchronized cell division in heat-treated Tetrahymena has been the relative asynchrony of DNA synthesis which persists following the synchronizing treatment. This phenomenon has been well documented in the past and is perhaps most simply demonstrated by the fact that only about 50 per cent of the cell nuclei label with thymidine between the end of the heat-treatment (EHT) and the first synchronous division (SD)¹. Because spectrophotometric measurements of the DNA content of EHT cells have shown that they contain two to three times the DNA found in an average log cell², the reasons for additional DNA synthesis after EHT have been even more obscure. In the past it has usually been suggested that DNA replication is complete at EHT and that this further synthesis in some cells merely represents redundant DNA accumulation by "mature" nuclei3 known to be polyploid4.

We have recently employed hydroxyurea in an effort to study this problem quantitatively. Hydroxyurea was chosen after a search for an antibiotic which would effectively inhibit DNA synthesis in the absence of a sig ifficant effect on RNA synthesis. The other inhibitors examined—for example, phleomycin, mitomycin c, cytosine arabinosides and fluoro-uridine deoxyriboside—all showed a substantial inhibition of RNA synthesis at levels producing the desired inhibition of DNA synthesis. Because the synthesis of RNA5, and particularly mRNA $^{6.7}$, is thought to be necessary for synchronized division in Tetrahymena pyriformis, significant inhibition of RNA synthesis would obscure the meaning of the experiment. To study the effect of hydroxyurea on DNA synthesis we added varying amounts of the drug to 2.0 ml. samples of a 12.0 ml. synchronized culture containing 100 μ c. of ³H-thymidine. In each case, the kinetics of synthosis were followed using the direct filter paper disk procedure which allows better quantitation than autoradiography. The effects of the two higher concentrations (500 and 1,000 μg/ml.) are seen in Fig. I. At 1,000 μg/ml. synthesis is completely inhibited after a lag period during which the

cells are capable of synthesizing about one-half the DNA attained in the control culture at the time of division (compare with Fig. 2). Lower levels of hydroxyurea than those shown resulted in minor degrees of inhibition. Cell viability was unimpaired at even the highest levels. Parallel experiments using ¹⁴C-amino-acids and ³H-uridine gave less than 5 per cent inhibition after 60 min. therefore chose $1,000 \mu g/ml$. hydroxyurea and studied its effect on the kinetics of synchronized cell division.

In Fig. 2 the cell counts obtained from a control culture and one containing 1,000 μg/ml. hydroxyurea are shown. All cell counts are the average of four values obtained at 15 min intervals using a model B Coulter counter. It may be seen that approximately 50 per cent of the treated cells fail to divide even once and the remainder show no further division after the first cycle. It is of particular interest that the number of cells completely inhibited (about 50 per cent) corresponds very closely to the number which ordinarily show nuclear thymidine labelling by autoradiography. In view of the good specificity of the action of hydroxyurea and the close correspondence between the percentage of inhibited cells and the percentage which will usually label with thymidine, it seems reasonable to conclude that the effect of hydroxyurea is based on its ability to inhibit DNA synthesis. It would therefore seem that despite the relatively large stores of DNA in these cells (compared with log cells) further replication is needed. The source of the delay in division induced in the treated cells which divide successfully is not as yet known.

These experiments are therefore consistent with the hypothesis that the small amount of DNA synthesis which occurs in about half the population of synchronized Tetrahymena is nevertheless essential for division in this fraction of the culture. Perhaps the most straightforward interpretation of these results is that the replicating DNA fraction codes for division-related proteins. Because the entire population can be prevented from dividing by actinomycin D^5 and is known to require mRNA synthesis during the early part of this period6,7, it is also possible that actual replication of this DNA fraction is necessary for its transcription to occur. The well documented polyploid nature of the *Tetrahymena* macronucleus⁴ strongly reinforces this latter interpretation because it



Time (min)

Fig. 1. Uptake of ³H-thymidine by synchronized Tetrahymena pyriformis GL. 100 µc, of ³H-thymidine was added to a synchronized culture and the culture divided into six equal portions in flasks containing sufficient hydroxyurea to yield 0, 1, 10, 100, 500 and 1,000 µg/ml. After removal of zero time controls the kinetics of uptake were followed in each flask using the filter paper disk assay. Shown here are the curves obtained from control (—●—) flasks and those containing 500 (—▲—) and 1,000 (—○—) µg of hydroxyurea/ml. Inhibition occurred with all values of hydroxyurea and was complete after a lag in the flask containing 1,000 µg/ml. It may be noted that the total synthesis attained with 1,000 µg/ml. approximates one-third that seen in the conrol at the time of synchronous division (about 90 min).

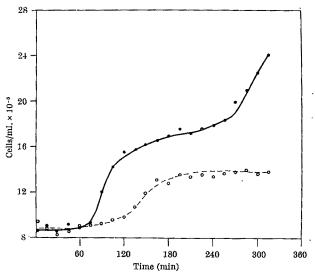


Fig. 2. Kinetics of synchronous cell division in the presence of hydroxyurea 1,000 μ g/ml. Approximately 95 per cent of the control culture has divided by about 90 min (— —). The culture containing hydroxyurea (—)—) shows division by about 50 per cent of the cells after a short delay. Following the first division no further increase in cell number is seen with hydroxyurea while logarithmic growth is resumed in the control flask.

eliminates the problem of gene dosage. Extensive morphogenetic studies have shown that the entire synchronized population is well advanced in the life cycle. These experiments are therefore also consistent with the hypothesis that replication of some division-related genes occurs late in the life cycle. These experiments suggest at least two possible interpretations. The first and most intriguing possibility is that a unique replicating site or sites may exist which is potentially capable of controlling cell division. Failure to replicate and transcribe this site might repress expression of division-related genes. Demonstration of such a site by more rigorous means would have great relevance to differentiation and malignancy. A second alternative, however, is that the cell possesses some recognition system which allows the physical processes of division (for example, mitosis, amitosis or cytokinesis or both) to proceed only when the entire genome has been copied (the late replicating sites not necessarily coding for division-related proteins). If this is the case, then the cells labelling under the conditions described may represent a portion of the mass population which has not yet completely copied their gene complement. We are now conducting experiments designed to distinguish between these two possibilities.

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MICROBIOLOGY

Inhibition of Haemaggregation by Lepromin and Other Mycobacterial Substances

Human erythrocytes suspended in glucose buffered at about pH 5–5·8 may aggregate and this aggregation can be detected by the rapid settling of cells in a tube or by the pattern of deposit in a tube or plastic haemagglutination plate. The haemaggregation is inhibited by sodium chloride and quite specifically by polioviruses, by influenza viruses¹ and by other biologically active substances including nucleic acids, mucoproteins, tuberculin and rhinoviruses². Old tuberculin and purified protein (derivative) inhibit the haemaggregation of untreated erythrocytes, but certain viruses inhibit only erythrocytes treated with trypsin. The inhibition caused by tuberculin is correlated with skin sensitizing activity and is inhibited by specific antibody³.⁴.

In view of the shortage of in vitro methods for detecting the biologically active components of Mycobacterium leprae and related organisms we have attempted to show

that they inhibit haemaggregation.

The materials for assay were dialysed against a mixture of distilled water and ion exchange resins to remove sodium chloride, and included the following: (a) four samples of integral lepromin prepared from the skin nodules of patients with lepromatous leprosy by Wade's modification of the Hayashi-Mitsuda method⁵, standardized to contain 1.6×10^{8} acid fast bacilli/ml.; (b) washed bacilli of M. lepraemurium, and attenuated M. tuberculosis strain H37 Ra; (c) cytoplasmic fractions of M. leprae, M. lepraemurium and M. tuberculosis (H37 Ra), obtained by rupture of washed bacilli in a French press or Mickle disintegrator and removal of cell walls by centrifugation; (d) washed cell walls of M. leprae, M. lepraemurium and M. tuberculosis (H37 Ra); (e) normal human skin prepared as (a), containing no acid fast bacilli. The materials and methods of the haemaggregation-inhibition plate test have been described in detail². For this study, fresh human group A erythrocytes were used throughout, because they gave clear patterns and high titres. The erythrocytes could be used for 3 days after venipuncture, and were treated overnight with 0·1 per cent trypsin in saline during the night before use and suspended in 0.454 molar glucose. Using WHO pattern plastic haemagglutination-inhibition trays the materials were diluted serially in 0.3 ml. volumes of buffer. Borax-succinic acid buffers were made with varying proportions of 0.1 molar stock solutions of borax and succinic acid—for example, 45 ml. of 0·1 molar borax and 55 ml. of 0.1 molar succinic acid for a solution of pH 5.5—but each mixture was checked and adjusted to the desired pH using a glass electrode pH meter. buffer for dilution was made up just before use by mixing 2 volumes of 0.908 molar glucose, 2 volumes of 0.1 molar succinic acid buffer and 1 volume of 0.375 per cent bovine serum albumin in 0.67 molar phosphate buffer pH 7 which had been heated at 56° C for 30 min and stood at room temperature for 60 min immediately before mixing. The value of heated albumin in obtaining clear patterns and reproducible results is emphasized. To each cup was added 0.2 ml. of a I per cent suspension of human erythrocytes treated with trypsin and the plates were placed at 4° C for 1 h. The patterns were recorded. On each day of assay a control test was performed using sodium chloride -1.125 g of sodium chloride dissolved in 100 ml. of 0.67 molar phosphate buffer-to inhibit haemaggregation. Triplicate cups with a range of buffers from 5.0 to 5.7 with and without sodium chloride indicate the highest pH at which definite inhibition of haemaggregation is observed (Fig. 1). From this control test it may be concluded that aggregation is incomplete at pH 5.6, and that it is completely inhibited between pH 5.4 and 5.6.

Table 1. Inhibition of haemaggregation by mycobacterial substances and normal skin

Source	Organism	Preparation	Titre
Human lepromatous skin	M. leprae	Lepromin Mitsuda (1963)	*1,280
Human lepromatous skin	M. leprae	Lepromin Mitsuda (Japanese)	1.280
Human lepromatous skin	M. leprae	Cytoplasmic fraction	3,200
Infected rat liver	M. lepraemurium	Washed bacilli	80
Infected rat liver	M. lepraemurium	Cell walls	80
Infected rat liver	M.lepraemurium	Cytoplasmic fraction	160
Culture	M. tuberculosis (H37 Ra)	Washed bacilli	80
Culture	M. tuberculosis (H37 Ra)	Cell walls	20
Culture	M. tuberculosis (H37 Ra)	Cytoplasmic fraction	1,024

* This material agglutinated red cells to a titre of 320.

The results of a typical test are set out in Fig. 1 which shows that at the optimum pH of 5.6 the cytoplasmic fraction of M. leprae tested had an inhibitory titre of 1/3,200. It can be seen that it is important to use the optimal pH. It was also shown that the same titres were obtained with cells of blood group O and A, but that titres were up to four times higher or lower if the cells were not treated with trypsin; but the mean difference between titres of nine comparisons was zero. No inhibition of haemaggregation was seen with horse cells, and rat cells were too unstable to use. Further materials were tosted and some representative results are shown in Table 1. Several different mycobacterial preparations were active. The cytoplasmic fraction of M. tuberculosis was much more active than the cell wall preparation and whole bacilli; but less difference was observed between the cytoplasmic fraction of M. lepraemurium and washed bacilli and cell walls. An extract of normal skin was prepared, but agglutinated red cells too strongly for use in the test.

Because haemaggregation was inhibited by both crude skin extracts and highly purified bacilli and their products, it seems probable that the activity of lepromin preparations is caused by a component of the leprosy bacillus, although further proof is necessary. It may be possible to show, as

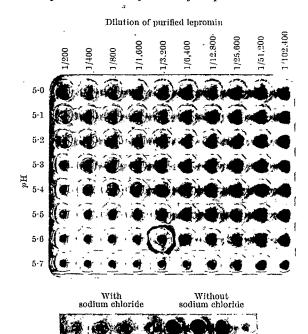


Fig. 1. The appearance of a representative test. The lower part shows that haemaggregation at three pHs has been inhibited by sodium chloride. The upper plate shows that serial dilutions of purified lepromin have inhibited haemaggregation; the highest titre is seen using the most alkaline buffer and the cup showing the end point is marked by a ring.

has been done in the case of tuberculin, that the inhibitory titre is proportional to the skin reacting activity, and also to adapt the test for the measurement of antibodies. This preliminary report is made so that others who may be able to obtain suitable material can explore the significance and possible applications of the phenomenon.

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Isolation of Cyanophages from India

Using the methods of Safferman and Morris¹, who isolated the first phycovirus, strain LPP-1, and propagated it on the blue green alga, Plectonema boryanum, five strains of cyanophages2 have been isolated from local polluted pond Two of these differ in host range and plaque morphology while the remaining three bring about lysis of LPP-1 host, P. boryanum, although showing variation in growth behaviour and plaque size. The first two strains infect either spore forming and/or heterocystous blue green algae and are inactive on P. boryanum. One of these which has been isolated on a species of Cylindrospermum, forming heterocysts at both ends of its filaments and subterminal chains of spores, has a restricted host range. It does not infect other strains of Cylindrospermum with a similar morphology or Anabaena doliolum, another heterocystous and spore forming blue green alga. The second isolate causes lysis of three spore forming blue green algae, Anabaenopsis raciborskii, A. circularis and Raphidiopsis indica, of which the first two are also heterocystous. The first isolate is designated C-1 and the second AR-1 following the terminology of Safferman and Morris¹ for LPP-1, which is based on the first letter of the genus name of the host algae. Strain C-1 causes fast lysis of the host in liquid medium and on agar plates. Plaques appear late but their size increases very fast and the entire algal growth on the plate is lysed within a few days. AR-1 plaques are irregular in comparison with LPP-1. Their size increases with longer incubation. After 16 days plaque size varies from 5 mm to 14 mm (Fig. 1). The margin of the plaque is frayed because of the filamentous nature of the host algae. The notable characteristic of strains C-1 and AR-1 is that they do not lyse spores and heterocysts which remain unaffected in plaques or lysates (Figs. 3 and 4). The gas vacuoles of the algae possessing them also remain unaffected by the strain

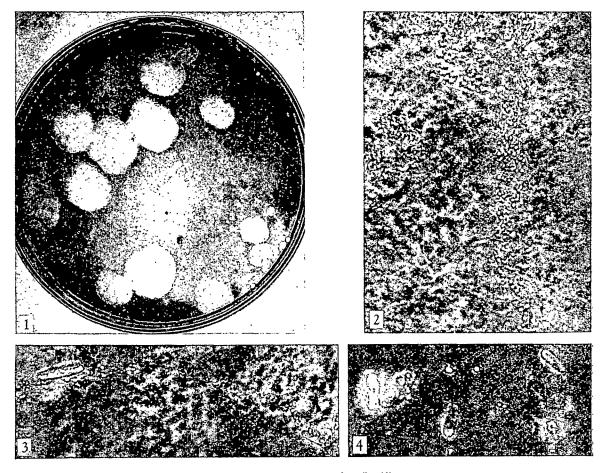


Fig. 1. Plaques of Anahaenopsis racihorskii.

Figs. 2-4. Photomicrographs of lysates. (×800, phase optics.)

Figs. 2-3. Anahaenopsis circularis showing trails of unlysed gas vacuoles and spores.

Fig. 4. A. racihorskii showing unlysed heterocysts and gas vacuoles.

AR-1, leaving trails of gas vacuoles behind (Figs. 2, 3

The isolates on P. boryanum have been designated P-2, P-3 and P-4 on the basis of their restricted host range and differences in plaque morphology and growth behaviour in comparison with strain LPP-1. The size of plaques in P-2 is large (8-12 mm after 12 days) and the host is lysed in about 4 days. Lysis occurs late with P-3 and P-4 and size of plaques varies from 1 mm to 4 mm after 12 days. Lysis with these isolates as with LPP-1 is Cyanophage LPP-1 resistant strains of P. boryanum (our unpublished results) are not resistant to viral isolates P-2 and P-3.

Safferman and Morris³ screened a large number of blue green algae including heterocystous and spore forming genera, Anabaena, Anabaenopsis, Cylindrospermum, Nodularia, Nostoc, Fremyella, Calothrix and Tolypothrix with their BGA virus strain LPP-1, and found it to be inactive. It is therefore the first report of isolation of cyanophages active on heterocystous and spore forming blue green algae. Besides raising questions of taxonomic grouping of these algae their behaviour towards the cyanophages C-1 and AR-1 provides thought to the basic problems concerning the nature of enigmatic structures like heterocysts and gas vacuoles and reproductive structures like spores, which remain unaffected by these viruses. These structures are also insensitive to lysozyme (our unpublished results). The chemical composition of their walls seems therefore to be different from the walls of ordinary vegetative cells which are dissolved by treatment with lysozyme. The other possibility is that DNA of heterocysts and spores may be in a non-replicative state and their metabolic inactivity suggests that they are insensitive to the virus strains described here.

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Integration of Energy Conversion and Biosynthetic Processes in Bacterial **Photosynthesis**

Many species of photosynthetic bacteria can grow in the absence of molecular oxygen (anaerobically) using organic compounds as the sources of cellular carbon1,2. These organisms, however, differ strikingly from nonphotosynthetic anaerobes in that they seem to be unable to obtain sufficient energy for growth from dark fermentative catabolism of organic substrates. Whatever the carbon source, the photosynthetic bacteria seem to require light for multiplication under anaerobic conditions2,3. Through the activity of the photochemical system, light energy is converted to chemical energy in the form of adenosine triphosphate (ATP)^{4,5}, the key fuel for biosynthesis in all types of cells. It is possible that at least part of the energy requirement for anaerobic growth of photosynthetic bacteria can be satisfied by exergonic catabolism of particular substances, but so far this has not been shown. Our search for alternative (dark) anaerobic processes capable of generating ATP at significant rates led us to investigate the intimacy of biochemical coupling between the photochemical energy conversion apparatus and biosynthetic metabolism in the photosynthetic bacterium Rhodopseudomonas capsulatus. The initial aim

was to determine the length of the time period over which the biosynthetic machinery can function at a normal rate following cessation of illumination. The experiments reported here indicate a very tight coupling between the light-dependent ATP generating system and overall biosynthesis, and also describe an experimental approach which should prove useful in exploring the general problem of how energy conversion and biosynthetic processes are integrated.

The initial experiments were based on two assumptions, namely, that: (a) because the photochemical reactions involved are extraordinarily rapids and the subsequent dark reactions associated with ATP formation seem to be completed within only 0·1-0·15 sec (ref. 7), the in vivo rate of ATP production at high light intensity is likely to be fast in comparison with the rates of the enzymatic processes responsible for biosynthesis of cell materials; and (b) during a light period of some particular length, presumably quite short relative to the mass doubling time in continuous light, cells must accumulate sufficient ATP to drive overall biosynthesis at an optimal rate over a subsequent dark period of comparable length. Accordingly, the growth rate with some suitably programmed regimen of intermittent illumination should be equal to that observed in continuous light. Consider now a regimen of equal light and dark times in which the period is much longer than the 'magic' length. It is evident that in these circumstances biosynthesis (growth) will proceed during the light periods, but owing to lack of a continuing energy supply it will be virtually suspended during the dark periods. If growth resumes without lag at the start of each light period and if degradation of essential cell components does not occur to an appreciable extent durin; the dark periods, the overall average growth rate of the cell population should be approximately half the control value. These predictions were tested as follows.

Freshly inoculated cultures of Rps. capsulatus were grown in continuous light until they had entered the logarithmic growth phase. They were then exposed to intermittent illumination, each light period being followed by a dark period of equal length. Growth kinetics with light: dark cycles of different duration were determined by measuring turbidity of the cultures. Typical results showing the relationship of growth rate to length of the light: dark cycle are shown in Table 1.

Table 1. GROWTH RATES OF Rhodopseudomonas capsulatus in continuous AND INTERMITTENT LIGHT

Length of light : dark cycle (sec) 0 (continuous light)	Doubling tim (h) 2·4
10·0 20·0 40·0 60·0 120 240 600 1,200	7·2 9·2 9·0 8·8 8·8 8·8 6·0

A synthetic 0.4 per cent DL-malate + 0.1 per cent ammonium sulphate medium similar to the one specified by Ormerod et al. was used, with thiamine hydrochloride (1 mg/l.) added in place of blotin. Inoculated cultures, in completely filled screw-cap test tubes of 17 ml. capacity, were incubated in continuous light until the bacterial density was approximately 50 Klett-Summerson photometer units (filter No. 68). The tubes were then subjected to repeating light: dark cycles as indicated; in each cycle. light and dark times were equal. Growth rates are given in terms of time required for the bacterial mass to double as indicated by photometer measurements; the shift from continuous to intermittent illumination did not induce a significant lag, and the overall growth kinetics were logarithmic over several doublings. Light source: 30 W reflector flood lamps. Light intensity: 400 ft.-candles (as measured with a Weston Illumination meter model 756), which is essentially saturating; increase of Intensity to 550 ft.-candles decreases the doubling time in continuous light to 2·0-2·2 h. Temperature: approx. 30° C.

It can be seen that even with a cycle as short as 10 sec (that is, 5.0 sec light: 5.0 sec dark), the mass doubling time is significantly longer than the control value of 2.4 h observed in continuous light. The doubling time with even shorter cycles may well approach that of the control more closely, but this has not yet been investigated. Inhibition of growth was particularly pronounced with

cycle lengths between about 20 sec and 4 min, and this range is therefore of special interest. The constancy of the growth rate over this rather wide range suggests that a characteristic lag, occasioned by dark to light transitions, in some critical process is not the primary cause of the inhibition. The expected result with relatively lengthy light and dark periods was not observed with cycles as long as 10-20 min, but a separate series of experiments showed that the doubling time with cycles of 30 min or longer was indeed close to twice that of the control. The data of Table 1 indicate that in Rps. capsulatus there is a very close coupling between the activity of the photochemical energy converting system and the biosynthetic apparatus of the growing cell. It also seems that during photosynthetic growth at high light intensity in a malate + ammonium sulphate medium, the bacteria do not store reserve materials which can be rapidly mobilized to provide energy in the absence of light. The integration between the photophosphorylation system and biosynthesis is evidently markedly disturbed when energy flow is repeatedly interrupted at certain frequencies. From the results of the growth experiments we infer that in the particular medium used, a period of the order of several minutes is required for completion of a key energy dependent process, necessary for optimal balanced growth of Rps. capsulatus. It may be that interruption of energy flow for a significant time during this period is attended by alteration or partial degradation of precursors of a "key product" and that the cumulative effect of such repeated interruptions is an inhibited growth rate.

As a first approach to studying the nature of the derangement caused by intermittent illumination of short cycle, we have analysed cells grown under different regimens for their contents of protein and ribonucleic acid (RNA). With continuous saturating light (550 ft.-candles), the protein/RNA ratio in the cells is about 3. After several mass doublings in intermittent light with cycle lengths between 30 and 120 sec, the ratio increases substantially (for example, to 4.6 with a 60 sec cycle), chiefly because the RNA content decreases significantly. On the other hand, the protein/RNA ratio of cells grown with light: dark cycles longer than 5 min approximates the value characteristic of organisms cultivated in continuous light. This suggests that with longer cycle times, biosynthesis proceeds during the light periods at a nearly optimal rate and is essentially "frozen" during the dark periods. The changes observed in the protein/RNA ratio are doubtless related in some way to the growth rates under the several conditions. It is well known with certain non-photosynthetic bacteria that, over a particular range of doubling times, growth rate (with continuous energy supply) is proportional to total cellular RNA contents. Because the growth rate and ribosome content of the cell are similarly correlated9, inhibition of ribosome synthesis may possibly be one of the major early consequences of interference with energy

Studies¹⁰⁻¹² on a number of non-photosynthetic bacteria of different physiological types have shown that the synthesis of one g dry weight of cell material requires the regeneration of about 100 millimoles of ATP from adenosine diphosphate (ADP) and it is likely that this also holds for photosynthetic bacteria. The size of the steady state pool of adenylate nucleotides (adenosine monophosphate (AMP), ADP, and ATP) present in growing bacteria—for example, about 0.015 millimoles per g dry weight in the photosynthetic bacterium *Chromatium* 13—is clearly insignificant in terms of the energy requirements for net growth and quite small in respect to the amounts necessary for appreciable nucleic acid synthesis. Adenylate nucleotides exert regulatory effects on the activities of a number of enzymes¹⁴ and it is possible that the pool functions primarily as a signal device for control of energy flow and biosynthetic reactions directed toward macromolecular syntheses. Although the total size of the adenylate nucleotide pool may not vary greatly under different

metabolic conditions, recent experiments of Atkinson et al. 15,16 show that the activity of certain enzymes which utilize ATP is strongly affected by changes in the relative concentrations of the nucleotide species. The composition of the pool in Rps. capsulatus subjected to intermittent illumination presumably oscillates between limiting states of high and low "energy charge", a concentration ratio control parameter defined as (ATP+0.5ADP)/(ATP+ ADP + AMP). Other significant oscillations must occur, such as in the ratio reduced/oxidized pyridine nucleotide, and the net effect of intermittent illumination on growth will obviously be the resultant of a number of interdependent changes in a very complex network of biochemical reactions. Experiments now in progress are aimed at evaluating the working hypothesis that operation of the biosynthetic machinery in Rps. capsulatus is dominated by the rate of ATP generation and the "energy charge".

In view of their relatively rapid growth rates and other metabolic properties, and the ease with which the activity of the energy conversion system can be experimentally manipulated, photosynthetic bacteria lend themselves to investigation of a number of general problems, especially the control mechanisms which govern integration of energy yielding and biosynthetic processes. The technique of programmed illumination is essentially a means of pulsing ATP formation in vivo and, as such, obviously can be exploited for study of several important aspects of metabolic regulation.

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IMMUNOLOGY

"Species Specificity" of Interferons: a Misnomer?

In 1961, there were two reports of monkey interferon protecting human cells1,2 and one of human interferon protecting monkey cells3, but in none of these was the extent of the cross-reaction measured. In view of the usual strict "species specificity" of interferons (for example, chick interferon is not active in mouse cells, human cells4 or duck cells5), it seemed worthwhile to examine the cross-reactivity of human and monkey interferons.

For our present work, we have used a cytopathic effect-inhibition assay with tube cultures of either diploid human embryo lung cells or primary monkey kidney cells (Erythrocebus patas or Cercopithecus aethiops). The assay tubes were incubated with the interferon for 24 h at 37° C, and the interferon was removed and the tubes were washed twice with fresh medium before challenge. The purpose of this was to remove inhibitors other than interferon which may have been present in the preparations^{6,7}. The challenge virus was 100 TCD_{50} of HGPrhinovirus which had similar growth characteristics in both monkey kidney and diploid human lung cells, and the test was read after 3 days at 33° C. Interferons were prepared by infecting monkey leucocytes (Macaca nemestrina) or human leucocytes with Sendai virus, and collecting the culture fluid after 18 h. Virus was destroyed by keeping the fluids at pH 2 for 5 days, and a potent Sendai antiserum was added before assay.

Four batches of monkey and human interferons were assayed and each gave high heterologous titres (Table 1). With the monkey interferon, the titre in human cells was usually greater than that in monkey cells.

Table 1. THRE OF HUMAN AND MONKEY LEUCOCYTES INTERFERON IN

		110344511 211137	DIOLILLIA CLI	200	
	Monkey i			Human i	
Batch	Monkey cells	Human cells	Batch	Monkey cells	Human cells
1	26	40	1	20	85
2	55	100	2	165	400
3	60	126	3	500	600
4	1,000	1,000	4	3,000	2,000

Monkey interferon was made from leucocytes of Macaca nemestrina. Batches I-3 were assayed on primary kidney cells of Cercopithecus aethiops, and batch 4 on primary kidney cells of Erythrocebus patas. The human cells were embryonic lung.

Using the most potent interferon sample (batch 4), the respective homologous and heterologous activities were shown to be unaffected by low pH, ultracentrifugation and treatment with ribonuclease, but they were destroyed by trypsin. There was some reduction in titres when Sindbis virus was used as challenge, and neither type of interferon showed any activity in mouse L cells (Table 2). From this it seems that the high cross-reactions of monkey and human interferons are genuine heterologous activities.

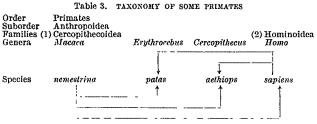
Table 2. THE CHARACTERIZATION OF MONKEY AND HUMAN INTERFERON HETEROLOGOUS ACTIVITIES

	Human interferon		Monl	cey interfe	eron	
Assay cells→	Human diploid	Monkey kidney	Mouse L	Human diploid	Monkey kidney	$_{\rm L}^{\rm Mouse}$
Treatment of interferon						
None	2,000	3,000	ND	1,000	1,000	ND
pH 2, 5 days	2,000	3,000	ND	1,000	1,000	ND
40,000 r.p.m., 3 h, 4° C	2,000	3,000	ND	1,000	1,000	ND
0.5 mg/ml. of trypsin, 1 h, 37° C	30*	30*	ND	30*	30*	ND
0.05 mg/ml. of RNase, 1 h, 37° C	2,000	3,000	ND	1,000	1,000	ND
Untreated but Sindbis challenge	2,000	2,000	16*	560	560	16*

ND, Not done.
* Highest concentration tested.

The present results show that interferon made from one

genus of monkey-Macaca-is active in cells from two other genera—Čercopithecus and Erythrocebus. monkey interferon does not show "species specificity"



The cross-reaction of interferons is shown by arrows.

or even "genus specificity". These three genera belong to the family Cercopithecoidea (Table 3) and, because interferon from Macaca is active in human cells, and human interferon is active in Cercopithecus and Erythrocebus cells, there seems to be complete cross-reaction at the level of the taxonomic family. Until the extent of this type of cross-reaction is more clearly defined, perhaps it would be preferable to abandon the term specificity" and instead state the cell types in which heterologous interferon activity is or is not known.

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Factors influencing Lysis of Whole **Blood Clots**

LITTLE is known about the mechanism by which blood clots or fibrin monolayers usually lyse. The enzyme plasmin (fibrinolysin) has been assumed to play a key part in this mechanism and for this reason plasmin, its substrates fibrinogen and fibrin and its naturally occurring inhibitors have all been extensively studied and characterized1. The biological importance of these studies has depended on the assumption that the plasmin system serves to maintain the homeostatic balance between the polymerization of fibrin and the dissolution of this polymer. Although much is known about the activation of the polymerization phase of this homeostatic system, there is little information about the mode of activation of the clot dissolving or plasmin system in the intact blood clot. We describe here in qualitative terms some of the factors and steps involved in the lysis of a clot from whole blood diluted 1:10 with phosphate buffer2.

A sample (1 ml.) of whole blood was drawn from the antecubital vein of the forearm and immediately diluted 1:10 with phosphate buffer (0.034 molar, μ 0.072, pH 7.4) at 4° C. At this temperature and dilution the sample will not clot spontaneously. Inhibitors, that is, antisera and cobra serum, were then added to the diluted whole blood. Samples (2 ml.) in 15×100 mm test tubes of treated and untreated diluted blood were then coagulated at 4° C by the addition of 1 U of thrombin obtained from Parke-Davis, Detroit, Michigan. After 20 min at 4° C, the clots which formed were transferred to a water bath at 37° C and observed for retraction, shedding of red cells and time of complete lysis.

The requirement of platelets for the lysis of these clots is shown in Table 1. Samples of diluted blood (2 ml.) were centrifuged at 0-1,800g for 10 min before they were decanted into a second tube and thrombin was added at

Table 1. RELATIONSHIP OF RED BLOOD CELL AND PLATELET COUNTS TO CLOT DISSOLVING TIME

('entrifugation	Clot dissolv	ving time	Supernatani	cell counts	
of diluted blood	Supernatant	Remixed	Platelet	Red blood	
for 10 min	alone	samples	count/mm ³	cells/mn13	
at 4° C (g)	(h)	(h)	$(\times 10^{3})^{*}$	$(\times 10^{3})$	
0	4.3	4.3	25.0	513.3	
200	4.1	4.3	18.2	0.26	
1,200	4.6	4.3	8.1	0.29	
1.800	>12 >24	4-3	1.1	0.05	

Values are the average from studies on five healthy adults at 9.00 a.m. * Platelet counts carried out with a phase microscope.

4° C. Removal of red and white cells was virtually complete at 200g with no appreciable effect on clot retraction or time of lysis. When the platelet count was lowered to 1,100/mm³, however, retraction was reduced and clot lysis was prolonged. The normal times of clot lysis were restored on resuspension of the platelet buttons. That the platelets have to be structurally intact at least for the initial phase of clot lysis was demonstrated by sonication. Some samples were sonicated 15 min before and some 15 min after addition of thrombin. Those samples in which platelets were destroyed by sonication before thrombin was added did not retract and the lysis time was prolonged from 4 to 14 h. Those samples in which platelets were destroyed by sonication after thrombin was added retracted and lysed normally.

The requirements of plasminogen, YM-globulin and of the third (C'3) and the fourth (C'4) components of complement for normal clot lysis were demonstrated by the inhibition of retraction, shedding of red cells and clot lysis which resulted from the addition of specific antibodies to the assay system. Rabbit antisera to human albumin, α_2 -macroglobulin, transferrin, γG -, γM -globulin and γM cold agglutinin were obtained commercially from the Behringwerke Company, Marburg/Lahn, Germany, and antisera to human plasminogen3, C'3 (ref. 4) and C'4 (ref. 5) were prepared as described previously. The gamma globulin fractions of these antisera were prepared by precipitation with ammonium sulphate according to the method described by Kabat and Mayers. These fractions were made up to the original serum volume and samples of 0.25 ml. were then added to the 2 ml. aliquots of diluted, normal, whole blood 5 min before the addition of thrombin. Table 2 and Fig. 1 show that antisera to γM, C'3, C'4 and plasminogen inhibited clot retraction and lysis, whereas the antisera to albumin, a2-macroglobulin, transferrin and γG -globulin did not affect clot retraction or lysis. A highly purified protein from cobra venom7, which has been shown to inactivate selectively C'3, also caused inhibition of clot lysis. Addition of 20 to 40 μg of homologous antigen—that is, purified γM, C'3, C'4 or plasminogen—to these systems restored normal lysis, provided that they were added before the thrombin. All preparations of the complement components and of γM were tested for plasminogen activity but none was found.

Platelets, γM and complement components seem to be involved in clot lysis in vitro, which suggests that there is a functional association between platelets and these serum factors. In order to examine this possibility, platelets were collected from 10 ml. of whole blood diluted 1:10 with phosphate buffer at 4° C by centrifuging at 700g for 20 min and then collecting the plateletrich supernatant. These platelets were washed twice in phosphate buffer and resuspended in buffer to give a final count of 300,000/mm³. At this concentration there was no platelet aggregation or viscous metamorphosis. A sample (0·1 ml.) of the platelet suspension was mixed with 0·05 ml. of various dilutions of antisera listed in Table 3 and examined for agglutination after 30 min with the aid of a hand lens. A 1:10 dilution of platelets,

Table 2. EFFECT OF SPECIFIC ANTIBODIES TO HUMAN SERUM PROTEINS, AND OF COBRA FACTOR AND TEMPERATURE ON RETRACTION AND LYSIS OF BLOOD CLOTS

Inhibiting substance	Retraction (0-4+)	Lysis time (l
Phosphate buffer (0.25 ml.)	4+	4
Anti-yM-globulin (0.25 ml.)	Ō.	> 24
Anti-C'3 (0.25 ml.)	2+	14
Anti-C'4 (0.25 ml.)	2+	12
Anti-C'3 and C'4 (0.25 ml.)	1+	> 24
Anti-plasminogen (0.25 ml.)	3+	24
Anti-albumin (0.25 ml.)	4+	3
Anti-a, macroglobulin (0.25 ml.)	4+	4
Anti-transferrin (0.25 ml.)	4+	4
Anti-yG-globulin (0.25 ml.)	4+	$\frac{4}{3}$
Cobra factor (0.25 ml., 60 µg)	2+	$1\overline{2}$
Assay at 37° C at all times	2+	> 24
Assay at 4° C until thrombin wa		
added, then at 37° C	4+	4

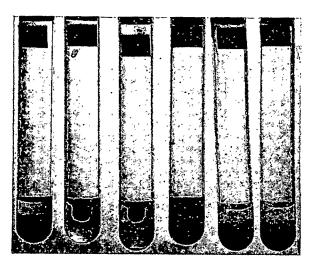


Fig. 1. Diluted whole blood clots. 0·25 ml. aliquots of partially purified rabbit antibodies have been added to: (1) albumin; (2) C'4; (3) C'3; (4) γM; (5) γG; (6) a₃-macroglobulin. Photograph taken 4·5 h after addition of thrombin. Samples 1, 5 and 6 have undergone complete lysis. Samples 2 and 3 have retracted but have shed few red cells and have not lysed. Sample 4 has not retracted, shed red cells or lysed.

whereas a 1:10 dilution and less of antisera to albumin, α_2 macroglobulin and transferrin did not cause agglutination. The antisera to γ G- and γ M-globulins caused 0 to 1+ aggregation, respectively. Purified γ M, C'3 and C'4 inhibited agglutination when added to the antisera before mixing with platelets.

before mixing with platelets. Although γM , C'3 and C'4 seemed to be associated. with platelets, the question remained to be answered whether the complement components were specifically adsorbed onto the platelets and whether they were adsorbed in vivo or in vitro. A 2 ml. sample containing 1×10^{10} platelets was tested for immune adherence by incubation with 0.1 ml. of 1×108 red blood corpuscles from the same subject. Immune adherence to erythrocytes is known to depend on the presence of specifically bound C'3 on the surface of the adhering particles. A 2+ reaction for immune adherence was noted, indicating that at least some of the C'3 was adsorbed specifically and in active form. In answer to the second question, blood platelets, drawn at 37° C and diluted with phosphate-sodium chloride buffer to give an ionic strength of 0.15, were examined for bound C'3 and C'4 by agglutination using specific antisera. In these conditions the agglutination reactions were significantly weaker than those seen with platelets of blood collected at 4° C and diluted in phosphate buffer with an ionic strength of 0.072 (see Table 3). Normal clots and clots to which antibodies to YM-globulin, C'3 or C'4 had been added were processed, sectioned and stained for microscopic examination of the relationship of platelets to the fibrin network. Clots examined 30 min after addition of thrombin revealed platelets adsorbed onto the fibrin strands, whereas the platelets had fused and were no longer discernible as individual elements in clots sectioned 2 h after addition of thrombin (Fig. 2A). The addition of antisera to YM, C'3 or C'4 inhibited fusion of the platelets as shown in Fig. 2B where platelets were still present and intact after 2 h in a clot containing anti- γ M-globulin.

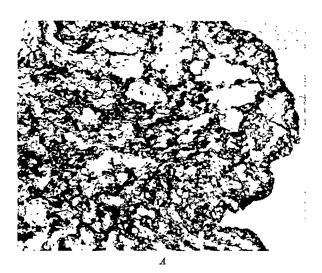
Table 3. AGGLUTINATION OF PLATFLETS BY ANTISERA TO SOME HUMAN PLASMA PROTEINS

Agglutination (0-4+) Agglutination (0-4+)

	Agginination (0-4+) (4° C, μ 0.072)	(37° C, μ 0·15)
Phosphate buffer	0	0
Anti-yM-globulin	2+	0
Anti-C'3	4+	1+
Anti-C'4	4+	1+
Anti-plasminogen	0	0
Anti-albumin	0	0
Anti-a ₂ -macroglobulin	0	0
Anti-transferrin	0	0
Anti-γG-globulin	1+	0
Platelets were washed at	4° C. 40.072, and at 37°	C. u 0.15.

There is a class of acquired haemolytic anaemias which is associated with the occurrence of cold agglutinin in the blood and the presence of C'3, C'4 on the surface of the erythrocytes¹⁰. We studied two of these patients with Dr Hugh Fudenberg of the University of California, San Francisco, and found that they had a short clot lysis time (2 h compared with 4-6 h for normal controls) and markedly positive agglutination reactions of their platelets when these were tested with antisera to \(\gamma M\)-globulin, C'3 or C'4. Patients with low platelet counts (10,000/mm³) were found to have rates of clot lysis twice as slow as normal, whereas patients with high platelet counts (800,000/mm³) had very rapid rates of clot lysis, as seen in studies with Dr Richard Creech of the University of Pennsylvania. One patient with Glanzmanns thrombasthenia (an abnormality of clot retraction and platelet agglutination in presence of adenosine diphosphate) had markedly inhibited clot lysis. The latter study was made in collaboration with Dr M. Zucker of New York University.

In conclusion, the preceding data suggest in qualitative terms what factors might be involved in one mechanism for the lysis of diluted whole blood clots in vitro. tentative concept of the underlying mechanism may be formulated in the form of the following working hypothesis. Platelets of diluted whole blood at 4° C adsorb



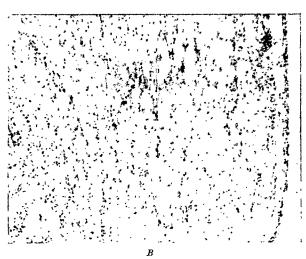


Fig. 2. A, Photograph of diluted whole blood clot 2 h after addition of thrombin. Note that platelets have fused and that there is contraction of the platelet-fibrin elements. B, Diluted whole blood clot with 0.25 ml, of antihuman yM serum 2 h after addition of thrombin. Note that platelets have not fused and that there is no retraction of platelet-fibrin elements.

γM-globulin (possibly 19s cold agglutinin) which triggers a complement reaction involving at least the components C'1, C'2, C'3 and C'4. The platelets bearing these proteins on their surface are then incorporated into the thrombin induced fibrin network, possibly by a reaction which resembles immune adherence. The protein coat may facilitate, if not initiate, fusion of the platelets, and activation of plasminogen may then be caused either by the enzyme activities associated with the complement components on the platelet surface or by enzymes released from the fusing platelets. The possible relevance of the observations made in this artificial system to in vivo and clinical situations is supported by observations on various clinical cases with selected abnormalities including patients with accelerated fibrinolytic states associated with acquired haemolytic anaemia characterized by the presence of cold agglutinins, high YM-globulin levels, 19s cold agglutinins and adsorption of C'3 and C'4 to erythrocytes and platelets under physiological conditions.

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Search for I Antigen in Human Tissues

THE majority of human red cell cold agglutinins have a specificity for an antigen termed I which is present in the red cells of the vast majority of adults1. Only very occasionally does an adult lack the I antigen. The high titre anti-I cold agglutinins found in association with chronic autoimmune haemolytic anaemia and, transiently, in association with Mycoplasma pneumoniae infection are therefore autoantibodies. In human infection with Myco-plasma pneumoniae the cold agglutinin seems to be directed not against the infecting organism but against the host red cell2. The present study was undertaken to investigate whether I antigen can be detected in human tissues other than erythrocytes and hence to determine whether anti-I cold agglutinin is an organ specific or a non-organ specific antibody.

Organ homogenates, prepared from three cases (adults) at post-mortem examination, were tested for their ability to absorb anti-I cold agglutinin at 4° C. Twentyeight organs were examined and sixteen of them were obtained from more than one case (Table 1). The anti-I cold agglutinin used was from a patient with chronic autoimmune haemolytic anaemia; it had been purified by

repeated absorption onto and elution from group OI red cells by a technique previously described and had a cold agglutinin titre of 256 at 4° C. Tissue samples, weighing approximately 10 g, were cut into small pieces with scissors and washed in several changes of normal saline until there was no blood staining of the washings and they were then homogenized in cold saline using a Griffin and George mechanical homogenizer. The organ homogenates were washed twice by suspending them in an excess of normal saline followed by centrifugation at 3,000 r.p.m. for 15 min and 25 per cent suspensions (v/v) of them were made in normal saline. In addition, 25 per cent suspensions were made of washed red cells from (a) each post-mortem case, (b) a healthy adult of group OI and (c) a healthy adult of group Ai (i denotes lack of I antigen). In the absorption tests, 0.4 ml. of each antigen suspension was pipetted into a 1 x 6 cm plastic tube, the antigens were packed by centrifugation at 3,000 r.p.m. for 10 min and the supernatants were removed, leaving in the tubes 0.1 ml of packed antigen, each of which was mixed with 0.4 ml. of anti-I reagent and incubated for 2 h at 4° C. The tubes were shaken at intervals of 30 min. At the end of the incubation period the supernatants were separated after centrifugation at 3,000 r.p.m. at 4° C for 10 min and they were then tested for residual anti-I activity, that is, their cold agglutinin titres were determined by a micro technique which has been previously described2. At the same time, the cold agglutinin titre of unabsorbed anti-I reagent was determined. Each titration was performed in duplicate. The inhibition score for each tissue was the number of "tubes" by which the anti-I titre was reduced after absorption, for example, a score of one indicates a two-fold reduction and a score of two indicates a four-fold reduction in titre. In one of the post-mortem cases (of blood group B) the ability of the stomach homogenate to absorb anti-B was tested using the same v/v ratios of antigen to antibody; an anti-B serum with a titre of 128 was used.

The mean anti-I absorption scores of red cells from the post-mortem cases and from the healthy adult were 4.6 and 4.3, respectively (representing a sixteen-fold reduction in anti-I titre), compared with a score of zero with the i red cells; the mean scores of all the other tissues tested were between zero and one. Because a two- or even a four-fold difference in cold agglutinin titre is within the experimental error of this technique, it is

Table 1. INHIBITION OF ANTI-I ACTIVITY BY HUMAN ORGAN HOMOGENATES

AND REI	CELLS		
Organ	No. of cases tested	Mean ir Score*	hibition (Range)
Pharyngeal mucosa	1	0	
Oral mucosa	â	ŏ∙7	(0-2)
Bronchial mucosa	ĭ	0.5	(0-1)
Lung	2	0.4	(0-1)
Skin	9	0.2	
Stomach	ى 9	0.3	(0-1) (0-2)
Liver	o o	0.1	(0-2)
Gall bladder	3	0.1	
Colon	ī		(0-1)
Ileum	ខ្ម	0.5	(0-2)
Trant	1	1	
Heart	ğ	0	(0.0)
Aorta	3	0.5	(0-2)
Pectoral muscle	3	0.5	(0-2)
Adipose tissue	1	0.5	(0-1)
Kidney	3	0∙3	(0-2)
Lymph node	2	Ō	
Spleen	1	0	
Brain	2	0	
Spinal cord	1	. 0.5	(0-1)
Submaxillary salivary gland	3	0	
Thyroid	2	0	
Pancreas	3	0.2	(0-1)
Oesophagus	1	0	
Bladder	2	0.2	(0-1)
Uterus	1	0.5	(0-1)
Ovary	1	0	
Testis	ī	Ó	
Prostate	ĩ	Õ	
Red cells from post-mortem cases	ã	4.6	(2-7)
Control group OI red cells	ĭ	4.3	(3-6)
Group Al red cells	1313333131333313212132312111311	ō	,- v,

^{*}Inhibition score denotes number of "tubes" fall in anti-I titre after absorption with antigen at 4° C. Duplicate titrations were performed for each antigen preparation; the mean score was therefore representative of two to six readings.

doubtful whether any absorption of anti-I had occurred with any of the organ homogenates. Furthermore, the incubation of a gross excess of these organ homogenates with anti-I reagent (0.8 ml. of packed antigen with 0.2 ml. of anti-I reagent) did not result in significant change in anti-I titre. The group B stomach homogenate, however, completely absorbed the anti-B antibody from the serum, yielding a score greater than 6. Absorption tests using an anti-I serum from a patient with Mycoplasma pneumoniae infection and homogenates of lung, liver, brain and erythrocytes from one of the post-mortem cases gave results comparable to those in Table 1 (results not shown).

In a further experiment, a search for soluble I antigen in the organs was made by incubating 25 per cent suspensions of the organ homogenates at 4° C overnight and adding 0.2 ml. of their supernates to an equal volume of anti-I reagent. After incubation for 1 h at 4° C there was no loss of anti-I activity, which indicates that there was no detectable I antigen in soluble form. Because I antigen seems to be localized to red cells anti-I cold agglutinins can be considered as organ specific auto-antibodies.

We thank Dr W. J. Jenkins for supplying us with group Ai red cells and Drs P. Scheuer and Anne Stuart for the post-mortem material. This work was supported by grants from the Royal Free Hospital and the Medical Research Council.

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Immune Cross-reactivity of Penicillin and Cephalothin

WE recently described positive direct Coombs tests in patients receiving sodium cephalothin. Because most normal sera caused the *in vitro* agglutination of red cells coated with cephalothin, we concluded that some of the positive direct Coombs tests must be related to non-immune binding of a serum protein-cephalothin complex to the red cell surface. We also suggested the possibility that some of these test sera contained anti-penicillin agglutinins.

Despite the similarity in molecular structure between penicillin and cephalothin, very few instances of allergic reactions have been reported among patients known to be allergic to penicillin who were receiving cephalothin²⁻⁴. In patients with a history of penicillin allergy, the association of positive haemagglutination with the more severe forms of allergy is striking, but not absolute⁵. In patients with benzyl-penicilloyl (BPO) specific skin sensitizing antibodies, there is an association with circulating BPO specific antibodies. In normal patients without a history of penicillin allergy, however, and who even have negative skin tests, almost 10 per cent have a significant haemagglutination titre against penicillin treated cells. Furthermore, some patients who have never received penicillin have positive haemagglutination tests7. The correlation between clinical allergy and haemagglutination reaction is not therefore absolute. We have studied the serological cross-reactivity of penicillin and cephalothin in a haemagglutination system.

Red blood cells were coated with penicillin and tests for anti-penicillin agglutinins were done by the method of Ley*: agglutination tests with cephalothin were

performed as previously described1. Eluates prepared by the heat method of Marcuse⁹. Sera from two patients with anti-penicillin antibody who had never received cephalothin agglutinated red blood cells coated with penicillin in the indirect Coombs test (cells+serum+ anti-globulin reagent) in titres of 1:256 and 1:1,024. The same two sera, when tested against red blood cells coated with cephalothin, had titres of 1:256 and 1:512, respectively. The two sera directly agglutinated (cells+ serum) group O red blood cells coated with penicillin in titres of 1:64 and 1:128; group O red blood cells coated with cephalothin were directly agglutinated in titres of 1:64 and 1:32, respectively. The direct haemagglutination of red cells coated with penicillin and red cells coated with cephalothin by the anti-penicillin sera was inhibited by prior incubation with penicillin. Inhibition of the indirect Coombs activity required greater amounts of penicillin than were necessary for inhibition of direct agglutination. Cephalothin also inhibited the haemagglutinating activity of these sera against red cells coated with penicillin and red cells coated with cephalothin in both the direct agglutination and the indirect Coombs

Seventy-five blood samples from patients receiving cephalothin were studied. All patients had a history of prior penicillin therapy. Fourteen of these people had agglutinins against cells coated with penicillin and thirteen of the fourteen had positive direct Coombs tests while receiving cephalothin therapy. Forty-one of the seventy-one patients who did not have demonstrable anti-penicillin agglutinins had positive direct Coombs tests while on cephalothin. It was also observed that the positive Coombs tests in the latter group were not as strong as in the former. Twenty eluates were prepared from samples of red blood cells from patients with positive direct Coombs tests while on cephalothin, including six from patients with anti-penicillin agglutinins. Six eluates gave positive results in the indirect Coombs tests against cells coated with cephalothin and all six were from the patients with anti-penicillin agglutinins. Two of these eluates were tested against cells coated with penicillin and gave the expected positive indirect Coombs tests.

These studies demonstrate that sera with anti-penicillin antibody react in vitro with red cells coated with cephalothin and penicillin to almost identical titres. Studies of blood samples from patients receiving cephalothin demonstrate the same type of cross-reactivity. The eluate studies demonstrate the relationships between anti-penicillin antibody, penicillin and cephalothin, and identify the importance of anti-penicillin antibody in the pathogenesis of some of the positive direct Coombs tests in patients receiving cephalothin. The antibody to penicillin seems to bind specifically to cells coated with cephalothin. This represents another mechanism of action other than non-immune, non-specific binding of protein associated with cephalothin on red cells. specific antibody to cephalothin has not been demonstrated.

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GENETICS

Pentosuria in a North American Indian

PENTOSURIA, one of Garrod's original four inborn errors of metabolism1, is usually believed to occur almost entirely among people of Jewish descent, whose antecedents can be traced to eastern Europe2. The only well authenticated cases of this disorder to be reported among individuals of other ethnic origin have been in four Lebanese families3,4. We wish to report a case of essential pentosuria in a North American Indian.

During a search for metabolic abnormalities among the patients of a large hospital for mental defectives, simple urinary screening tests disclosed a child whose urine gave a strongly positive reduction of Benedict's solution. When the urine was subjected to unidimensional paper chromatography for sugars in ethyl acetate: pyridine: water (12:5:4), and the chromatograms were developed with a p-anisidine spray reagent⁶, only one sugar was present. This matched authentic xylulose in both its migration rate and its colour. The patient, a severely mentally retarded 10 year old boy, is an Athabascan Indian from a reserve on the British Columbia-Yukon Because there was no evidence that he had border. Caucasian forebears, it seemed important to prove that his urinary sugar was indeed xylulose.

Approximately 500 mg of the sugar was isolated from a specimen of the patient's urine by a modification of the method of Touster et al.7. Urine was passed successively through columns of 'Dowex' 50W-x2, and 'Duolite' A-4. to remove cations and anions, and was then treated with urease to destroy urea. After lyophilization, sugar was extracted from the residue with 95 per cent ethanol. The ethanolic extract was concentrated to a small volume on a rotary evaporator at room temperature, and the concentrate was streaked on to sheets of Whatman 3MMThese were then chromatographed unidimensionally in the ethyl acetate: pyridine: water solvent, and the bands from each chromatogram, shown to contain presumptive xylulose, were cut out, and the sugar eluted from the paper with 95 per cent ethanol. By this technique, small amounts of xylose, which may be produced from xylulose as an artefact on passage through the ion exchange columns, as well as other trace impurities. were removed. Finally, the combined cluates were taken to relative dryness on a rotary evaporator at room temperature, and the resultant thick yellow syrup was further dried over phosphorus pentoxide.

The isolated urinary sugar co-chromatographed precisely on paper with authentic D-xylulose in five different solvent systems. Phenylosazones were prepared from authentic D-xylose and from the isolated urinary sugars. Each had a melting point of 157°-158° C and, when equal portions of the two osazones were mixed, the mixed melting point was elevated to 190° C. In addition, the urine reduced qualitative Benedict's solution within a few minutes at room temperature. These findings support the identifica-

tion of the urinary sugar as xylulose.

The amounts of xylulose present in the urine were quantitated both by paper chromatographic comparison with appropriate standards of xylulose, and by the reduction of quantitative Benedict's solution, as described by Lasker and Enklewitz⁹. Results of both methods were in good agreement, and during two different periods of 24 h the child was found to excrete 7.2 and 4.5 g of xylulose. These figures are higher than the 1.0-4.0 g of xylulosc/day usually excreted by pentosurics2. When the patient was given orally 10 g of p-glucuronolactone, a metabolic precursor of L-xylulose, his rate of urinary excretion of xylulose was tripled for several hours.

Urine specimens were examined from four of the patient's seven siblings, as well as from his mother. None contained xylulose. One sibling, who was found not to have pentosuria, was also mentally defective, and it seems that the patient's mental defect and pentosuria are not causally related.

The presence of essential pentosuria in an Indian from north-west Canada suggests that the gene mutation for pentosuria has occurred more than once in widely diverse human populations, and that the disorder can be expected to be found in other persons who are not of eastern

European or eastern Mediterranean or gin.
We thank Dr O. Touster for a sample of authentic D-xylulose, S. Hansen and D. Love for technical assistance, and Dr B. Tischler, R. Bunting and Sister R. Herle for help with the patient and his relatives. This work was supported by grants from the Medical Research Council and the Department of National Health and Welfare, Canada.

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Duplicate Genetic Activities affecting Meiotic Chromosome Pairing at Low Temperatures in Triticum

Common wheat, Triticum aestivum (2n=42), is an allohexaploid with the genomic constitution AABBDD. One of its parents was a tetraploid, like T. dicoccum, with twenty-eight chromosomes and with an AABB genomic constitution. The A, B and D genomes—each of which contains seven chromosomes—were derived from closely related diploid species. Consequently, there are considerable levels of genetic duplication in T. dicoccum and of triplication in T. aestivum. This is formally recognized by the classification of the chromosome complements of both species into seven homoeologous groups2. Homoeologous chromosomes are genetically corresponding members of different genomes the relationships of which are presumed to stem from their evolutionary origin from the same chromosome of the common progenitor of all three diploid ancestors. Twenty-one bivalents are usually formed at meiosis in T. aestivum and there is disomic inheritance. T. dicoccum has a similar diploid-like pattern of chromosome pairing with the regular formation of fourteen bivalents. Several distinct genetic activities are known to affect the regularity of meiotic pairing of T. aestivum³⁻⁵ and it has been inferred that some of these activities are duplicated—being performed at more than one locus^{5,6}. This communication is concerned with the first unequivocal demonstration of such duplication and with the demonstration that different components of the system are responsible for the maintenance of meiotic regularity at low temperatures in different Triticum

Meiotic chromosome pairing is normal in euploid forms of T. aestivum var. 'Chinese Spring' over the temperature range 12°-28° C. There is, however, pronounced pairing failure at 12° C and 15° C in the absence of chromosome 5D,

although pairing is normal at 20° C and 28° C (refs. 5 and 7). Consequently, chromosome 5D performs a genetic activity which stabilizes chromosome pairing against the effects of low temperatures. There is no difference in the extent of meiotic pairing over the temperature range 12°-28° C in AABB tetraploid forms of Triticum, like T. dicoccum⁵. Consequently, because 5D is not present, either some other chromosome must take over the activity performed by 5D in the hexaploid or else normal pairing takes place without this activity at low temperatures in tetraploid To determine the nature of the tetraploidhexaploid difference, T. dicoccum and T. aestivum var. 'Chinese Spring' were compared by experimental breeding.

Hybrids were produced between these species in which chromosome 5D of T. aestivum was either entirely absent or was present in the disomic condition. The 5D-nullisomic hybrids with thirty-four chromosomes resulted from the cross T. aestivum monosomic $5D \times T$. dicoccum; while the 5D-disomic hybrids with thirty-six chromosomes resulted from the cross T. aestivum tetrasomic $5D \times T$. dicoccum. At meiosis, the 5D-nullisomic F_1 plants usually had fourteen bivalents, representing A or B genome pairs, and six univalents, representing the remaining unpaired D genome chromosomes. Meiosis in the 5D-disomic hybrids with thirty-six chromosomes was like that in the 5D-nullisomic hybrids except that there were fifteen instead of fourteen bivalents, the additional bivalent being the 5D pair. These hybrids were allowed to self-pollinate so that F_2 progenies could be obtained. In the 5D-nullisomic F_2 progenies, derived from the hybrids with thirty-four chromosomes, chromosome 5D was never present. By contrast in the 5D-disomic F_2 progenies, derived from the hybrids with thirty-six chromosomes, chromosome 5D was always present in double dose. The F₂ progenies were grown in controlled environment rooms in conditions of continuous light at 15° C. The numbers of chiasmata were recorded in ten pollen mother cells at first metaphase of meiosis in fifty-eight 5D-nullisomic and in eighty-two 5D-disomic plants. Because of the irregular segregation of the six D genome chromosomes which had been unpaired in the F_1 hybrids, the chromosome numbers of these progenies extend from twenty-eight to forty in the 5D-nullisomic F_2 and from thirty to forty-two in the 5D-disomic F_2 . In order to remove differences in chiasma frequencies that simply reflected differences in chromosome number, chiasma frequencies were expressed as mean chiasmata per chromosome for each plant.

In the resulting data there is a striking difference between the progenies with and without chromosome 5D (Table 1). All the 5D-disomic F_2 plants have fifteen or more bivalents and means of more than 0.75 chiasmata per chromosome (Fig. 1). By contrast, in the 5D-nullisomic F_2 there is segregation into two classes in one of which pairing is normal (≥0.75 chiasmata per chromosome) while in the other there is reduced pairing with many univalents and few bivalents (<0.17 chiasmata per chromosome). When F_2 plants which had reduced pairing at 15° C were transferred to temperatures between 20° C and 25° C, meiotic pairing became normal after five days. The ratio of plants in the 5D-nullisomic progeny with normal : reduced synapsis at 15° C is not significantly different from 3:1 $(\chi^2 = 0.0075, P > 0.95)$, but it does not fit a 15:1 or a 9:7 ratio.

From this it can be concluded that, in the 5D-nullisomic F_2 , the disjunct distribution of chiasma frequencies depends upon the segregation of alleles at a single locus. The activity of the dominant allele apparently duplicates

Table 1. Mean chiasmata per chromosome in 5D-nullisomic and 5D-disomic F_a progenies from the cross T. $aestivum \times T$. dicoccum at 15° C (ten cells/plant)

	Mean chiasmata/chromosome		
F_2 type	Reduced pairing (≤0·17 chiasmata)	Normal pairing (≥0.75 chiasmata)	
	(=50-17 cinasmata)	(>0 10 omasmaca)	
5D-nullisomic	14	44	
5D-disomic	0	82	

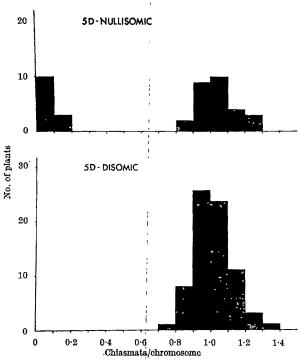


Fig. 1. Histograms showing the distributions of plants over a range of mean chiasmata in each chromosome in F_2 progenies from the cross T. aestivum $\times T$. dicoccum when chromosome 5D was either nullisomic or disomic.

that performed by chromosome 5D of T. aestivum 'Chinese Spring' because its segregation was not revealed phenotypically in the 5D-disomic progeny. It is proposed to call this locus "low temperature pairing" (ltp). The regularity of meiotic pairing at low temperatures in T. dicoccum presumably depends upon the presence of the dominant allele (Ltp Ltp), while T. aestivum 'Chinese Spring' carries the recessive allele (ltp ltp). The chiasma frequencies of F_2 plants in the $5\bar{D}$ -disomic progenies are not strikingly different from those of the high frequency class in 5D-nullisomic progenies. Thus the effects of Ltp and of chromosome 5D are not additive so that the threshold necessary for normal pairing at low temperatures can be attained by a single dose of either Ltp or chromosome 5D. As one activity relates to the long arm of chromosome 5Dit seems likely that Ltp is located on the long arm of a homoeologue. Chromosome 5A of T. aestivum 'Chinese Spring' has been shown to carry out an activity like that of 5D but with much less efficiency. It may therefore be that the alleles by which T. aestivum 'Chinese Spring' and T. dicoccum differ are on chromosome 5A and that l l p is active though less efficient than L t p. The next task is to locate and map Ltp, for when this has been done an assessment will be possible of its relationship with the better known genetic properties of chromosome 5B by which pairing specificity is restricted in *Triticum*.

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BIOCHEMISTRY

Isolation of a New Type of Cross Link from the Hinge Ligament Protein of Molluscs

THE two best known elastic proteins are elastin and resilin. So far, elastin has been isolated only from vertebrates although the presence of elastin-like material has been reported in several invertebrates1,2. Resilin has been found only in arthropods3. Apart from their amino-acid composition, resilin and elastin differ in the way they are cross linked into three dimensional networks. Thus resilin contains di- and tri-tyrosine formed oxidatively from tyrosine residues^{4,5} and elastin contains desmosine and isodesmosine⁸ together with a small amount of lysinonorleucine⁷, all formed from lysine residues. The presence in foetal elastin of dityrosine has recently been reported. During an investigation of elastic proteins from other phyla, I noticed that molluscan hinge ligaments contain phenolic compounds, which in several respects resemble dityrosine (Fig. 1). One of these compounds (compound A) has now been isolated and identified as 3,3'-methylene-bistyrosine (Fig. 2). The isolation procodure and the reasons for ascribing the structure in Fig. 2 to the compound are described in this communication; a more detailed description of the properties of the compound will be published later.

> dityrosine 3,3'-methylene-bistyrosine OH OHOHOH CH_2 ĊH2 ĊH2 $\dot{\mathrm{CHNH}}_2$ CHNH, CHNH, CHNH, COOH COOH COOH COOH Fig. 1. Fig. 2.

Whole ligaments of Mytilus edulis were decalcified in 1 molar acetic acid for 48 h and then hydrolysed by refluxing with 6 molar hydrochloric acid for 22 h. The hydrolysate was evaporated to dryness in vacuo over solid sodium hydroxide and thereafter fractionated, as described for hydrolysates of resilin5, on a column of cellulose phosphate (1.6×55 cm). Elution was performed by means of a linear sodium chloride gradient, established by running 1,000 ml. of 1 molar NaCl in 0.2 molar acetic acid into a mixing chamber containing 1,000 ml. 0.2 molar acetic acid. The absorption of the effluent was recorded automatically at 280 mu and fractions of 10 ml. were collected.

One of the major peaks showing absorption in the ultraviolet was located in the region where dityrosine is eluted and this region was taken for further purification. After being evaporated to about 5 ml. it was fractionated on a column of 'Bio-Gel P-2' (1.6 × 55 cm) by elution with 0.2 molar acetic acid. Phenolic compounds are adsorbed to the polyacrylamide matrix of 'Bio-Gel' and are therefore eluted after the inorganic salts, so that the fractionation results both in a purification and a desalting of phenolic compounds. The sample from the ligaments gave two well separated peaks which were eluted after the inorganic salts. The first (compound A) showed maximum absorption in the ultraviolet at 278 mu in acid solution and at 298 mu in alkaline solution; the other compound (compound B) showed maximum absorption at 292 mu in acid solution and at 317 mu in alkaline solution. Both compounds seemed to be homogeneous according to thin-layer chromatography on silica gel with n-butanol-

acetic acid-water (3:1:1 v/v/v) as solvent and according to low voltage paper electrophoresis at pH 2 and 5. At pH 2 both compounds migrate towards the cathode and at pH 5 both appear neutral. They give colour reactions as a-amino-acids and as phenols and they are non-fluorescent. Partial dinitrophenylation according to Silaev et al. showed that both compounds contain two amino groups and because they are neutral at pH 5 it can be assumed that they also contain two carboxylic groups. Spectrophotometric titrations indicated that compound A contains two phenolic groups having pK'-values of 8.0 and 12.5, and that compound B contains two phenolic groups having pK'-values of 7.8 and 11.2. Neither of the compounds will form complexes with boric acid, in contrast to catechol and dityrosine.

Table 1. Major peaks of the mass spectra of the trifuluoroacetylated and methylated derivatives of dityrosine, compound a, and synthetic 3,3'-methylene-bistyrosine

Dityrosine Relative		Compound A Relative		3,3'-Methylene- bistyrosine Relative	
m/e	abundance	m/e	abundance	m/e	abundance
608	0.9	622	2.0	622	$1 \cdot 2$
594	11.2	608	9.7	608	5.3
535	2.4	549	1.3	549	1.0
481	12.9	495	13.2	495	12.5
410	100	424	100	424	100
350	13.4	364	7.6	364	9.8
336	2.9	350	2.0	350	7.9
		318	5.8	318	5.0
297	13.9	311	14.9	311	14.2
		304	3.9	304	6.4
265	2.9	279	7.7	279	7.5
		258	8-8	258	6.5
		244	7⋅6	244	9.1
225	5.0	239	8.6	239	4.9
211	10-6	225	6.4	225	8.4
194	3.7	208	3.3	208	2.5
		121	16.8	121	5-1
		120	13.2	120	4.3
		119	$21 \cdot 2$	119	11.9

Compound A and dityrosine were compared by mass spectrometry. To make them sufficiently volatile the compounds were first trifluoroacetylated and methylated by diazomethane. The results are shown in the first two columns of Table I. Both derivatives are apparently a mixture of the mono- and di-methyl ethers with the monomethyl ether dominant, as the fully methylated derivative of dityrosine has a molecular weight of 608 and the monomethyl ether has a molecular weight of 594. That only one of the phenolic groups is easily methylated must result from the presence of a hydrogen bond between the phenolic Such a hydrogen bond will also explain the unusual acidity found for one of the phenolic groups both in dityrosine⁵ and in compound A. It can also be seen from Table 1 that for each major peak present in the dityrosine spectrum a corresponding peak is present in the spectrum of compound A but located fourteen mass units higher, indicating that compound A might be a methylene derivative of dityrosine. The peaks in the mass spectrum of compound A which have no counterparts in the spectrum of dityrosine can be explained by cleavages occurring between the two aromatic rings. As such a cleavage does not occur in the case of dityrosine and as compound A does not have the biphenolic structure of dityrosine according to the ultraviolet spectra, the most reasonable structure to suggest for compound A will be that of 3,3'-methylene-bistyrosine. This structure is similar to some of the intermediates (novolaks) in the preparation of bakelite from phenols and formaldehyde 10. I therefore attempted to synthesize methylene-bistyrosine by treating 1 mmole of N-acetyltyrosine ethyl ester with 0.5 mmole of formaldehyde in a mixture of 5 ml. methanol and 5 ml. of 2 molar hydrochloric acid. The mixture was refluxed for 5 h and then evaporated to dryness and hydrolysed with 6 molar hydrochloric acid at 110° C for 16 h to remove the blocking groups. The hydrolysate was fractionated on cellulose phosphate in the same way as the ligament hydrolysate. Apart from tyrosine, only one compound was found which emerged in the same position as compound A and which had the same ultraviolet spectrum and the same mass spectrum (Table 1) as compound A, confirming that this compound is identical with 3,3'-methylene-bistyrosine.

The complete structure for compound B has not yet been obtained, but it resembles that of compound A in several respects, such as containing two aliphatic side chains with terminal carboxyl and α-amino groups. This structure makes both compounds well suited as cross links between peptide chains. As the hinge ligament in Mytilus has been reported to be strongly cross-linked11 it can thus be assumed that the function of the compounds is to link the protein chains in the ligament together in a three dimensional network.

The compounds do not only occur in the ligament of Mytilus: both of them are also found present in the inner ligament of Spisula solidissima, and 3,3'-methylenebistyrosine is present in the inner ligament of Pecten maximus. The structure of compound A indicates that it is formed in vivo by linking two tyrosine residues together by means of a one carbon unit. This represents a novel way of forming selerotized proteins which is strikingly different from the formation of both resilin and insect exocuticle.

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Metabolism of ³H-Myoglobin

PERKOFF¹ has recently emphasized the paucity of information on myoglobin metabolism and the importance of this haemoprotein in the myoglobinurias. Previous attempts to study the turnover of myoglobin by administering ¹⁴C-glycine-2 to rats met with limited success because of the poor incorporation of isotope and non-specific labelling of the molecule (that is, haem and globin). We now describe a new technique for preparing 3H-myoglobin labelled with haem and studies of its turnover and degradation are reported.

Radiochemically pure ³H-Δ-aminolaevulinic acid-3-5 (³H-ALA-3-5), specific activity 400 mc./mmole, was prepared by enolization exchange² and 100 µc. was administered intraperitoneally to hamsters. At intervals, hamsters were decapitated, their musculature was excised and homogenized, and the homogenate was subjected to lead acetate and phosphate precipitation according to the method of Morgan3. The dialysed crude myoglobin which remained in the supernatant was purified by chromatography on DEAE-'Sephadex' (equilibrated and developed with 0.05 molar tris, pH 8.6 (ref. 4)), and on 'Brushite' (developed with phosphate buffers of increasing ionic strength, pH 7.0) columns. The concentration of myoglobin was estimated from the optical density at 420 mu

Table 1. Specific activities of 3H-myoglobin* labelled with habm

Days after injection	Specific activity of haem in myoglobin† (d.p.m./µg)	Specific activity of haem spllt from myoglobin ‡ (d.p.m./µg)	
1	Not done	234	
3	Not done	160	
10	159	157	
19	140	158	
26	154	169	

* Each value represents the average of separate determinations of myoglobin from the muscle of three hamsters injected with *H-ALA-3-5.

† The specific activity of haem in myoglobin is calculated by assuming that the haem content is 3.6 per cent¹⁰. Then d.p.m./µg myoglobin is 0.036 0.036 d.p.m./µg of haem in myoglobin.

of the cyanmet-myoglobin derivative ($E_{1 \text{ cm}}^{1 \text{ \%}} = 53.9$ at 420 mμ according to Cameron⁵ and our own unpublished data). Haem split from myoglobin and crystallized was determined as the pyridine haemochromogen ($E_{mM} = 34.4$ at 557 mu in 25 per cent pyridine?). Counting was performed on 0.5 ml. samples of solution in 15 ml. of Bray's solution⁸ in a Packard liquid scintillation spectrometer.

The ratio of the optical density at 410 to that at 280 m μ of ³H-myoglobin prepared as described was 5.0 (ref. 9) and the material was chromatographed to constant specific activity on DEAE-'Sephadex' and 'Brushite' columns. The specific activity of ³H-myoglobin expressed as d.p.m./ μg of estimated haem in myoglobin (haem = 3.6 per cent of the weight of myoglobin¹⁰) was nearly identical to the specific activity of haem split from 3H-myoglobin (Table 1). In contrast, less than 3 per cent of the radioactivity was present in crude solutions of globin prepared from 3Hmyoglobin. The highest specific activities were obtained in animals killed 24 h after administration of ³H-ALA-3-5 (Table 1). After an initial sharp drop, relatively constant specific activities were observed. Measurable radioactivity persisted in myoglobin obtained from hamsters killed up to 6 months after injection. 3H-Myoglobin was administered intravenously to rats prepared with ligated renal pedicles and cannulated bile ducts. Rats, rather than hamsters, were used for this purpose because of the comparative ease of surgical manipulation. Approximately three-quarters of the administered radioactivity rapidly appeared in bile (Fig. 1), and 80-90 per cent of the radioactivity in bile was identified by crystallization¹¹ as 3H-bilirubin.

These results establish that radiochemically pure, 3Hmyoglobin labelled with haem with specific activities more than 1,000 times greater than those previously achieved with ¹⁴C-glycine-2 can be prepared in vivo in hamsters. They indicate that myoglobin turnover in the hamster is slow and they provide the first direct demonstration that the prosthetic group of myoglobin can be converted to hilirubin.

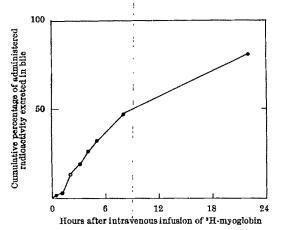


Fig. 1. Excretion of radioactivity in bile after intravenous administration of °H-myoglobin labelled with haem. Data from one representative study are shown. Recipient animals were prepared as described in text.

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Effect of Ultraviolet Light on RNA and Protein Synthesis in Differentiated **Epidermal Cells**

STUDIES of the effects of microbeam ultraviolet light on growing tissue culture cells have shown that nucleolar RNA synthesis decreases rapidly but protein synthesis is either not affected1 or is decreased as a result of inhibition of nuclear RNA synthesis2. The influence of ultraviolet light energy on formation of macromolecules in more differentiated cells, however, has not been adequately studied. In the present study we examined the effects of heterochromatic ultraviolet light on RNA and protein synthesis in granular cells of human epidermis in vivo. These cells are differentiated epidermal cells which do not replicate, but do actively synthesize RNA and protein.

Three sites of the upper arm in eighteen volunteers were exposed to a mild erythemal dose of ultraviolet light from a hot quartz, high-pressure Hanovia contact lamp $(5\cdot1\times10^6 \text{ to } 10\cdot2\times10^6 \text{ ergs/cm}^2)$. Ten μ c. of ^3H -cytidine (specific activity $3\cdot6$ c./mmole), ^3H -histidine (specific activity 4 c./mmole) and 3H-leucine (specific activity 5 c./mmole) diluted in 0.1 ml. of sodium chloride solution was injected intradermally in separate sites at 1, 3, 6, 24, 48 and 72 h after ultraviolet irradiation. Non-irradiated control sites were injected with these substances in a similar manner. Biopsies were secured at 15 min after injection of 3H-cytidine and at 30 min after injection of the amino-acids. Tissues were prepared for autoradiography using NTB-2 emulsion and 3 weeks' exposure time, followed by haematoxylin and eosin staining.

In non-irradiated sites we observed incorporation of ³H-cytidine and ³H-histidine in almost all cells in the basal and spinous layers, but, in the granular layer, 17.7 per cent of cells did not label with 3H-cytidine and 10.4 per cent did not label with 3H-histidine: these results are to be published. Epidermal cells seem to synthesize RNA and protein continuously as they move towards the surface from the basal layer to the spinous and granular layers. Some granular cells, however, stop the synthetic process at one of the terminal differentiation stages just before they become horny cells. After irradiation the number of granular cells synthesizing RNA and protein decreased rapidly as shown in Fig. I. Within 1 h 80·1 per cent and 47.1 per cent of granular cells were not labelled after injection of 3H-cytidine and 3H-histidine, respectively. Percentage of unlabelled granular cells increased to 94 per cent and 83.1 per cent at 3 h after irradiation, and by 6 and 24 h almost all granular cells did not label with injected 3H-cytidine and 3H-histidine. Morphologically, these granular cells did not show any significant changes during the first 24 h after radiation, whereas some cells in the Malpighian layer became degenerative with pyknotic nuclei and densely eosinophilic and homogenized cytoplasm by 6 h. Synthesis of RNA and protein was markedly inhibited in the degenerated Malpighian cells, but basal cells appeared morphologically unchanged and continued to synthesize RNA and protein.

These observations were confirmed by comparing grain counts in the granular layer and basal layer of the epidermis after injection of ³H-histidine and ³H-leucine. A Whipple eyepiece micrometer disk was placed at the border between the epidermis and dermis for determination of the number of grains in the basal layer, and at the upper edge of granular cells for examination of grain counts in the granular layer. Because the number of grains actually appearing in the section varied considerably from one individual to another, ratio of counts obtained in 200 squares in both layers was computed, taking the count in the basal layer as one. In nonirradiated epidermis the concentration of grains in the granular layer was four times greater after injection of ³H-histidine and 1.5 times greater after injection of ³Hleucine as compared with that of the basal layer. After irradiation the ratio of grain counts was reversed, being higher in the basal layer (Figs. 2 and 3). This indicated that protein synthesis in granular cells was markedly inhibited.

Biochemical studies have indicated that ultraviolet light immediately inhibits RNA and protein synthesis in the skin3. Morphological changes in the skin, however, have not been reported to occur before 12-24 h after irradiation4.5. In the present study it was possible to correlate the early biochemical changes in epidermal cells with their morphology by means of autoradiography. The results demonstrated that ultraviolet light energy inhibited both RNA and protein synthesis in granular cells which are highly differentiated long before the structural changes reported to date. Reduction of incorporation of amino-acids was observed within 1 h. It takes more than 1 h for newly synthesized RNA to move from the nucleus to the cytoplasm, and so the decrease in protein synthesis observed in granular cells seems to be a result of a direct effect of ultraviolet light on cytoplasmic protein synthesis rather than of decrease in RNA synthesis. Cellular degeneration and RNA and protein syn-

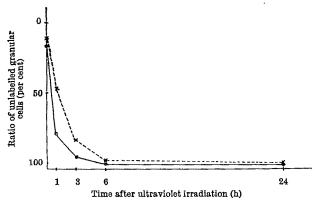


Fig. 1. Effect of irradiation on RNA and protein synthesis in granular cells. ×---×, After injection of ³H-histidine; •—, after injection of ³H-cytidine.

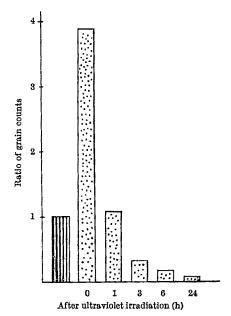


Fig. 2. Effect of irradiation on grain counts in the granular (stippled columns) and basal (hatched column) layers of the epidermis after injection with *H-histidine.

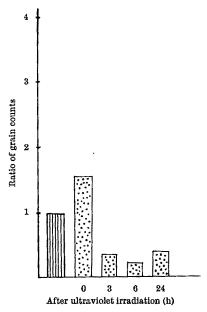


Fig. 3. Effect of irradiation on grain counts in the granular (stippled columns) and basal (hatched column) layers of the epidermis after injection with ³H-leucine.

thesis inhibition were noted in Malpighian cells by 6 h after irradiation. In contrast, basal cells appeared morphologically unchanged and continued synthesis of RNA and protein.

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Haptoglobin acting as a Natural Inhibitor of Cathepsin B Activity

Observations in this laboratory on various homogenates and on tumour ascites fluid1,2 have suggested that a component in blood plasma acts as a powerful cathepsin In order to study this interesting finding, cathepsins B and D were first separated by gel filtration from the lysosomal pellet of calf liver homogenates and subsequently studied in their partially purified form. For the colorimetric assays edestin denatured with urea was used as a substrate in 0·1 molar acetate buffer at pH 5.0 according to Ottoson and Sylvén¹. Both 0.004 molar cysteine and 0.05 EDTA must be added for maximum cathepsin B activity. Without their addition, or with added cysteine alone, extinction increments which were too small and erratic were obtained. By means of this system the cathepsin B and D activity changes could then be studied separately after the addition of small amounts of all the different available purified plasma protein fractions from normal human blood. One fraction only prepared from Cohn's fraction IV-b containing the haptoglobins exhibited a marked inhibitory effect on the cathepsin B activity. This inhibition was noted even at weak haptoglobin concentrations amounting to a quarter of the amount of enzyme. No inhibition was found on addition of other highly purified serum proteins such as transferrin, ceruloplasmin or $\alpha_{\text{\tiny I}}\text{-acid}$ glycoprotein, nor by addition of pure sialic acid, which is a component of haptoglobin. The cathepsin D activity, however, remained totally uninfluenced by all of the plasma fractions.

Through the courtesy of the KABI Co., a sample of higher purity (human haptoglobin RFE 76) was supplied containing more than 90 per cent haptoglobin. preparation showed a much stronger inhibition at the same concentrations as the first fraction (Fig. 1). inhibition was reversible. When a certain amount of enzyme and haptoglobin were mixed and a dilution curve of the activity of this sample was determined, a non-linear curve was obtained showing a downward curvature suggesting the presence of a dissociable inhibitor-enzyme complex. Preincubation of haptoglobin and cathepsin B for different lengths of time did not abolish the effect. Further analyses were made using various substrate and haptoglobin concentrations; evaluation in a Lineweaver-Burk diagram³ showed that the kinetics of the enzyme reaction behaved as expected for a non-competitive type of inhibition. A study of the pH-dependence showed

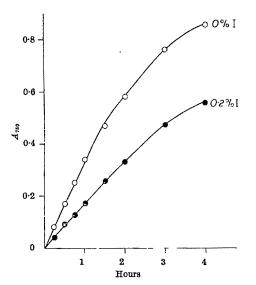


Fig. 1. Hydrolysis of urea-denaturated edestin by purified cathepsin B at pII 5·0. Each sample of 50 μ l. contained 600 μ g edestin, 20 μ g cathepsin B. I=0, no haptoglobin added; I=0·2, 20 μ g haptoglobin added; cysteine+EDTA present.

further that the inhibition occurred over the whole pHrange of enzyme activity. In addition to the protein substrate edestin, haemoglobin denatured with urea was also investigated and the same result was obtained. When the artificial substrate BANA (N-benzoyl-DL-arginine- β -naphthylamide) was used, however, according to Goldbarg and Rutenburg⁴, no enzyme inhibition was found. This may be because of the hydrophobic character of BANA.

Experiments were further designed to separate a mixture of haptoglobin and cathepsin B on a 'Sephadex G-200' column. The two components could be separated and the original activity of cathepsin B was regained. In the separation diagram, however, the curve of cathepsin B seemed distorted when compared with a diagram where cathepsin B alone had been filtrated. This is to be expected when the small molecule of cathepsin B is dragged along with the much larger haptoglobin molecule before they dissociate.

Trypsin was investigated in the same way, but no inhibition was found when haptoglobin was added. There is thus a considerable difference in behaviour between cathepsin B and trypsin toward haptoglobin. enzymes split peptide chains at the same amino acids. Trypsin splits the haptoglobin molecule easily, but cathepsin B cannot hydrolyse haptoglobin. It seems that the inhibitor is reversibly bound to cathepsin B in a manner which alters the enzyme structure so that its active group cannot work.

It seems, from the literature, to be very difficult to obtain a haptoglobin free from contaminations of other proteins. The haptoglobin we used carries less than 10 per cent of contaminating substances. Results so far obtained strongly suggest that haptoglobin or one of its major components (?) is the cathepsin inhibitor. Because part of the cathepsin B activity extends above pH 7 the presence of a physiological plasma inhibitor would seem of importance for the protection against active proteolysis. It is also noticeable that conditions such as inflammatory processes, tumour diseases and major tissue injury where cathepsin B will be liberated are associated with increased amounts of haptoglobin in the blood plasma6.

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Improved Multi-enzyme Analyser

By a relatively simple adaptation of the Technicon autoanalyser system, it was possible to develop a technique for the automatic assay of groups of enzymes1. reaction mixture—for example, a tissue homogenate, buffer and any co-factors common to all the enzymes in the group—flows continuously through the analyser. Different substrates are then introduced through the automatic sampler, so that the various enzymes in the homogenate which react with these substrates are assayed in sequence.

This system, in a relatively simple form, has been used to study oxidases, phosphatases, NAD-linked dehydrogenases and a variety of ferricyanide reductases1-3. Experience with these simpler systems, however, revealed two important difficulties. In the first place, it was difficult to obtain a suitable concentration of enzyme mixture (for example, the tissue homogenate) which gave optimum rates for all the enzymes in the group. This was because the enzymes were not present in equal amounts in the mixture, and the least active enzymes required protein concentrations in the assay which were far too high for the most active enzymes. Second, the simpler multiple enzyme analysis system previously used only provided a single time of incubation for each assay, as is the case for the majority of Technicon enzyme assay systems. As a result, it was impossible to establish whether the enzymes had been assayed in conditions in which the rate of reaction was linear with respect to time.

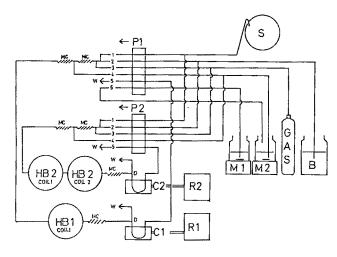


Fig. 1. Manifold for multi-enzyme analyser. B, Buffer plus any cofactors common to all enzymes in group; C1, C2, continuous flow colorimeters; D, debubblers; HB1, HB2, variable temperature heating baths; M1, M2, magnetic stirrers; MC, mixing coils; P1, P2, proportioning pumps; R1, R2, recorders; S, sampler table, containing substrates; W, waste. Channel 1 consists of P1, HB1, C1 and R1 and channel 2 of P2, HB2, C2 and R2. Stock enzyme is placed in mixer 1 and pumped through line 6 of pump 1 into mixer 2, which contains a suitable diluent. The diluted "enzyme gradient" is pumped out of mixer 2 by line 4 in each pump. Time of incubation in each channel depends on flow rates of lines (see Table 1).

By incorporating a simple gradient-making device into the system, the assays have now been performed with a continuously increasing protein concentration in the reaction mixture. Thus the relationship between reaction rate and protein concentration is obtained automatically. Moreover, a double analytical system is now used, so that two incubation times are provided for each assay. In this way it is possible to check that the reaction is linear with respect to time. The principles of the method are illustrated by experiments with ferricyanide reductases of yeast.

The analytical system is shown in Fig. 1. It consists essentially of two channels. Channel 1 contains a proportioning pump, P1, a heating bath with one time delay coil, HB1, and a colorimeter, C1, connected to a recorder, R1. The second channel is identical (P2, HB2, C2 and R2) except that the heating bath contains two time delay coils. Substrates are placed in cups in the automatic sampler, S, and the stream of substrates is split and fed into both channels. Similarly, buffer and any common co-factors are placed in the vessel B and fed into both channels. The substrate and buffer streams are segmented by a suitable gas which is fed into both channels from a common cylinder. The enzyme mixture is placed in a mixing flask, stirred by a magnetic stirrer, M1. The flask is

kept at 0° C either by a temperature jacket connected to circulating ice-bath, or more conveniently by standing i in ice in a container of low thermal conductivity, such a an 'Igloo' container. The enzyme solution is then intro duced into a similar vessel on a second mixer, M2, by means of line 6 in the first pump. The second vessel con tains a suitable volume of diluent, and as the concentrated enzyme solution drips into it, the diluted solution is pumper out into the two channels by line 4 in each pump. The shape of the "enzyme gradient" can be altered by using lines of different flow rates, and depends on the relative rates of filling and emptying of vessel M24. If line 6 in pump 1 has the same volume as line 4 in pumps 1 and 2 the rate of emptying is exactly twice the rate of filling and a linear gradient results. The slope of the gradient is determined by the rates of filling and emptying, and by the volume of fluid originally present in the vessel M2 Provided that enough solution is present for the assay, the volume of fluid in vessel M1 need not be determined accurately.

If the substrates are placed in repeating groups in the sampler table, it is possible to obtain a series of readings at different protein concentrations for each enzyme. As an example, the assay of a group of four dehydrogenases in yeast will be described. These are NADH dehydrogenase, L (+) lactate dehydrogenase, D (-) lactate dehydrogenase and succinate dehydrogenase. All the enzymes are followed by measuring the reduction of potassium ferricyanide at 4200 Å, in phosphate buffer pH 7.4 and with a low concentration of neutral detergent ('Triton X-100') which serves to reduce the turbidity of the suspensions, activate any latent enzymes and also improve the bubble pattern in the autoanalyser. Using sampler type I there are places for forty cups on the sampler table, and they are filled as follows. All even numbered cups contain water, in order to provide efficient washing between samples. (If the improved sampler II is used, this is not necessary.) The substrates, and water to provide blanks, are arranged as follows: (a) water in cups 1, 11, 21 and 31; (b) NADH in cups 3, 13, 23 and 33; (c) sodium L (+) lactate in cups 5, 15, 25 and 35; (d) sodium D (-) lactate in cups 7, 17, 27 and 37; and finally (e) sodium succinate in cups 9, 19, 29 and 39. In this way, there is a water blank between each group of assays, and the most active enzymes are assayed first, in the first group. The composition of the buffer and the enzyme source are given in Table I, together with the flow rates of the lines in the manifold. The results of a typical assay are given in Fig. 2, and for clarity only the

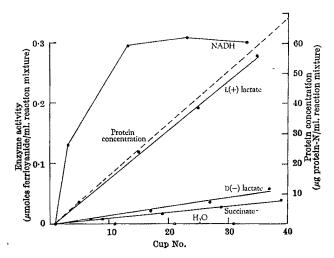


Fig. 2. Results of multiple enzyme analysis of ferricyanide reductases. Manifold is given in Fig. 1 and details of assay conditions in Table 1. Protein concentration (---) was calculated from protein concentration in the stock enzyme suspension, and from a calibration curve obtained by pumping mmolar potassium ferricyanide through the gradient-making system (see ref. 2). Results given for channel 1 only, with 7-3 min of incubation.

Table 1. ASSAY CONDITIONS FOR MULTIPLE ENZYME ANALYSIS OF FOUR DEHYDROGENASES

Channel	Line	Description of lines Description of line	Flow rate			
No.	No.		(ml./min)			
1	1	Sample	0.60			
	2	Buffer	2.00			
	3	Gas	1.20			
	4	Enzyme (from mixer 2)	1.20			
	5	Waste	2.50			
	6	Enzyme (from mixer 1)	1.20			
2	1	Sample	0·60			
	2	Buffer	2·00			
	3	Gas	1·20			
	4	Enzyme (from mixer 2)	1·20			
	5	Waste	2·50			
Reaction mixture						
Line Buffer Buffer Buffer Sample Sample Sample Sample Gas	10 mmolar sodium L $(+)$ lactate (cups 5, 15, 25 and 35)					

Gradient started with 100 ml. of 0·3 molar mannitol in mixer 2 and 100 ml. of yeast homogenate (strain D243) containing 0·28 mg protein-N/ml. in 100 ml. of 0·30 molar mannitol. Bath temperature 37°, sampler rate 40/h with water in even-numbered cups and cups 1, 11, 21 and 31. Measurements at 4200 Å. Times of incubation were 7·3 mln for channel 1 and 11·6 min for channel 2.

values for the first channel, with 7.3 min of incubation, are given.

It can be seen from Fig. 2 that the method automatically reveals the assay conditions in which enzyme activity is linear with respect to protein concentration. Thus the values for NAD-ferrievanide reductase are far from linear, and a most reliable estimate of the specific activity would be obtained from the readings at the beginning of the enzyme gradient. The other enzymes, however, give reasonably linear curves. Also the least active enzyme, succinate dehydrogenase, is scarcely measurable at the beginning of the gradient, but a more reliable value can be obtained at the highest protein concentration. The initial slope of the curves in Fig. 2 gives the best estimate of the enzyme activity in each unit of enzyme protein. The values in Fig. 2, however, are only obtained from a single incubation time. If the initial slopes of the similar family of curves obtained from channel 2 are calculated, it is possible to plot the reaction against time, as shown in Fig. 3. It can be seen that all the activities calculated in this way seem to have been reasonably linear with respect to time.

The method of expressing the results in Figs. 2 and 3 is convenient for comparing the relative activities of the

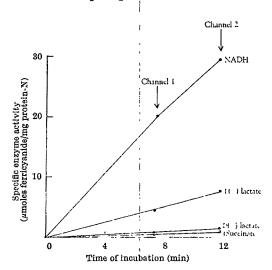


Fig. 3. Progress curve from dual channel analyser. The initial slopes of the plots of enzyme activity against protein concentration (Fig. 2) were calculated for the four enzymes, using results from both channels. Value in channel 2 for NADH-ferricyanide reductase is 8 per cent below value for linearity.

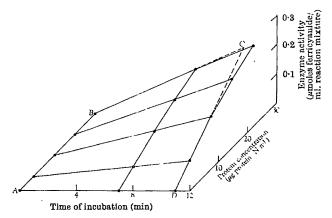


Fig. 4. Three dimensional plot relating incubation time, protein concentration and enzyme activity. Results with D(-) lactate ferricyanide reductase. Assay conditions as in Table 1, except that a respiratory deficient yeast (strain 45 $ac \times 188 up$) was used (see ref. 3). Stock enzyme suspension in mixer 1 had a concentration of 0·12 mg protein-N/ml. Surface ABCD represents an ideal assay in which the enzyme activity is both linear with respect to time, and proportional to protein concentration.

enzymes in the group. It is also of interest, however, to combine all the measurements made with an individual enzyme in one three dimensional diagram, in which the enzyme activity is plotted along the X axis, time along the Y axis and protein concentration along the Z axis. Examples of such plots from two other experiments are given in Figs. 4 and 5. These diagrams reveal the assay conditions under which the enzyme activity is both proportional to protein concentration and linear with respect to time. Conditions of perfect linearity are given by the surface ABCD in Fig. 4, and deviations from these conditions can readily be seen in the three dimensional diagrams.

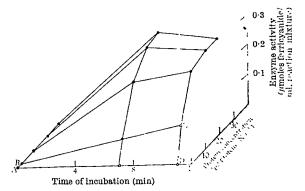


Fig. 5. Three dimensional plot with severe deviation from linearity. Results with NADH ferricyanide reductase. Assay conditions as in Table 1 except that yeast strain 40×41 was used. The stock enzyme suspension had a concentration of 0-15 mg protein-N/ml. Note that results are only linear in the surface ABCD.

By combining a dual channel system with the use of a gradient of protein concentration (see refs. 5, 6 for similar systems for single enzymes), the multi-enzyme analyser described here can thus provide results of precision and validity comparable with manual assays based on progress curves carried out over a range of protein concentrations. Clearly, by combining suitable gradient mixing devices with the flexibility of the Technicon system, it would be relatively easy to develop systems for the automatic study of such parameters as K_m , K_i , pH optima, heats of activation and thermal stability not only with individual enzymes, but also with groups of enzymes which have some common feature in their assay. There is no limit to the complexity of analytical systems which can be set up with modern automated equipment for studying enzyme catalysed reactions, and this system is a compromise between such complexity and ease of operation.

I thank Miss J. Jones for technical assistance and Dr D. Wilkie for supplying strains of yeast. I am also grateful to the Medical Research Council and the Science Research Council for financial aid.

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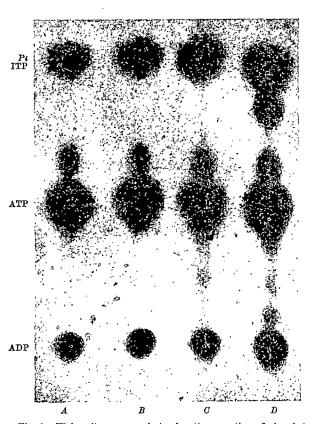
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Phosphate Esters of Human Erythrocytes: Synthesis of ITP-14C from Inosine-8-14C

THE recent finding of relatively large concentrations of inosine triphosphate (ITP) ($44-51~\mu g$ of phosphorus/g of haemoglobin) in erythrocytes obtained from two members of a family led to a closer investigation of the nucleotide composition of normal erythrocytes. A detailed analysis of several samples of fresh human erythrocytes revealed the presence of small amounts of ITP in an average concentration of 1.8 µg of phosphorus/g of haemoglobin². From these studies it was concluded that ITP is a normal constituent of human erythrocytes.



High voltage paper electrophoretic separation of phosphate om fresh erythrocyte haemolysates obtained from four different subjects, and treated with **P-orthophosphate.

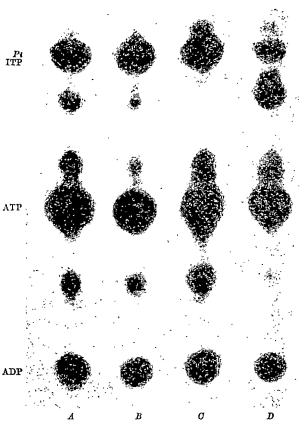


Fig. 2. High voltage paper electrophoretic separation of phosphate esters from inosine incubated blood obtained from the same subjects illustrated in Fig. 1. Following incubation, the washed crythrocytes were haemolysed and treated with *2P-orthophosphate.

While preparing sedoheptulose-1,7-diphosphate labelled with phosphorus-32, by incubation of whole blood with inosine and labelled orthophosphate2,3, analysis of the S-1,7-P "zone", obtained by high voltage paper electrophoretic separation of acid-soluble phosphate esters labelled with phosphorus-32, disclosed in a few samples the presence of labelled ITP in concentrations significantly higher than 1.8 μg phosphorus/g haemoglobin, the average concentration of ITP in normal fresh erythrocytes. The increase in concentration of ITP (up to 3-9 µg phosphorus/g haemoglobin) found in the conditions of the experiment suggested that in a few cases the erythrocyte can synthesize ITP in vitro from added inosine. Moreover, it points out the difference between samples of blood in their ability to synthesize ITP from inosine.

The rapid isotope equilibrium exchange between 32Porthophosphate, ATP and added nucleoside triphosphate carried out by haemolysates in the cold4 can be used as a qualitative test for detecting ITP in erythrocytes. For this purpose red cells obtained from fresh blood or blood previously incubated with inosine and phosphorus were haemolysed with a solution of 32P-orthophosphate. mixture was allowed to stand for 0.5-1 h, and the acidsoluble phosphate esters obtained4 were then separated by high-voltage paper electrophoresis3.

The electrophoretic patterns of 32P-labelled phosphate esters of fresh erythrocytes and erythrocytes from inosine incubated blood, respectively, are shown in Figs. 1 and 2. Fig. 1, sample D, shows an additional phosphorus-32 spot corresponding to ITP. This sample was obtained from the blood of one of the two siblings found to contain high ITP concentrations. Fig. 2 shows the same blood samples as in Fig. 1 after incubation with inosine and treatment with 32 P-orthophosphate. In samples A and B 32 P-ITP spots of

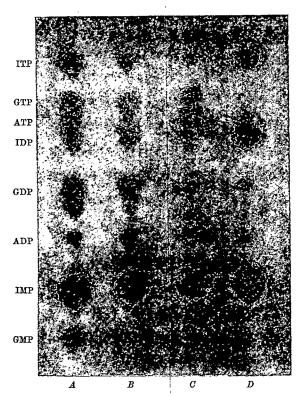


Fig. 3. High voltage paper electrophoretic separation of phosphate esters from inosine-8-¹⁰ incubated blood obtained from the same subjects illustrated in Figs. 1 and 2. This particular sheet was exposed for 6 months to facilitate photographic reproduction.

different intensities appeared; in contrast, sample C shows no detectable ITP spot.

To confirm the in vitro synthesis of ITP from inosine, experiments were carried out with inosine-8-14C. The high voltage paper electrograms of the acid-soluble phosphate esters were exposed to 'No-screen' medical X-ray film for 3-6 weeks. In these circumstances distinct spots corresponding to radioactive Hx, Is, IMP, ITP (and occasionally IDP) are seen. In addition, several other minor spots can be recognized which migrate at rates similar to those of the guanosine and adenosine mono-, di- and tri-phosphates, respectively. Their identity was confirmed by paper chromatography in three different solvent systems⁵. As seen in Fig. 3, inosine-8-¹⁴C was incorporated in ITP (and IDP), in addition to IMP, the extent of incorporation depending presumably on the ability of the particular erythrocytes to synthesize ITP from inosine. The ratio of counts ITP/IMP falls essentially into three groups: 0.14-0.21 for the erythrocytes with high ITP concentration; 0.01-0.1 for erythrocytes which can synthesize ITP; and 0.001-0.003 for erythrocytes with low concentration of ITP, and which do not show increase in concentration of this nucleotide on incubation with inosine.

There is no evidence in the literature for the formation of ITP from inosine in human erythrocytes. The studies of Lowy et al.⁸ with inosine-8; ¹⁴C show that most of the labelled compound is found in the form of IMP with a fraction of the counts in GTP and ATP. Analogous results were obtained by Bishop' when studying the degree to which hypoxanthine is incorporated into the acid-soluble nucleotides of whole human blood. Kleins, working with an active haemolysate preparation from human erythrocyte, found that adding hypoxanthine to the system gave rise only to IMP. Similar results were reported by Mager et al.9. That ITP was not detected by these workers in their particular systems may be because there is a low percentage of individuals with erythrocytes capable of synthesizing ITP, and that ITP itself is present in erythrocytes in relatively low concentrations. The biosynthesis of ITP and the possible genetic link between high ITP erythrocytes and ITP synthesizing erythrocytes is under investigation.

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Phylogeny of the Neurohypophysial Hormones

VERTEBRATE neurohypophysial hormones have a common structural pattern characterized by a chain of nine aminoacid residues with a disulphide bridge connecting the amino-acids in positions 1 and 6. There are usually two hormones in each species, the active principles rarely varying from one species to another within a given class. Thus oxytocin and arginine vasopressin have been chemically identified in five species of mammals belonging to the orders Primates, Artiodactyla, Perissodactyla and Isotocin (Ser₄-Ile₈-oxytocin) and vasotocin (Args-oxytocin) have up to now been isolated from six species of bony fish belonging to the families Gadidae, Scombridae and Cyprinidae. The structural variations which occur between one vertebrate class and another are confined to one or two amino-acid substitutions in positions 3, 4 or 8 (for a review of this subject see ref. 1).

There seems to be a particular neurohypophysial hormone heterogeneity in the cartilaginous fish, although the existing evidence for the three principal groups, the rays, sharks and Holocephali, is contradictory. It was originally thought that there was only one hormone in several elasmobranchs². Nevertheless, two active principles were shown to be present by exchange ion chromatography in two species of ray, Raia clavata3 and Raia batis4, in one shark, Squalus acanthias3,5, and in one of the Holocephali, Hydrolagus collei⁵. It now seems probable that the cartilaginous fish, like most of the vertebrates, have at least two hormones. These two principles, however, are present in most unequal proportions: vasotocin, which according to the pharmacological data seems to be one of these factors, is present in only a very small amount, in contrast with the relatively high amount found in the bony fish. The hormone present in greatest quantity, glumitocin (Ser₄-Gln₈-oxytocin), was identified in the rays and isolated from the hypophyses of Raia clavata3 and Raia batis4, while its presence in Raia naevus and Raia fullonica has been identified during current research.

216 mg of pituitary acetone powder has been prepared from sixty-eight Raia naevus hypophyses and 125 mg from fourteen glands of Raia fullonica. The respective titres for the oxytocic activity, determined by the Holton method with a physiological solution without magnesium, were 10 mm U.S.P./mg and 3.5 mm U.S.P./mg; 100 mg of material was extracted twice with 8 ml. of 0.01 normal sulphuric acid at 4° C for 24 h, the insoluble residue was removed, the extract concentrated to about

4 ml. and the pH adjusted to 3.9. Ten mg of beef neurophysin was added and three consecutive precipitations of the neurophysin-hormone complex made with sodium chloride at 15 per cent (18 h), 10 per cent (4 h) and 6.5 per cent (18 h), each precipitate being dialysed and redissolved in 4 ml. of 0.5 molar acetate buffer, pH 3.9 (refs. 8 and 9). The final precipitate was dialysed, redissolved in 4 ml. of 0.25 per cent acetic acid and the complex was dissociated with 5 per cent trichloroacetic acid which precipitated the neurophysin but left the active principles in solution in the supernatant. This solution was de-acidified by passing it through a column of 'Amberlite IR 45' $(1 \times 6 \text{ cm})$ and it was then freeze-dried. The hormones were separated and purified by paper chromatoelectrophoresis. Electrophoresis was carried out in a pyridine acetate buffer, pH 3.7, for 2 h at 60 V/cm, followed by chromatography in an n-butanol-acetic acid-water (4:1: 5 v/v/v) system for 18 h. Development with dilute ninhydrin (0.01 per cent in alcohol) usually detects only one peptide, but a more sensitive pharmacological method was used which consisted of cutting the sheet into 3 cm pieces, eluting with 1 per cent acetic acid and determining the oxytocic, pressor is and hydro-osmotic activities. By this method the presence of two hormones was found in each species of ray.

Table 1. AMINO-ACID COMPOSITION OF RAY GLUMITOCIN

Amino- acid	Raia clavata molar ratio using Asp as reference	Raia naevus molar ratio using Asp as reference	Raia fullonica molar ratio using Glu as reference	Number of residues per mole
Asp	1.00	1.00	0.76	1
Ser	0.84	1.07	0.84	1
Glu	1.12	1.17	1.00	1
Pro	0.95	0.76	0.76	1
Gly	1.34	1.19	1.00	1
Tle	0.95	0.89	0.83	1
Tyr*	0.58	0.53	0.30	1
Cvs*	1.52	0.86	1.22	2

*Tyrosine and cystine undergo partial destruction when the peptide is hydrolysed after elution on paper. Cystine was determined in a separate sample after oxidation to cysteic acid with performic acid. The preparations contain small quantities (less than 0·2 residue) of Ala, Thr and Leu.

A first principle was demonstrated with chromatographic and electrophoretic migrations similar to those of vasotocin and also with oxytocic, pressor and hydro-osmotic activities. Its chromatographic migration compared with alanine as reference was 0.50-0.60. The amount present was too small to determine the amino-acid composition of this hormone. With alanine as reference, the second principle had a migration of 0.85-Only its oxytocic activity was appreciable. When determined in the presence of magnesium' the oxytocic activity was seen to be increased some ten times. After hydrolysing with 6 normal hydrochloric acid in vacuo in a sealed tube at 105° C for 48 h, the product was analysed for amino-acids in a Spinco 120 \bar{B} automatic analyser12. The amino-acid composition resembled that of glumitocin isolated from Raia clavata³ and Raia batis⁴ (Table 1). The quantities used for analysis were of the order of 0.02-0.05 μmole which corresponded approximately with 0.15-0.50 units of oxytocic activity. A study of the structure of the peptide isolated from Raia clavata showed that it was Ser₄-Gln₈-oxytocin². The products obtained from the other three species took up the same position during chromatoelectrophoresis (electrophoresis at pH 3.7 in a pyridine acetate buffer; chromatography in n-butanol-acetic acid-water solvent) and had the same amino-acid composition. It could be concluded from this that glumitocin was present in all four species of ray and that the ray group shows a certain uniformity in this respect. According to Perks and Sawyer¹³, however, Raia ocellata has a hormone which is pharmacologically distinct from those found in the other species of ray.

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Pink Spot, p-Tyramine and Schizophrenia

Boulton et al.1, using monodimensional paper chromatography, confirmed the presence of the pink spot in urines of some schizophrenic and Parkinsonian patients and claimed that it was not caused by 3,4-dimethoxy-βphenylethylamine (DMPEA) but by p-tyramine. It has been suggested2 that the much discussed pink spot may have been caused by p-tyramine and, furthermore, that this compound may be causally related to schizophrenia or Parkinson's disease. With regard to these suggestions, it might be pertinent to consider some of our findings which were obtained with urines from normal subjects and from chronic schizophrenic patients.

Using a bidimensional thin-layer chromatographic technique³ (sec-butanol: formic acid (100 per cent): water in proportions 40:1:6 and isopropanol: ammonia (15 normal): water in proportions 8:1:1), we routinely distinguished the spot caused by p-tyramine from that tentatively identified as DMPEA. Both DMPEA and p-tyramine produced a pink spot when treated with ninhydrin followed by Ehrlich's reagent. Co-chromatographic techniques confirmed the presence of p-tyramine

in all control and schizophrenic subjects tested.

Our thin-layer technique did not allow a precise quantitation, and so a number of 24 h urine samples were assayed for p-tyramine by a fluorometric procedure. No significant difference was found in the excretion of p-tyramine between chronic schizophrenics and normal subjects (Table 1). The amounts of p-tyramine excreted varied considerably, in both schizophrenic and control subjects. Determinations of both p-tyramine and nitrogen in 24 h urine collections from twenty-three schizophrenic subjects showed a fair correlation (r = 0.62, P < 0.005) between the two (Fig. 1), indicating that tyramine excretion is, at least in part, a function of the amount of protein ingested. This relationship is supported further by the observation that patients fed glucose (no protein) have a markedly diminished excretion of tyramine. In consideration of

Table 1. URINARY EXCRETION OF p-TYRAMINE IN CHRONIC SCHIZOPHRENICS AND NORMAL SUBJECTS

	No. of subjects	No. of samples	$\mu \mathrm{g}/24~\mathrm{h}$	Range
Schizophrenics Controls	28 12	37 24	496 ± 27* 593 ± 55	(210–908) (247–1,240)

* Average ± S.E.

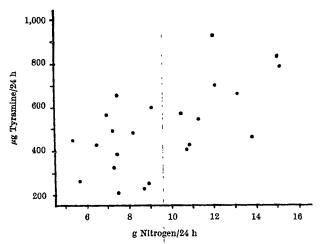


Fig. 1. p-Tyramine and nitrogen excretion in 24 h urine samples from twenty-three schizophrenic subjects.

these observations on p-tyramine excretion, it would be difficult to attribute significance to the finding by Boulton et al.1 of what they call an abnormally high concentration of p-tyramine in the urine of one schizophrenic subject. In any case, our results do not support the hypothesis of a relationship between schizophrenia and urinary excretion of p-tyramine.

The amount of p-tyramine found by Boulton et al. in their sample from a schizophrenic patient is approximately 30-200 fold greater than the amounts of DMPEA reported by other investigators3,4,6-11 to be present in the urine of schizophrenics and healthy controls. p-Tyramine is a normal urinary constituent found in all individuals by paper chromatographic as well as by fluorometric methods^{5,12,13} and so its absence in a large percentage of schizophrenics (fifty-six out of sixty-seven) and in some Parkinsonian patients (four out of twenty) as reported by Boulton et al. 14.16 does not agree with the incidence of p-tyramine excretion described here and by other investigators.

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Instability of Amitriptyline Base

AMITRIPTYLINE hydrochloride is an antidepressant which produces mild tranquillizing side effects (Fig. 1a). During an investigation into a death caused by the ingestion of amitriptyline it was noticed that the free base, a colourless oil prepared from a tablet for reference purposes, became yellow on standing. Thin-layer chromatography (silica gol G.F.; methanol: acetone 1:1 v/v) showed that the yellow material travelled further than the free base and unlike the base showed no colour reaction with potassium iodoplatinate. Gas liquid chromatography of the fresh base showed a single peak. On standing for 2 days, a second peak appeared, presumably corresponding to the yellow breakdown product. Some of this yellow compound was separated by thin-layer chromatography and analysed by infrared spectrophotometry. Comparison between the spectrum of the impurity and that of the pure base suggested that the aliphatic double bond had been oxidized to yield a ketonic product. The strong aliphatic-N-(CH₃)₂ stretching frequencies at 2,760 and 2,800 cm⁻¹ were absent and there was an increase in the relative intensities of aromatic over aliphatic bands.

Approximately 1 g of amitriptyline hydrochloride was then oxidized with 5 per cent potassium permanganate. The resulting solution was extracted with chloroform and a dark yellow oil was obtained from the solvent. On elution from a silica gel column with ethyl acetate, a yellow band moved quickly through the column and was Evaporation of the ethyl acetate yielded a collected. yellow oil which seemed to be on the point of crystallizing, but attempts to produce crystals from various solvents failed. The infrared spectrum of this oil between potassium bromide plates was identical with that previously obtained and both spectra were shown to be consistent with the Sadtler Index spectrum of 5H dibenzo (a,d) cycloheptene 5-one 10,11 dihydro (Fig. 1b). The nuclear magnetic resonance spectrum of this yellow oil in deuterochloroform measured against tetra methyl silane was also consistent with this structure (Fig. 2)four benzyl protons (3.1 p.p.m.) and eight aromatic protons (7.2 and 7.8 p.p.m.).

Although we have so far failed to detect 5H di-benzo (a,d) cycloheptene 5-one 10,11 dihydro in the urine of patients taking amitriptyline, the presence of a substantial amount of a neutral metabolite is indicated and work is being continued to identify this compound. Because of the rapid deterioration of the free base, a knowledge of this

$$CH_{2} - CH_{2}$$
 $CH_{2} - CH_{2}$
 $CH_{2} - CH_{2} - N$
 CH_{3}
 CH_{3}
 CH_{3}

Fig. 1. a, Amitriptyline hydrochloride; b, 5H di-benzo (a,d) cycloheptene 5-one 10,11 dihydro.

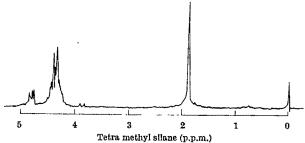


Fig. 2. Nuclear magnetic resonance of product of decomposition of amitriptyline base.

breakdown product would be useful when amitriptyline is extracted during a toxicological examination.

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BIOLOGY

Water Economy of the Ostrich

STUDIES of the physiology and water relations of desert birds have dealt with a few carinate species1, but practically nothing is yet known of the adaptations of the ostrich Struthio camelus L. to hot, dry environments. Because it is large, the ostrich cannot obtain shelter from the rigours of its environment in the manner of smaller birds, but we have found it to possess salt-excretory nasal glands which enable it to utilize saline water. At least one subspecies, S. camelus massaicus, frequents the neighbourhood of salt and soda lakes2. It has been suggested that ostriches can exist on dry or succulent food without needing free water, but no experiments have previously been carried out to confirm this. therefore investigating the water economy of S. camelus in the Sudan.

Results obtained so far show clearly that the ostrich must have access to drinking water, even in winter when the temperature in the shade varies between about 10° and 30°C with a relative humidity of 10-30 per cent. In these conditions, half-grown ostriches (about 13 kg) lost weight steadily when deprived of water, although they were given either dry food (millet) or green vegetables. They easily withstood a loss of 25 per cent of their body weight which was reached after 9 days of dehydration (the lethal point was evidently about 34 per cent). A maximum of 2.4 l. of fresh water could then be ingested at one time by the birds, which represents about 96 per cent of the total loss in body weight during the period of dehydration. During dehydration, the daily intake of food was reduced from an average of 300 g to about 170 g of millet; the water content of the faeces was reduced by 22.4 per cent and the body temperature tended to rise. Cloacal temperatures ranged between 37.7° and 40.0° C in control birds and between 38.8° and 41.0° C in dehydrated birds.

When fresh water was not available, ostriches drank dilute sea water and solutions of sodium chloride. They maintained their weight on 20 per cent sea water or 0.2 molar sodium chloride solutions, but lost weight steadily when given higher concentrations. In the Sudan, ostriches live in desert and semi-arid regions where some water or succulent foods are available. In the summer, they migrate to places where succulent food is abundant, even when water is not available in the immediate neighbourhood.

These observations indicate that ostriches can survive dehydration to a greater extent than can gazelles3 and more closely resemble the camel in this respect².

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Respiration in Polyzoa (Ectoprocta)

THE accepted view that zooids of the Polyzoa (or Ectoprocta) are small enough for gaseous exchange to be achieved by diffusion has recently been challenged. Mangum and Schopf¹, having measured oxygen consumption in Bugula turrita, concluded that diffusion alone was insufficient to supply oxygen to all parts of the zooid. They proposed a circulation of body fluid, brought about by the periodically repeated process of retraction and protrusion of the lophophore and tentacles, which is observed in these organisms. This, they supposed, would cause the transfer of oxygen-rich coelomic fluid from the region of the lophophore and tentacle sheath to the lower part of the zooid.

Mangum and Schopf's calculations can be criticized on biological grounds. First, they assume mixing of the lophophoral fluid with that of the main coelom: this is unlikely. The coelomic lumen of each tentacle opens into a ring coelom surrounding the mouth, these components together making up the lophophoral coelom or mesocoel. The only connexion between the mesocoel and the metacoel is through a transversely elongated pore beside, and nearly closed by, the nerve ganglion^{2,3}. While some movement of fluid between mesocoel and metacoel is obviously possible, the morphological arrangements do not suggest that this could be other than on a very small scale.

Second, acceptance of their figures for oxygen consumption invokes the unlikely premise that the internal viscera have the same kind of oxygen demand as the ciliated cells of the tentacles, which are beating continuously to create feeding currents. Such arguments do not disprove their hypothesis, but they render it less attractive.

Finally, however, Massaro and Fat4 have re-examined the data by applying standard mathematical techniques to the analysis of a diffusion system containing a chemical reaction. They found that the oxygen consumption of the polypide could be completely accounted for by diffusion alone.

Neither of the recent contributions on this subject1,4 has, however, considered the position in other Polyzoa. Whereas Bugula zooids can be considered as semi-cylinders with a flat surface which is membranous and readily permits gaseous exchange, the zooids of many other Polyzoa have all their walls calcified and display certain adaptations which seem to facilitate gaseous exchange by diffusion.

In the Cyclostomata, the zooid consists of a long cylindrical tube, the distal opening of which is closed by a membrane. Protrusion of the tentacles is achieved in a different manner from the Cheilostomata, to which Bugula belongs. The fact that the tentacle sheath does not protrude beyond the orifice of the zooid, as it does in the Cheilostomata, will largely preclude its functioning as an organ of respiration, while the less pronounced movements of the polypide, plus the presence of an annular septum in the metacoel, make extensive mixing of the coelomic fluid unlikely. (For a full description of the zooid and the method of protruding the tentacles, Borg's paper should be consulted.)

The body wall in cyclostomes comprises an epidermis (lined by a peritoneum), calcareous layer and cuticle. The skeletal calcareous layer is perforated by "pseudopores", filled with living tissue. It has been supposed^{2,3} that these permit gaseous diffusion through an otherwise impermeable wall. Borg³ even noted the paucity of pseudopores in the vicinity of the terminal membrane, which must be sufficiently permeable to gases to render the pores superfluous. The gonozooid, which contains large numbers of developing embryos, bears about twice as many pseudopores per unit area of surface as the normal zooid. It seems clear that this must be to satisfy the high oxygen requirements of the embryos, which can obviously be met only by diffusion, because here there is no polypide to promote circulation.

A recurring evolutionary trend in the Cheilostomata is the protection or elimination of the frontal membrane found in Bugula and other genera classified as Anasca. In the Ascophora, the front wall of the zooid is calcified and the hydrostatic function of the frontal membrane is performed by the ascus, a thin-walled sac opening directly to the exterior, into which water can flow as a decrease in coelomic volume forces out the tentacles (for diagrams, see ref. 6). In addition to its role as a compensation sac, the ascus may be the principal respiratory organ in asco-The rhythmic movements withdrawing and protruding the tentacles will keep emptying the ascus and refilling it with fresh, oxygenated water.

The frontal wall of ascophorans also often contains pseudopores. They may permit gaseous exchange to some extent, but this is probably not their primary func-Unlike cyclostomes, most ascophorans deposit secondary calcification on the outside of the initial skeletal layer. How this is achieved, remotely from the epidermis, has never been satisfactorily explained, but the tissues in the pseudopores are clearly involved. Thus, when the pseudopores are restricted to the margin of the zooid, secondary calcification spreads from the margin centripetally (producing a pleurocyst); when they cover the primary wall, secondary calcification takes place all over the surface (producing a tremocyst) (for diagrams

It is concluded that anascans and non-calcified Polyzoa have no diffusion problem; in cyclostomes it is met by pseudopores, varying in frequency of occurrence, which permit diffusion of oxygen from outside. In the ascophorans, pseudopores are again present, but often only at the margin of the zooid, and they seem to fulfil a different function; the ascus is perfectly adapted as an efficient respiratory organ, and the behaviour of the organism seems to ensure that it does so.

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Diurnal Activity of Hermatypic Gorgonians

THE natural food of gorgonians, or any other soft corals, inhabiting coral reefs is unknown, but net photosynthesis to respiration ratios from 2 to 5 have been reported for isolated colonies of five species of Caribbean reef gorgonians in laboratory experiments^{1,2}. This photosynthesis is attributed to the dinoflagellate symbionts living in the

Table 1. NUMBER OF GORGONIAN COLONIES WITH EXPANDED OR CONTRACTED POLYPS IN DIFFERENT CONDITIONS OF LIGHT

Cloudy day (ncon ±1 h) Bright sun Twilight (noon ±1 h) (7 p.m. ±1 h) Night (9-10 p.m.) Polyps expanded Polyps contracted 166 (81) 39 (19) 100 (44) 129 (56) 427 (85) 73 (15) 85 (57) 63 (43)

Numbers in parentheses are percentages of all colonies observed in the specified condition.

gastrodermal cells of these and other so-called hermatypic corals. There is also evidence that members of the gorgonian family Xeniidae in the Red Sea draw most of their nutrition from this photosynthesis3. Observations reported here suggest that gorgonians from Caribbean reefs may also depend for nutrition on their algal symbionts.

Equipped with self-contained underwater breathing apparatus and with a hand-tally in either hand, I observed every gorgonian I could find in six patches of corals on reefs less than 7 m deep off the upper Florida Keys during 3 weeks in June 1966. I made these observations in full sunlight, in complete overcast, in twilight and at night. I counted gorgonian colonies whose polyps were expanded on one tally and colonies whose polyps were contracted on the other. The few colonies which were partially expanded were not counted.

Table 1 shows the pooled results of these observations. The polyps of these hermatypic gorgonians on these reefs tend to be expanded during the day and contracted at night. No attempt was made to make such counts for individual species, but my impression was that the sea fans, Gorgonia, seldom varied from this condition, while the abundant members of the genus Pseudopterogorgia seldom showed this trend but tended to be expanded at night and contracted in the daytime.

In the same conditions of light, I collected plankton over the reefs with a 20 mesh/cm net of 25 cm diameter towed within 1 m of the sea surface from a skiff travelling at 1-3 knots. None of the tows lasting 20 min in daylight caught more than twenty or thirty organisms, but the net became clogged and the 50 ml. collecting jar overflowed with plankton within 5 min during every night

Close-up moving pictures of several gorgonian species taken on the reef in bright sunlight and at night by artificial light show (a) the trend of gorgonian polyps other than those of Pseudopterogorgia to be expanded in the daytime and contracted at night and (b) the paucity of visible plankton in daytime and its abundance at night. Stony corals such as Montastrea cavernosa and Diploria clivosa seem to be mostly contracted in daytime, but at night they are expanded and actively catching and ingesting swarming zooplankton.

The fact that the polyps of most gorgonian species I have observed expand in the daytime when zooplankton is scarce and when conditions are right for photosynthesis leads me to suggest that many of these species on shoal Caribbean reefs may draw most of their nutrition from the photosynthesis of their algal symbionts. The predominantly contracted state of these polyps at night when zooplankton is abundant suggests that these gorgonians do not feed extensively on zooplankton.

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Overwintering in Pemphigus bursarius (L.)

Алтноиси many aphids overwinter in the egg stage, several species can overwinter in the adult stage as parthenogenetic morphs (Aphis pseudobrassicae Davis1, some Dysaphis Börner spp.², Aphis gosypii Glover³, Pemphigus betae Doane⁴, etc.). The physiological mechanisms involved in overwintering by the parthenogenetic morphs of aphids have not been widely studied. I have used Pemphigus bursarius L., which can overwinter as adult virginoparae even in the absence of the host plant⁵, to show that overwintering adults are physiologically distinct from virginoparae living on lettuce roots during the summer.

'Webb's Wonderful' lettuce seedlings, germinated in a modified Hoagland's nutrient solution, were transferred to plastic pill boxes lined with damp filter paper. Some of these cages were placed in a room supplied with artificial light giving a long photoperiod (17 h light and 7 h dark) and kept at about 18° C, and others were placed in cabinets equipped with lights to give the same photoperiod and kept at a temperature of 10° C. Single virgino-parous adults of *P. bursarius* from a laboratory culture initiated by winged emigrants from poplar galls collected in Harpenden, Hertfordshire, were introduced to cages at both temperatures and were allowed to produce three to six offspring. The adults were then removed. At 18° C. offspring became adult virginoparae after 13-14 days. (Some offspring became sexuparae at this temperature; these results will be discussed elsewhere.) The adults started to reproduce the same day, or the day after the adult moult. Offspring born at 10° C took about 20 days to become adult but did not reproduce, although they were kept for up to 56 days after the adult moult at this temperature.

Adults reared at 10° C were retained at that temperature for varying periods, then removed to fresh seedlings and kept at 18° C. Reproduction did not commence immediately after removal to the higher temperature. The length of delay before the onset of reproduction in adults reared in the cold was about 3 weeks for aphids retained at 10° C for 4–9 days after the final moult and 2 weeks for those retained in this way for 33 days.

Adults reared at 18° C and 10° C were fixed in alcoholic Bouin after the final moult, embedded in 'Ester Wax 1960' and sectioned at 7μ (Figs. 1 and 2). The overall size of these adults was similar but there were marked anatomical differences. In adults reared at 18° C, the embryos were larger at a later stage of development and more numerous than the embryos of adults reared at 10° C. The fat body was, however, more extensive in adults reared at 10° C than in those reared at 18° C.



Fig. 1. Longitudinal section of adult virginopara of *P. bursarius* reared from birth at 10° C showing extensive fat body (F) and retardation in development of embryos (M).

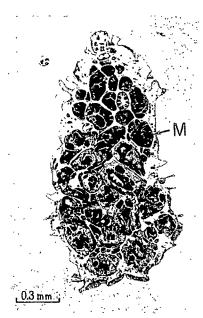


Fig. 2. Longitudinal section of adult virginopara of $P.\ bursarius$ reared at 18° C with numerous well developed embryos.

The failure of adult virginoparae, reared at 10° C, to reproduce without delay when transferred to 18° C, the failure of embryos to develop and hypertrophy of the fat body, all suggest that these adults were in a state of reproductive diapause. Whether adult virginoparae of P. bursarius reared at 10° C are considered to be in a state of reproductive diapause or quiescence, they are physiologically distinct from summer virginoparae and as such are separate morphs, termed hiemalis. The hiemalis are adapted for survival rather than reproduction, as the large food reserves (fat bodies) show. The few small embryos may be associated with a low rate of respiration. It is possible that the occurrence of such morphs, specially adapted for overwintering, is widespread among other species of the Aphididae which have facultative or obligatory anholocycles.

The economic importance of overwintering colonies of aphids is still to be investigated. It seems, however, that these colonies could build up more rapidly in the spring on host plants of commercial importance and cause damage earlier in the season than colonies initiated by winged emigrants from winter host plants.

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Phototactic Response of Euglena gracilis to Polarized Light

Phototaxis (translational movement in response to a light stimulus) in the single cell micro-organism *Euglena gracilis* can be used as a means for studying sensory perception at the molecular level. This process seems to be controlled by a primitive visual system consisting of a

photoreceptor, at the base of the flagellum, and a pigmented shading device called an eyespot¹⁻³. Euglena rotates about its long axis as it swims, and thus in the presence of light from one side the photoreceptor will be periodically shaded by the eyespot. It has been suggested that this shading causes a succession of phobic responses (shock reactions) which act to point the organism towards the light source. Once the organism is properly oriented, a continuous shading of the photoreceptor results and no further phobic response occurs. (It is possible that the inverse is true, that is, that the shock response occurs on shading the photoreceptor. There is, however, some evidence against this type of mechanism, for example, negative phototaxis in eyespotless Euglena and the polarized light effect described in the present work.)

Evidence has been presented which indicates that Euglena has a greater motility in linearly polarized light than in ordinary light. Furthermore, many species of animals, mostly arthropods such as the crab Cardisoma, are capable of detecting the orientation of the plane of polarization of light, and germinating spores of the fungus Botrytis and the fern Osmunda orient their growth relative to the polarization plane of a light stimulus. Thus we felt it would be of interest to determine whether polarized light has an effect on the phototactic response in

Euglena.

Euglena gracilis, strain Z, was grown as in our earlier work⁶ using a lactic acid medium described by Wolken². White light (maximum intensity about 140 ergs cm⁻² sec⁻¹) from a 500 W tungsten projection lamp was used in all experiments^{6,7}. Heat was removed using watercooled infrared absorbing glass. Polarized light was obtained by placing a sheet of 'Polaroid' in the stimulating beam. Phototaxis was measured using a "phototaxigraph" designed in this laboratory. Basically, this instrument is a chopped double beam turbidimeter which uses near infrared light in the measuring and reference beams and which photoelectrically records the accumulation of organisms within a lighted region. A diagram of the optical configuration of the phototaxigraph is shown in Fig. 1. All other experimental details were as previously described^{6,7}.

In Fig. 2, "phototaxigrams" showing the effect of plane polarized light on phototactic accumulation are given. When the plane of polarization of the stimulating light (a collimated beam about 2 mm wide) was oriented so that its electric vector was perpendicular to the long axis of the sample tube (a cylindrical cuvette approximately 10 cm in length), a normal phototaxigram was obtained (Fig.

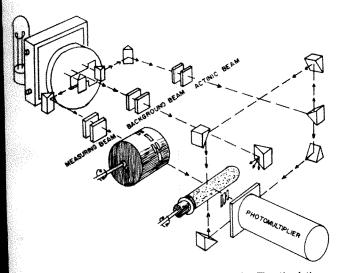


Fig. 1. Optical configuration of the phototaxigraph. The stimulating (actinic) light is incident on the sample cuvette from above and below. The measuring beam passes through the same region of the cuvette but at right angles to the actinic beam.

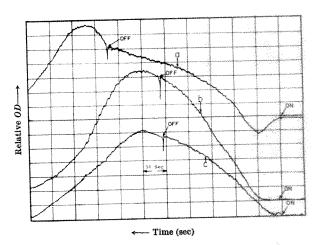


Fig. 2. Comparison of phototaxigrams obtained using polarized stimulating light and unpolarized stimulating light. a, Polarized light with E-vector parallel to the long axis (axis of rotation) of the tube; b, unpolarized light; c, polarized light with E-vector perpendicular to thong axis (axis of rotation) of the tube. Curves represent optical density at approximately 8000 Å in the illuminated zone (ordinate) against time (abscissa; sequence of events from right to left). Optical density proportional to number of organisms. Downward spike when light is turned off is merely a marking device.

2b, c). The lower rate and extent of phototaxis in curve 2c, as compared with curve 2b, is the result of the lower intensity of stimulating light.

Rotating the plane of polarization so that the electric vector was parallel to the long axis of the sample tube produced a distinctly different phototactic behaviour of the Euglena (Fig. 2a). Intermediate positions of the polarizer produced phototaxigrams of intermediate shape. The characteristic features of the polarized light effect (curve 2a) are an initial negative phototaxis occurring immediately on turning the light on, and a sudden influx of Euglena into the illuminated zone when the light is turned off.

In the absence of any orientation of individual Euglena with respect to the sample tube, a mechanistic explanation for different phototactic responses to different orientations of plane polarized light is difficult to envisage. We therefore looked for such orientation in our experimental system. In the phototaxigraph, the sample tube is rotated at 10 r.p.m. about its long axis so as to keep the Euglena from settling and to expose all sides of the tube to the same amount of light'. Photomicrographs of Euglena suspensions in the rotating and non-rotating tube were taken and are shown in Fig. 3. It can be seen that when the tube is not rotating the organisms are randomly oriented, but when the tube is rotating the organisms are mostly aligned perpendicular to the axis of rotation, probably in response to the flow of medium. consistent with the observation that free-swimming protozoa orient themselves against a current*.

This finding not only allows an interpretation of the phototactic behaviour but also gives information about the molecular geometry of the eyespot. If we assume that the pigment molecules in the eyespot* are ordered with respect to the plane of the organism, then the initial negative phototaxis can be interpreted as a shock reaction produced when actinic light which is polarized so as not to be absorbed by the eyespot pigments (that is, so that the plane of polarization is perpendicular to the molecular electronic transition moment) illuminates the photoreceptor. This shock reaction is experienced by those Euglena directly in the stimulating beam and causes a decrease in the number of organisms within the illuminated zone, probably by causing a reversal of swimming direction

^{*} It is difficult to formulate an explanation of our results, particularly the switch from positive to negative phototaxis as the plane of polarization of the stimulating light is rotated, based on an ordering of pigment molecules in the photoreceptor and not in the eyespot.

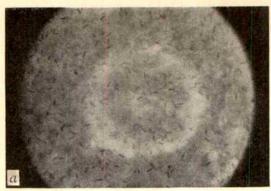




Fig. 3. Photomicrographs of Euglena gracilis in a non-rotating (a) and a rotating (b) tube $(\times 60)$. Axis of rotation of tube is coincident with its long axis, which is oriented vertically within the plane of the paper.

at the dark-light interface. This type of response would be analogous to the negative phototaxis observed with eyespotless mutants9, which is probably the result of direct stimulation of the photoreceptor.

Those Euglena outside the illuminated zone are exposed to scattered light, which will have lost much of its polarization. Thus, as shown in Fig. 2a, after an initial period of negative phototaxis caused by the shock reaction, a positive slope corresponding to positive phototaxis is observed.

There is usually a 15 sec lag time after the unpolarized light is turned off, caused chiefly by inertia in the swimming motion of the Euglena, during which the organisms will continue to accumulate within the previously illuminated zone9. With polarized light, as in Fig. 2a, the rate and extent of such accumulation are much greater than with unpolarized light. This is probably produced by a combination of the normal inertial lag and a cessation of the opposing shock reaction to polarized light.

A specific statement concerning the geometry of the pigment molecules within the eyespot can be made by a consideration of the fact that only light polarized with its plane parallel to the axis of rotation of the sample tube, and thus perpendicular to the long axis of the aligned Euglena, produces the negative shock reaction. If our interpretation of the negative response is correct, the pigment molecules must be aligned in the eyespot so that their molecular transition moments are parallel to the long axis of the organism. The pigment molecules in the eyespot are known to be carotenoids3. The molecular transition moment associated with the long wavelength absorption band of carotenoids is parallel to the plane of the extended conjugated double bonds. The carotenoids, in the eyespot of Euglena, must therefore be arranged with the long axes of the molecules parallel to the long axis of the organism. (This interpretation implies that the pigment molecules in the photoreceptor are either not oriented, are oriented differently from those in the eyespot (if they are

carotenoids), or are not carotenoids and have differently polarized transitions.)

The ordering of pigment molecules in the phototactic organelle is reminiscent of similar situations in the arthropod eye4, in the mammalian retina10 and in the chloroplast11. One wonders whether this is an accidental result of the necessity of having a high pigment concentration in a shading device, such as the eyespot, or whether the ordering has physiological significance in providing Euglena with a mechanism for orienting with respect to the plane of the polarized light in its environ-

This work is taken in part from a thesis submitted by K. E. Bound to the University of Arizona for the degree of M.Sc. We are grateful to the Air Force Cambridge Research Laboratories for research support, to Dr D. Lindes for helpful discussions and to Mr D. Morisky for technical assistance.

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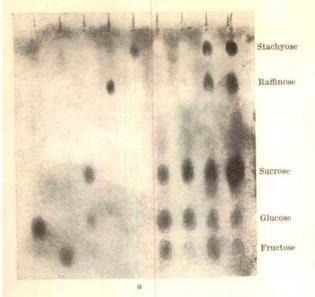
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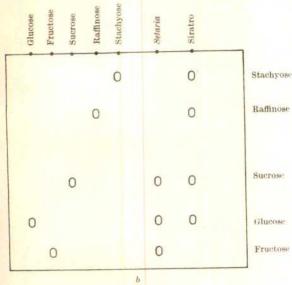
Soluble Carbohydrates in the Seeds of Tropical Pasture Species

TAXONOMISTS have used the composition of the soluble carbohydrate fraction of grass seeds in the biochemical differentiation of grass species1,2. Sucrose is usually the most abundant sugar, but the presence of the raffinose group in the seeds of certain ten perate species of grasses and legumes has been established. McLeod² found raffinose and stachyose in the seeds of Brachypodium spp. whereas Bromus spp. contained neither, and of twenty two temperate species of Gramineae examined she found raffinose in 70 per cent and stachyose in 45 per cent.

We have examined the seeds, extracted in 80 per cent ethanol, of the tropical grass species Cenchrus ciliaris (Buffel), Chloris gayana (Rhodes) and Setaria sphacelata and the tropical legume species Stylosanthes humilis (Townsville lucerne), Glycine javanica and Phaseolus atropurpureus c.v. siratro, by paper chromatography in ethyl acetate-pyridine-water (10:4:3) with 3-4 per cent p-anisidine hydrochloride in butan-1-ol-ethanol-water (4:1:1) as spray reagent. Raffinose and stachyose were present in the seeds of all three legume species but were absent from the grass species (Fig. 1a and b).

In all the tropical grass and legume species examined, fructan (identified by alcohol-resorcinol test and isolation) was found in the deproteinized, cold-water extracts of the seeds previously extracted with ethanol. It seems therefore that de Cugnac's classification of the Gramineae into levulifers and sacchifers based on the presence or absence of fructan cannot be applied to the seeds of tropical species.





We suggest that the presence or absence of the raffinose roup in the seed may provide a means of distinguishing etween tropical grass and legume species.

Fig. 1.

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APPLIED SCIENCE

Effects of Particle Size Range in the Differential Thermal Analysis of **Powders**

ALTHOUGH the technique of differential thermal analysis s widely used in the study of materials in powdered form, here seems to be considerable uncertainty as to whether he particle size characteristics of the material influence he thermograms obtained. The general consensus of

opinion would seem to be that the mean particle size might have some effect; the possible effect of the spread of particle size in the sample appears, however, scarcely to have been considered.

In connexion with research in which we are engaged, it is necessary to study the peaks arising from the transformation of extremely small quantities of material and, in consequence, it is essential that due allowance should be made for the effects of any differences arising from the particle size characteristics of the different samples. In order to investigate this question, forty-seven tests have been carried out on samples of quartz sand, from a single source; the samples being prepared, from the natural sand, by sieving between sieves of known aperture.

The size distribution of the samples is defined in terms of two parameters

 $M = \frac{d_2 + d_1}{2}$

and

$$D \, = \, \frac{2(d_2 - d_1)}{(d_2 + d_1)}$$

where d_1 and d_2 are the apertures, in μ , of the finer and coarser sieves, respectively. Thus M is the mean particle size of the sample, and D is the spread of the sample. the latter being expressed in terms of the mean particle size of the sample.

The element of the thermogram which has been studied is the peak corresponding to the α/β transformation of quartz. The area of the peak is defined in the way shown in Fig. 1; the base of the peak area being obtained by the projection of the line CD before the peak. This procedure is justified because an independent analysis shows that the slope of this portion of the thermogram is independent of the particle size characteristics of the samples of quartz used.

The equipment used was a 'Standata 625' apparatus (Stanton Instrument Co.), fitted with a 'Sintox' block head. The weight of the samples used was 285 mg ± 1 mg and the samples were lightly tamped with a stainless steel rod.

The test results have been analysed on the basis of a double linear regression and the following relationship obtained.

$$A = 0.160 + 2.4 \times 10^{-6}M - 1.4 \times 10^{-2}D$$

where A is the area of the peak in square inches. statistical analysis of the data then reveals that, at the 95 per cent confidence level, there is: (a) no significant correlation between the area of the peak and the mean particle size of the material $(0.02 < M \le 2 \text{ mm})$; and (b) a significant correlation between the area of the peak and the spread of the particle size of the sample (0.2 < D < 2).

The present conclusions definitely relate only to the α/β transformation of quartz and to tests carried out with a particular apparatus. Thus, possibly, they are inapplicable to peaks relating to other types of transformation, and to tests made with apparatus of different design.

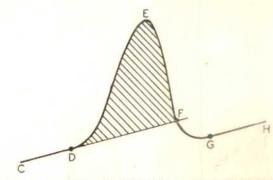


Fig. 1. Construction used for defining the area of the peak of the thermo-

In view of the paucity of information relating to this question, however, this communication is published in order to draw attention to errors which could arise when the results of tests carried out on samples of different particle size range are compared.

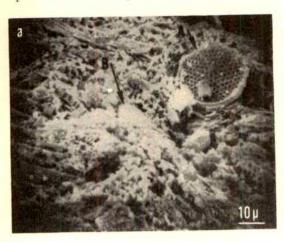
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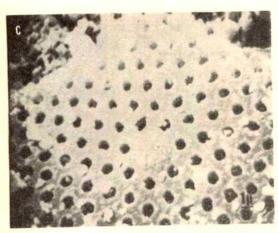
Powder Science Laboratory, Department of Mechanical Engineering, King's College, University of London. Received October 25, 1967.

GENERAL

Diatomaceous Earth: Scanning Electron Microscope of 'Chromosorb P'

DIATOMACEOUS earth, as mined in Lompoc, California, and other sites in the south-west United States, is made up of the siliceous skeletons of diatoms which lived in the Miocene and Pliocene periods. The mined diatomite is crushed, blended, pressed into brick and fired at 900° C for use as high temperature insulation. When ground, this material, known as 'Chromosorb P' (Johns-Manville Corp.), is sold as a support for the liquid used in the gas chromatographic separation of organic compounds. Some of the diatom skeletons were found to be intact after this rigorous treatment. It has been estimated that as many as 10,000 species of diatoms may be found.



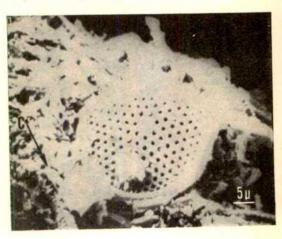


Individual diatoms and composites have been examined by optical and electron microscopy to try to obtain a better understanding of their function in the gas chromatographic column. Optical techniques lack depth of focus, so at best, only a small depth of material can be observed. The electron microscope provides additional resolution, but, because it is also a transmission instrument, it shows the composite of the whole specimen. The 'Stereoscan' scanning electron microscope provides a means of observing the surface of the specimen with considerable depth of focus, even at high magnification.

Many diverse samples can be examined with this instrument—for example, transistor terminals³ and soil micro-organisms⁴, and we are currently examining pollen and meteorite surfaces. An investigation of the solid support used in gas chromatography was suggested because questions have been raised about the physical features of the modified diatomite relative to the size of the pores and the size of the openings through which the gas would flow.

Previously, investigators have had to rely on indirect observations to establish the type of pores (mercury-intrusion method) and the surface area (Brunauer-Emmett-Teller, gas adsorption method). Early studies⁵, in which the pore size distribution was measured with and without varying quantities of stationary phase, concluded that the pore size distribution was between 0.4 and 2μ for 'Chromosorb P'. Verification by direct observation was desired to estimate the distribution and types of holes in 'Chromosorb P'.

Part of the surface of a particle of 'Chromosorb P' is shown in Fig. 1. The view shown in Fig. 1(a) shows the variety and complexity of the particle surface. The part



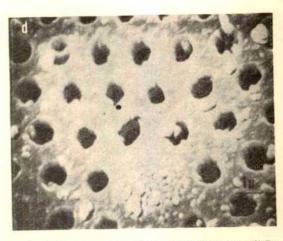
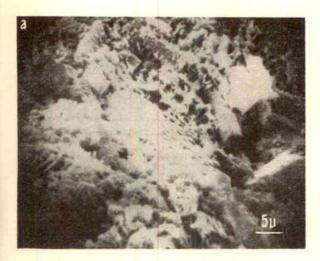
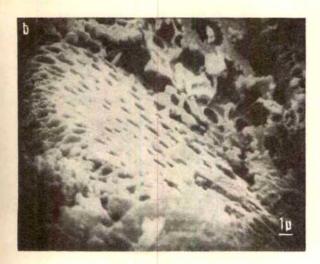


Fig. 1. Surface of 'Chromosorb P'. (a) Surface and diatom skeletons; arrow A. Coscinodiscus; arrow B. side view of a similar diatom; (b) Coscinodiscus, and arrow C. Plagiogramma; (c) and (d) enlarged views of Coscinodiscus.

of Fig. 1(a) indicated by arrow A is shown further in Figs. 1(b), (c) and (d). Similar diatom skeletons are seen throughout the area, either in valve view or at some angle to the surface. It can be seen that many of these are broken and the edges are clearly in view. These diatoms are believed to be of the genus Concinodiscus.





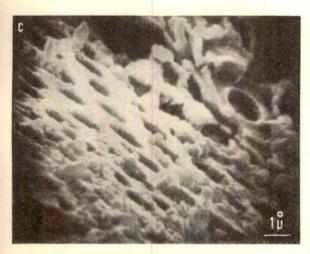


Fig. 2. Side view of the surface of Coscinodiscus.



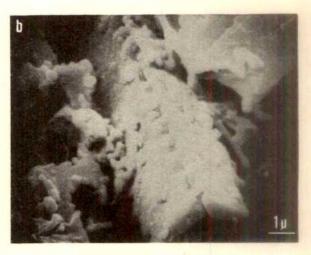


Fig. 3. Fragment of Plagiogramma.

The particle indicated by arrow B of Fig. 1(a) is shown in Fig. 2. Fig. 2(a) provides further information on the fragments surrounding the more complete skeleton. Figs. 2(b) and (c) show the height of the particles on the surface, hole dimensions and spacing. The surface particles are seen to be 0.2 to 0.5 μ in diameter and are up to 1 μ high,

The spacing between holes (primary structure) in the surface of the diatom skeleton in both Figs. 1 and 2 is about 0·7 to 1·1μ. These uniformly spaced holes are about 0·5 to 0·8μ in diameter. The immediate area around each hole appears to be slightly raised. The total diameter of the specimen is approximately 23 μ.

A second diatom skeleton is indicated by arrow C in Fig. 1(b). This is believed to be of an unidentified according genus. Such forms make up a small part of the population of diatoms in the particle. An enlarged view of this specimen is shown in Figs. 3(a) and (b). Although there is some slight roughness of the surface, the surface features of this skeleton are much less prominent than that of the Coscinodiscus of Figs. 1 and 2. The outstanding features of this specimen are the valve-like longitudinal protuberances. Fragments of other species are readily evident in Fig. 3(a) also.

Another area of the particle, showing many additional surfaces, can be seen in Fig. 4. Of particular interest is the girdle view of the broken diatom, believed to be similar to the *Coscinodiscus* of Fig. 1. This is of particular interest to workers in gas chromatography, for it relates to the spreading of the liquid phase. Figs. 4(a) and (b)

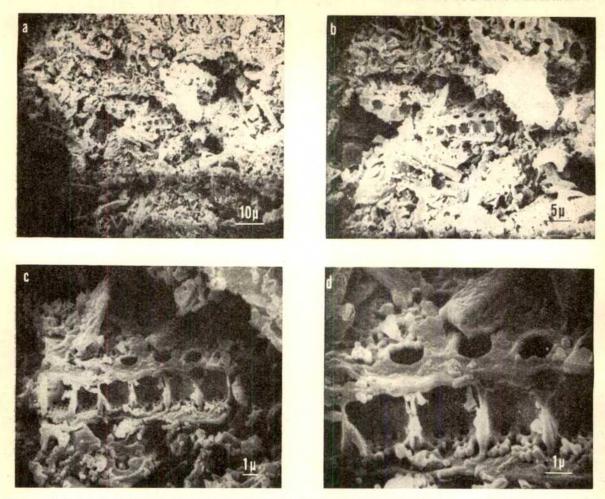


Fig. 4. Surface of a particle of 'Chromosorb P'; (a) and (b) 'Chromosorb P' fragments including area of (c) and (d) side view of diatom Coscinodiscus.

show the large number of these fragments which are present in some parts of the 'Chromosorb' particle and the inter-relationship of the broken diatom skeletons in the particle as a whole. Figs. 4(c) and (d) present a more detailed view of the surfaces which are similar to that of Figs. 1 and 2. These protuberances and the finely divided fragments probably account for the high surface area measured by Baker et al.5. The holes in this specimen are found to be similar to those of Figs. 1 and 2. The dimensions of similar shaped diatom skeletons reported by Ottenstein as viewed in thin-section were found to vary between 50 and 20µ. This lower value is in general agreement with our observations and may be peculiar to the particular species examined. The electron micrograph of Ottenstein, where the primary hole structure has dimensions of approximately 3µ, is also probably a different species because 0.5-0.8 u openings are observed in Figs. 1, 2 and 4. Furthermore, considerable variation in size from form to form may be found in any given species.

The holes in the lower portion of the Coscinodiscus were found to vary from 0·1μ to more than 0·4μ and are similar to the secondary structures indicated by Ottenstein which were approximately 0.5µ in diameter. The chambers seen in Figs. 4(c) and (d) are 1.5 to 2.0 u in diameter and 1.5μ in height, and have an estimated volume of 1.5 × 10-8 μl. Because such a small quantity of liquid would be required to fill these cells, the importance of the liquid distribution on supports used in gas chromatography is readily apparent. In the event that the cells were not filled the surface tension of the liquids would determine the degree and completeness of the filling or covering of the holes, both primary and secondary. It

would be expected that if any holes were to be closed the secondary ones would be filled first. It is evident that this microstructure can be the cause of tailing as observed in gas chromatography, especially where high carrier gas flow rates are used.

The large variation in the size of the pores and their random arrangement readily explain the high effectiveness of the diatomite as a filter aid. It is apparent that particles as small as 0·1μ might be expected to be retained when the solution is passed through a bed of calcined diatomaceous earth.

It should probably be emphasized that the kind of diatoms which are related to the location of diatomite deposits and the temperature and length of firing would all have some influence on the cell and pore sizes. These are therefore important considerations in the efficiency and use of 'Chromosorb P' as a gas chromatographic support.

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ceived September 25, 1967.

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BOOK REVIEWS

LAISSEZ-FAIRE, LAISSEZ DETRUIRE

Man and Environment

Crisis and the Strategy of Choice. By Robert Arvill. (A Pelican Original.) Pp. 332+13 photographs. (Harmondsworth, Middx.: Penguin Books, Ltd., 1967.) 8s. 6d.

MUCH that we admire in the English landscape—the trout stream, the parkland of the great estate, the grouse moor, the college garden, the mixed farm of arable and grassland, thick hedges and planted coverts-was created for the benefit of the few. It was created generally by an autocratic minority for their exclusive use and enjoyment in perpetuity and was deliberately designed, often with protective measures bordering on the ferocious, to exclude

Modern democratic principles insist, and rightly, that recreation in the widest sense and the enjoyment of the whole environment shall be the preserve of the whole nation. The increased leisure, the affluence, the train (as Wellington tactlessly pointed out) and latterly the car enable millions to travel to places where 50 years ago only a few could afford or were entitled to penetrate. I believe that the countryside will have to become, as the theatre and football match have become, a place of restricted entry unless it is to be destroyed by the multitude. Payment for entry to a National Park will have to become as commonplace as payment to enter any other place of entertainment.

The increase in population of the British Isles, or of other affluent countries for that matter, is only a trivial actor in the increase in pressure on the countryside. It s widely, but erroneously, assumed that were our population stabilized at, say, the 1900 figure no problems of vandalism, waste disposal, air and water pollution, housng pressures on land and all the other nastiness associated with industrial societies would arise. They would, in fact, all occur, but at a marginally lower rate. The principal ressures are industrial demands, ever increasing standards of individual affluence and leisure time, increased energy equirements, the spread of democratic principles and, bove all, the invention of the car.

Mr Arvill investigates in great detail the effect of these ncreased pressures on the air, the water and the wildlife opulations of our countryside. He places, I think, too such emphasis on the problem of population pressures nd insufficient on some of the other villains of the piece. Te makes excellent suggestions for immediate and future onservation and does not make the common mistake of elieving that conservation consists of putting a fence round certain animals and plants with a label saying Keep Out". He believes, as we must all come to believe, at conservation is the antithesis of preservation and nould comprise a mutual adaptation of the environment ourselves and ourselves to the environment. He does, think, less than justice to the planners in Britain, who, the past 20 years, have housed more people, accomodated more industry and permitted more exploitation the environment with less relative damage than virally any other country in the world. This is some compense for the unlimited and uncontrolled damage flicted in the 150 years up to 1939.

The book has an excellent bibliography with a catholic ste, a good index and a high standard of production roughout. I do not think that, with his condensed text d unlimited homework, Mr Arvill will achieve many nversions, but he will certainly educate, encourage and ovide ammunition for the converted. B. E. JUNIPER

HISTORIES OF TECHNOLOGIES

Technology in Western Civilization

The Emergence of Modern Industrial Society Earliest Times to 1900. Vol. 1. Edited by Melvin Kranzberg and Carroll W. Pursell, jun. Pp. xii+802. (London and New York: Oxford University Press, 1967.) 68s. net.

THE editors of this volume have attempted, with considerable success, to move forward from the tradition of encyclopaedias in the history of technology towards a viable textbook. The very definition of "technology" is nearly impossible, and whatever it is, it has complex relations with the whole of human activity and thought. so that a satisfactory history will require a great scholar as yet unknown. In default of that, the editors have at least produced a scheme of organization from which some historical understanding can be derived; and within its framework they have obtained essays of generally high quality from their specialist contributors. Implicitly acknowledging the contemporary interests of their audience (in the first instance, the US Armed Forces Institute), they assign bulk and detail in proportion to the historical proximity of the periods studied; thus the past two decades of the nineteenth century receive just as much space as all history to 1600. Within the limits of space of each period, they provide studies of the social. economic and cultural aspects of technological history. For the more recent periods, when the authors do not need all their space to review the well worn generalizations, this material can be illuminating.

But one is still left with a multi-layer sandwich. There are the good histories of particular technologies (divided up in a traditional scheme) and the good discussions of the preconditions and effects of technological change in the economic and social spheres. But, with the partial exception of the most recent period, there is little attempt to capture the flavour of the technical endeavour of any period. One cannot but compare this work with Klemm's History of Western Technology, which makes no claim to comprehensiveness, but which gives a vivid picture of the conception of technology in various milieu, through its carefully chosen extracts from original sources. textbook for an advanced course in the history of technology of recent times, this volume serves well; but for an introduction to this most important and most difficult sort of history, one will still fall back on Lilley, Mumford J. R. RAVETZ or Klemm.

FUNGI NEAR MR MacGREGOR'S GARDEN

Fungi, Wayside and Woodland By W. P. K. Findlay. Pp. xi + 202 + 50 plates. (London and New York: Frederick Warne and Co., Ltd., 1967.) 65s. net.

In the past fifteen years a considerable number of books. richly illustrated with coloured plates, have become available which help the field naturalist to identify the larger fungi. This book in the "Wayside and Woodland" series adds to their number, but it has a special quality because rather more than half of the forty or so coloured plates are the work of Beatrix Potter. It may come as a surprise to many that she was a serious and knowledgeable amateur mycologist, and indeed in the nineties of last century read a paper to the Linnean Society on the germination of agaric spores. But the environment of her books, which with the Bible and Shakespeare have played such a formative part in the literary style of my generation, is not so different from the habitat of toadstools. Peter Rabbit and his sisters who, it will be remem-"lived with their mother in a sandbank, under the root of a very big fir-tree" must have admired Russula emetica and many another in the autumn as they "went into the fields and down the lane" with strict orders to

avoid Mr MacGregor's garden.

To Beatrix Potter's coloured pictures, many of which have the authentic touch of genius, have been added almost an equal number of excellent plates by R. B. Davis and E. C. Large. Around these illustrations, and ten half-tone photographic plates the reproduction of which leaves much to be desired, Dr Findlay has woven a text. The first six chapters form a readable introduction to the larger fungi which the amateur will read with profit and which will give him added pleasure as he strolls through the woods in autumn casually trying to match the fungi he finds with the pictures in the book. The seventh to sixteenth chapters are devoted largely to the descriptions of individual species. The descriptions are simple and reflect Dr Findlay's profound knowledge of fungi. They are, however, clearly not intended for the more serious student and references to microscopic features and spore measurements have been avoided.

Perhaps the weakest parts of the book are the half-dozen black and white figures. For example, Fig. 3 gives a very poor and misleading picture of the beautiful microscopic structure of the hymenium in toadstools. It is easy, however, to criticize a work of this kind, but on the whole it is to be welcomed more on artistic and nostalgic rather than on scientific grounds, for all the time in appreciating Beatrix Potter's artistic skill in depicting a toadstool we can almost see Mr Jeremy Fisher or Squirrel Nutkin peeping round the corner. It is a pity that the book is so expensive that few individuals will be able to afford it.

C. T. Ingold

AN EXPANDING TECHNOLOGY

Progress in Microbiological Techniques

Edited by C. H. Collins. Pp. ix +231. (London: Butterworth and Co. (Publishers), Ltd., 1967.) 55s.

This collection of eleven papers reports contemporary developments in some of the more fascinating and sophisticated techniques used by microbiologists and those working in related fields. The editor of this volume, C. H. Collins, has selected a broad range of techniques for discussion, including several which are of direct relevance

to the applied microbiologist.

Clear, critical accounts are given of complement fixation, serological analysis of fungi and actinomycetes, fluorescent antibody methods and the freeze-drying of micro-organisms. Some specific needs of industrial and public health laboratories are considered in chapters on the microbiological assay of vitamins and amino-acids, standardization of vaccines, evaluation of yeasts and moulds for brewing and antibiotic production, phage typing of staphylococci and the examination of water supplies. This last chapter contains a particularly good appraisal of membrane filtration procedures and comments on the respective merits of currently available membranes. Most of the contributors direct their readers to sources of materials demanded by the methods described, whether they be items of equipment, reference cultures or serological reagents. One of the expressed hopes for this book is that it will be useful to microbiologists who may be required occasionally to apply any of the techniques discussed to their own peculiar problems. It is essential, therefore, to have a rapid and reliable reference to appropriate suppliers and this exigency is met almost to the full. Progress in Microbiological Techniques will also make valuable reading for teaching purposes and the chapter on the analysis of the bacterial cell gathers together an abundance of useful practical information. This book can be recommended strongly and any adverse criticism is slight and minor. One hopes that the producers of the present volume will be encouraged to think of it as the forerunner of others that will continue to survey, at a reasonable price, the development and application of new and specialist techniques.

ALAN T. BULL

LEARNING BY PROBLEM SOLVING

Problems and Solutions in Mathematical Physics

By Y. Choquet-Bruhat. Translated by C. Peltzer Translation edited by J. J. Brandstatter. (Holden-Day Series in Mathematical Physics.) Pp. ix+314. (Sar Francisco, London and Amsterdam: Holden-Day, Inc. 1967.) \$10.00.

This attractive book is aimed at introducing students of theoretical physics, applied mathematics, and engineering to some of the modern mathematical methods and concepts. It is not a book of rules of what to do, and it will appeal more to those people, both undergraduate and graduate, who care about what they are doing. The treatment is more rigorous than will appeal to some readers, though there is no undue emphasis on achieving the utmost generality; the work is essentially as simple as is compatible with ensuring the right answers.

There are 125 separate problems, many split into about five related parts, with full solutions. One of the first things to strike one is the great variety of topics covered. A list of the eight main section headings will give a rough idea of its scope: (1) Linear mappings. Operations on matrices. (2) Proper values and proper vectors. Reduction of matrices. (3) Scalar product and norm. Hermitian operators. (4) Vector calculus. Multiple integrals (5) Function spaces and operators. (6) Series expansions of functions. (7) Differential equations. (8) Partia differential equations. Some especially interesting features are the use of differential forms in parts of section 4, for example in a problem involving covariant derivatives and symmetries of the Riemann-Christoffel tensor, and the use elsewhere of the theory of distributions.

I found the problems interesting and well chosen Nearly all make a useful point or illustrate some importan principle. The solutions are helpful and seem generally though not universally, correct. Some of the English translation reads rather awkwardly, but there are few places where the meaning is not immediately clear. On certain pages there are quite a lot of trivial misprints—sixteen on page 217. In some cases these defects, or in complete explanations or definitions, make it difficult to interpret the question without first looking at the solution. The matter referred to in some footnote references for example, "... (see Problem Paris 1957)", on page 248, could not be found. Although the table of content lists every problem by name, a subject index would stick have been useful.

Even with the defects mentioned here, this seems to be a useful addition to the problem books of mathematical physics, and provides an enjoyable an interesting way of learning an important branch of the subject.

A. Weinmann

CHARGE CARRIERS IN ORGANIC SOLIDS

Organic Semiconductors

By Felix Gutmann and Lawrence E. Lyons. (Wiley Serie on the Science and Technology of Materials.) Pp. xvii 858. (New York and London: John Wiley and Son 1967.) 224s.

MODERN interest in the semiconducting properties organic crystals started about 20 years ago, with measurements on phthalocyanine crystals, first as powders, the as single crystals. The work was chiefly initiated

chemists for a variety of reasons, such as an interest in the light stability of dyestuffs, catalytic properties of conjugated compounds, or properties of polycyclic aromatic compounds in relationship to coal. The workers lent heavily on the broad theoretical ideas of the physicists, and still do. The physicists found germanium hard enough to understand, without branching out to consider organic substances, principally of ill defined purity. The additional stimulus of Szent-Gyorgyi's speculation on biochemical mechanisms in 1942 led to work on proteins and nucleic acids, and these biological aspects were first discussed at a Faraday Discussion at Nottingham in 1959. Around 1956 the relatively high conductivity of crystals of organic charge transfer complexes was discovered. An extra stimulus came in about 1960 when the Russian work on the semiconducting properties of heat-treated polyacrylonitrile appeared, and a great spate of work followed on polymers, of varying degrees of defined structure and purity. About this time also, precision pulse photoconduction measurements started on zone-refined anthracene and similar compounds. As a result of this work a good deal is now known about the way the various molecular energy states are involved in anthracene photoconduction, the effects of adsorbed oxygen and so on. Although a number of patents have been taken out in the field, many referring to electrophotography and one to possible superconduction, a major practical achievement is still awaited. On the biological side there are now a good many suggestions as to the involvement of electronic conduction in a number of biological processes, from photosynthesis to bone growth, without any conclusive proofs so far. (A discussion on the biological properties is planned for Nottingham in April 1968.)

With so much initial activity complete, we must extend a particular welcome to the present volume, by two Australian authors well known in the field. The treatment is as comprehensive as possible, and covers all the available literature, to the end of 1965; I detected only one or two gaps, in the Russian and Chinese references. An initial chapter outlines the basic notion of solid state physics which underlies the field of activity. Then follows an account of experimental methods and sample preparation. A fourth chapter deals with "Band Theory in Molecular Crystals", a fifth with excited states, and a sixth with ionized states. Finally, the tunnelling and nopping models are considered, the published data reviewed, parallels drawn between semiconductivity and structure, and the effect of space charges outlined. ast chapter comprises "Retrospect, Outlook and Speculation", and summarizes the present state of the art.

The book is to be praised for the comprehensive and accurate fashion in which it summarizes experiments and liscusses theories. The account is well balanced as well as encyclopaedic in character. No attempt is made critically to evaluate and distinguish the different approaches. and it may be argued that it is still too early for this to e a fruitful process. So far as can be judged the tables of data are reliable at all points. This is a book for all who contemplate research in the field. The authors leserve praise for their great effort of compilation, and he result is strongly recommended. D. D. ELEY

CARBONATE ROCKS

Carbonate Rocks

Origin, Occurrence and Classification. Edited by George Chilingar, Harold J. Bissell and Rhodes W. Fairbridge. Developments in Sedimentology, 9A.) Pp. 471. (Amsteram, London and New York: Elsevier Publishing Com-

HIS volume, together with Volume 9B in this series, is esigned to provide an overall treatment of carbonates.

Almost half (177 pages) of the book is devoted to two papers under the joint authorship of G. M. Friedman and J. E. Sanders ("Origin and Occurrence of Limestones" and "Origin and Occurrence of Dolostones"). Together these provide a valuable survey of much recent work within the area of reference. Some information is drawn from the authors' own experience (published and unpublished), but reference to 741 papers, from European, American and other sources, some of which are comparatively inaccessible, is an indication of their familiarity with, and presentation of, a very large volume of literature.

The same cannot be said of sonle other contributions. Fewer works are referred to by Y. Gubier, J. P. Bertrand. L. Mattavelli, A. Rizzini and R. Passega ("Petrology and Petrography of Carbonate Rocks"); due acknowledgement is not given to the dominant American influences on contemporary thought (economic and

academic) in this area.

A different criticism might be levelled at H. J. Bissell and G. V. Chilingar ("Classification of Sedimentary Carbonate Rocks"). The authors display a commendable lack of partiality, but a more systematic approach, such as that in one of the recent reviews of carbonate classification which they themselves recommend, would have been desirable. The new system proposed is not a radical departure, and is unlikely to meet with wide support. The article is nevertheless necessary to the volume and the authors provide many references and pertinent comments.

Other contributions by W. H. Taft ("Modern Carbonate Sediments") and J. W. Harbaugh ("Carbonate Oil Reservoir Rocks") also provide useful summaries. Harbaugh emphasizes the increasing commercial importance of both the physical-petrological and biological-ecological approaches to reservoir evaluation, and the interdependence of such factors with the environmental conditions in which the rocks were deposited.

"Carbonate Rocks and Palaecelimatology in the Biochemical History of the Planet" (R. W. Fairbridge) is concerned with the wider implications of carbonate sediments and environments. The geochemical, custatic and tectonic controls of carbonate sediments make them an exceptionally interesting field for large scale geological speculation.

In some ways the book is a disappointment: chapters are not all of the same standard, plates are not always relevant, and the introduction reviews at too great a length the subsequent chapters, themselves reviews. The surveys (particularly those by Friedman and Sanders) and references contained in them are, however, likely to prove of continuing use to the specialist and to those seeking a more general background to the ever-growing literature. C. J. R. BRAITHWAITE

SUBJECTIVE TIME

Psychological Time in Health and Disease

By John Cohen. (A Monograph in American Lectures in Living Chemistry.) Pp. xv+103. (Springfield, Ill.: Charles C. Thomas, Publishers, 1967.)

THE relationship between psychological time and physical or clock time has been the subject of philosophical speculation for many centuries. The study was put on a more scientific footing by the early experimental psychologists. but the area has been curiously neglected for the past 50 years. Professor Cohen's monograph may thus serve as a useful introduction to the psychology of time perception, either for those who are interested to discover what kind of work has been done or for those who are contemplating carrying out work in the area.

There is mounting evidence that many biological rhythms are controlled by internal "clocks" or "pacemakers" and, in his first chapter, Cohen examines the likelihood that the clusive time sense in man is mediated by such a clock. It does seem in fact that short-term changes in temperature or in arousal can modify the clock's speed and thus our estimates of time. Other factors such as attention, personality-type and emotion also seem to be involved, but few of the studies cited in the present book have been successful in assessing their relative roles.

Further chapters examine perception of small units of time and the broader concepts of past and future. The dependence of temporal judgements or the spatial position of stimuli is well illustrated by Cohen's own experiments. A chapter on aberrations of psychological time deals with the effects on mental illness, hypnosis and drugs and there is a chapter on subjective time in myth and literature which, if it does not give many scientific insights, has at least some pleasing illustrations. In a final chapter, Cohen integrates previous suggestions with his own ideas into a speculative model of time perception.

The strength of the monograph is that it is clearly and entertainingly written and may stimulate readers to seek out the original papers. Its weakness is that it is just too short and superficial to rate as a useful contribution to the scientific literature. The title Psychological Time in Health and Disease sounds comprehensive enough, but the "disease" aspect is dealt with in only four or five pages. In a creditable attempt to produce a readable book it seems to me that Cohen has thrown scientific rigour out of the window. Certainly there is a lack of good experimental studies on time perception, but Cohen seems to prefer anecdotes to such laboratory evidence as there is. Thus in the section on drug effects, although a number of experiments have been reported, Cohen quotes De Quincey and Walter de la Mare.

In summary, this is a lucid and entertaining book, but while it may be rated an admirable attempt to bridge the two cultures it does not take us much further in our search for the time sense.

F. I. M. Craik

OBITUARIES

Professor C. F. Davidson

CHARLES FINDLAY DAVIDSON was born at Monifieth, Angus, on July 16, 1911. From Morgan Academy he entered University College, Dundee, as second bursar and graduated at St. Andrews University in 1933 with first class honours in geology and mineralogy. Even before graduation he had published or had in hand five papers on the geology of his countryside and on an olivine-bearing conglomerate from Raasay in the Inner Hebrides.

In 1934 he was appointed assistant curator in the Museum of Practical Geology, and was responsible for the general displays on the ground floor of the new building in South Kensington to which the collections were being transferred before its opening in 1935. Private and official research in the period up to 1939 resulted in publication of "The Tertiary Geology of Rasasy" (1935), "A Contribution to the Geology of the Faroes" (with F. Walker, 1936), "Geology in the Museum" (with F. J. North and W. E. Swinton, 1941), several short papers such as "The Geology of the Coronation Stone" (1937), and his main petrological work, "The Archaean Rocks of the Rodil District, South Harris" (1942–43), which earned him the D.Sc. degree of St. Andrews.

Refused permission in 1939 to go on military service, Davidson was made responsible for the safe-keeping of the museum against war incident, the building having become the headquarters of Civil Defence, London region. In 1940, however, he was largely absorbed in the preparation of confidential reports to military and naval

intelligence on the topographical and surface conditions, the geology and the mineral resources of overseas areas of actual or potential warlike activity. Some of these areas became well known later in the war: the Möhne Dam, Yugoslavia, the flying bomb sites of northern France. In 1941 he was required to prepare a précis of all available information on the uranium resources of the world, and increasing demand for more, and more precise information led to the establishment of a Geological Survey unit to work on the location, examination and preliminary assessment of radioactive ores in collaboration with the Ministry of Supply and the US Atomic Energy Commission. For this work Davidson travelled widely to remote places ranging from the Canadian arctic, by equatorial Congo and Rhodesia, South Africa, southern India, to Australia. Thus he gained the field experience, the mineralogical knowledge, and the basic technical knowledge of prospecting and preliminary development which enabled him to train staff and to organize the laboratory facilities necessary for rapid quantitative assessment of samples, for mineral identification, and for development of an entirely new branch of prospecting science. In this task he was fortunate to have the support and advice of his director, W. F. P. McLintock. result of his experiences was the publication in 1949 of The Prospector's Handbook to Radioactive Mineral Deposits, which by the end of 1950 had sold more than 10,000 copies. The field work which he undertook in 1945 jointly with a South African and an American geologist led to the discovery of the uranium resources of the Witwatersrand bankets, to a meeting with Dr Jan Smuts, Prime Minister of South Africa, and to the inauguration in October 1952 of the new South African industry for the extraction of uranium. Davidson was in the forefront of the exploration and assessment of radioactive deposits in the Rhodesian copperbelt, at Radium Hill, South Australia, Blind River, Canada, and Travancore, India, and in the study of the rare elements in the granites and carbonates of many regions in Africa. While carrying out these field investigations he also supervised the development of new techniques for mineral identification and, in collaboration with physicists of the Atomic Energy Research Establishment, for the evolution of electronic instruments for mobile reconnaissance, as well as for detailed individual surveys, more precise laboratory evaluations of uranium and thorium content, and for borehole logging. For such outstanding service he was awarded the O.B.E. in 1953.

Later in 1953 he was invited to become professor of geology and mineralogy in his own university and accepted gladly. In his inaugural address he reviewed progress in the study of ore deposits since the time of Agricola and revealed the intensity of his concern with the search for minerals in the service of mankind. The change to academic life necessarily curtailed opportunity for practical work but afforded leisure—though leisure is not word properly applicable to Davidson-for reading and cogitation, and he devoted much time to the study of current Russian books and research papers on mineralogy ore genesis, and geochemistry, contributing longer and shorter critical reviews of them in Economic Geology, for the benefit of English-speaking geologists. His wide ranging study of the literature of modern field and laboratory investigations and his own experience combined to produce original conceptions of the origin of the gold and uranium in the South African bankets, of th accumulation of strata-bound ores through the agency of ascending brines, and of the genesis and epoch of the formation of diamonds in kimberlites. These were conceptions which provoked sharp controversy. Davidson publications, under his own name and in collaboration with his colleagues of the Atomic Energy division of the Ged logical Survey, on the association of uranium with hydre carbons and with phosphorites; on the application c isotope ratios to age determinations particularly relating

to the Pre-Cambrian rocks and of Recent fossil bones. and on the mineralogy of natural radioactive substances, are too numerous to itemize. But it is important to mention his numerous popular expositions of radiogeology and the place of geology in human progress, for his facility of lucid presentation rivalled that of the old masters of geology. Davidson's capacity for organization, his ready acceptance of responsibility, and his complete indifference to his own well-being in the course of duty were well known to his colleagues. They became apparent to an international gathering in the success of the recent symposium which he organized in St. Andrews at extremely short notice on behalf of the International Association on the Genesis of Ore Deposits. On the evening of his death he had been busy with proofs of the 50 papers and the proceedings of the meeting. Few men can have lived so full a life and so greatly enjoyed the living of it.

Professor James P. Todd

Professor Todd, who died on November 10, was appointed lecturer in pharmacy in the Royal Technical College, Glasgow, in 1921. In 1937 he became the first professor of pharmacy in the United Kingdom, in the same college. From modest beginnings, his work so prospered that he leaves to his successors a great school of biological sciences with more than four hundred students, a large staff qualified in a wide range of scientific disciplines, and eight academic chairs all tracing their foundation directly or indirectly to his inspiration.

A native of Glasgow, James Todd attended Albert Road School and received his early training in the old Glasgow and West of Scotland School of Pharmacy. His military service in the Medical Corps during the First World War in South Africa, India and Iraq gave him an insight into some of the problems of tropical medicine. After the war he resumed his studies and was awarded the qualification of Ph.C. in 1921. In collaboration with the late Professor Ellis, he then helped to establish the first degree course in pharmacy in Britain under the relevant ordinances of Glasgow University. The first students were enrolled in 1924. In the meantime, and often in difficult conditions, he commenced his personal research into the properties of the digitalis drugs which later led to his award of the degree of Doctor of Philosophy by Glasgow University.

His subsequent research always concerned practical problems of medical science and covered a wide field. During the Second World War the Department of Health for Scotland sought his help in preparing pyrogen-free saline infusion fluids, and this led to his contributions in the field of bacterial pyrogens. His work on blood transfusion led to the establishment of the West of Scotland Blood Transfusion Service. For many years he was

honorary director of this service.

War problems also attracted him to study the treatment of burns (in collaboration with the Medical Research Council, then established at the Royal Infirmary). This work, in turn, brought him into contact with the problems of plastic surgery. The establishment of the chair of bioengineering at Strathclyde can be traced directly to these early contacts with Mr T. Gibson, the plastic surgeon, who first sought his advice in attempting to find means of bringing science and engineering to bear on the practical work of the operating theatre.

After the war Todd served on a working party set up by the Minister of Food to examine the provision of facilities for the training of young people in the study of the properties and processing of foodstuffs. British vulnerability to food shortage had been highlighted during the war, and after consultations with the experts in this field Todd concluded that this subject merited designation as a scientific discipline in its own right. He established an associateship course (at honours degree level) and described the field of study as "Food Science". This was

the first course of its kind in the United Kingdom, and is believed to be the first use of this term applied to a degree level course in the world. This course led to the establishment of the chair of food science at Strathelyde in 1958, and the term food science is now used internationally and on both sides of the Iron Curtain.

For a man who has been the focus of so much initiative in pharmacy, food science, microbiology, pharmacology, pharmaceutical technology, biology and biochemistry, Todd was no academic tycoon. Unassuming in manner, his kindly, simple and tolerant ways disguised a fixity of purpose which was only apparent to those who knew him well, or who had the misfortune to oppose his vision. In these days when it is often difficult to distinguish a professor from a business man, he was one of the last of the great race of university characters. Of course, his unorthodoxy bred opposition. Time and again the ranks would close against him, only to find themselves outflanked by his mental and administrative dexterity. If his opponents were sometimes a little bewildered as to how he achieved his ends, they learned to respect him by his success.

He devoted countless hours to the Pharmaceutical Society, served on expert committees, worked as an examiner and, above all, made behind-the-seenes contributions to the establishment of the University of Strathelyde. This must wait for later appraisal, but it is pleasant to record that in 1964 his services to medicine were recognized by his election as an Honorary Fellow of the Royal College of Physicians and Surgeons, Glasgow.

J. HAWTHOEN

Professor Douglas McKie

ONE of Britain's leading historians of science died on August 28, 1967. The son of a Scot, Douglas McKie was born on July 15, 1896, in Tredegar, Monmouthshire, where he was educated at the Grammar School. Intending to be a regular soldier, he entered Sandhurst and served as a lieutenant on the Western Front until he was severely wounded in 1917. He became a chemistry student at University College, London, in 1920, graduated in 1923, and in 1927 obtained his Ph.D. under F. G. Donnan for research on gas adsorption.

Already interested in military history, McKie soon acquired a taste for the history of science, and in 1925 he became a part-time assistant in the new Department of History and Philosophy of Science (as it is now called) at University College, London. A full-time appointment followed, and eventually, from 1957 to 1964, he was

professor and head of the department.

McKie will be chiefly remembered for his studies of seventeenth and eighteenth century theories of heat, combustion and related topics; of the men responsible for the advances in this field; and of the institutions to which they belonged. Only his principal works can be mentioned here. In 1935, he published The Discovery of Specific and Latent Heats (with N. H. de V. Heathcote) and Antoine Lavoisier, the Father of Modern Chemistry, which contains a detailed account of Lavoisier's chemical researches. There was then no British periodical devoted to the history of science; McKie was a founder of Annals of Science, and as its principal editor from 1936 until his death he exercised a great and beneficial influence on the subject. Early volumes contain his masterly series of "Historical Studies in the Phlogiston Theory" (with J. R. Partington) and the first part of his long study of Black's unpublished chemical lectures, completed only in 1967. He was also a foundation member of the Society for the Study of Alchemy and Early Chemistry; his account of the combustion theories of Boyle, Hooke and Mayow is in Volume 1 of its journal, Ambix.

During the 1939-45 war, McKie was attached to the Department of Chemistry of University College, but he found time to publish in 1942 a valuable account of

Newton's chemical theories. He wrote several papers about Priestley, but in later years he concentrated on the French and Scottish schools. All aspects of Lavoisier's eareer are considered in Antoine Lavoisier, Scientist, Economist, Social Reformer (1952), and McKie was largely responsible for negotiations leading to the preservation in France of Lavoisier's apparatus. For this and other services to the Académie des Sciences he was appointed Chevalier de la Légion d'Honneur. The Royal Society is also indebted to him for a detailed discussion of its origins, published in 1960. A lover of books, McKie had a great knowledge of the bibliography of science, and he wrote scholarly introductions to reprints of works by Rey, Boyle and Lavoisier, and to a finely produced transcript of a set of notes taken during Black's lectures.

McKie was one of the first full-time historians of science in any country, and he lived to see the subject attain full academic and professional status. He received many honours, but he probably derived most satisfaction from the knowledge that former students of his department are now teaching the history of science in universities, colleges and schools throughout Britain and also overseas.

W. A. SMEATON

University News:

London

DR E. H. Andrews, reader in science of materials at Queen Mary College, has been appointed to the chair of materials tenable at that college.

Appointments

PROFESSOR MICHAEL ABERCROMBIE has been appointed director of the Strangeways Research Laboratory as from July 1, 1970. He will succeed Dame Honor Fell.

The following appointments have been made to the World Meteorological Organization Secretariat: Dr Kaare Langlo, at present chief of the Technical Division, is to be director of the Scientific and Technical Department; Dr Hermann Sebastian, at present chief of the Technical Co-operation Division, is to be director of the Technical Co-operation Department; Dr G. G. Tarakanov, at present special adviser on scientific matters to the secretary-general, is to be special assistant to the secretary-general for technical policies and programmes; Mr B. Zavos, at present chief of the Planning and Co-ordination Group at the National Environmental Satellite Centre, Environmental Science Services Administration, Washington DC, is to be special assistant to the secretary-general for World Weather Watch Management and Co-ordination.

Announcements

The following have been made honorary fellows of the Royal Aeronautical Society: Sir George Dowty, chairman and managing director of the Dowty Group Ltd., in recognition of his services to aviation, especially in the design and development of aircraft accessory systems; Professor H. J. Van Der Maas, professor of aeronautics at the Technological University of Delft, in recognition of his contributions to aeronautics, especially in advancing international co-operation and goodwill; Dr Barnes Wallis, chief of the Department of Aeronautical Research and Development, BAC (Operating), Ltd., and special director, BAC Weybridge, for his contributions to aeronautical design over many years.

The first Louisa Gross Horwitz Prize for basic research in biology or biochemistry has been awarded by Columbia University to Dr Luis F. Leloir, at present director of the Institute of Biochemistry of the Campomar Foundation and head of the Department of Biochemistry in the University of Buenos Aires. The prize of \$25,000 was given in recognition of his discovery of the nucleoside diphosphate sugars and the demonstration of their important role in the biosynthesis of sucrose, the plant starches, liver glycogen and other polysaccharides.

CORRESPONDENCE

No More Coal

SIR,—The comments on the coal industry in your issue of November 18 (Nature, 216, 627; 1967) may give a misleading impression of the future of this industry and of the attitude of those responsible for its leadership. One chronic problem facing the NCB is that while we are in the middle of a productivity and rationalization drive—which we are winning—we are continuously subjected to press comments which can only damage confidence in the industry's prospects.

We spend about £5 million a year on our research and development programme. There are some extremely exciting projects, especially involving new uses for coal and the up-grading of by-products and waste materials. To succeed in these projects we need not only a steady flow of good scientists, but also the interest of scientists outside the industry who may be able to help us.

We submitted a memorandum on these aspects of our research and development programme, covering our utilization and diversification activities, to the House of Commons Select Committee on Science and Technology. I enclose a copy of this and would be glad to supply one

to anyone who cares to write to me.

Your heading "No More Coal" is, of course, belied by the figures included in the White Paper Fuel Policy (now being revised because of devaluation), and Mr Richard Marsh, the Minister of Power, has himself always been at great pains to emphasize what a large industry this will be even in the light of the White Paper forecasts. For example, between now and the end of 1980 at least 1,500 million tons of coal will be required and the turnover of the industry will total something like £10,000 million, with our increasing diversifications. But the NCB does not accept the inevitability of the decline forecast in the White Paper You say that the White Paper figures on nuclear power "might almost convince Lord Robens". The figures to which you refer, however, are merely a new set of calcula. tions, admittedly of greater sophistication, but they are not based on any more positive evidence about the presumed performance of the AGR nuclear power stations Now that the Select Committee Report has been published our attitudes and arguments are fully available and would justify the closest consideration by all scientists and engineers. It is most important to appreciate that our case is not by any means related merely to social considerations, important—and expensive—though these We believe that coal can still be economically competitive throughout the 1970s.

Arguments for this belief and the reasons why previous studies have in our view been inadequate are complex. In the Select Committee Report there are three contributions from the NCB which set out the position. The last of these—and indeed the last page in the Report—ends as follows: "Conclusions. In view of the technical and economic doubts about the future performance of AGE stations, the NCB think that the proper course is now for an independent technical expert to make a full assessmen of the nuclear power programme, supported by as independent for a significance of the stations.

independent financial assessment."

We should obviously not be asking for such an examination if we were not fully confident that our views would be sustained.

Yours faithfully,

L. GRAINGER
Member for Science,
National Coal Board.

National Coal Board, Hobart House, Grosvenor Place, London, SW1.

FORTHCOMING EVENTS

* (Meetings marked with an asterisk are open to the public)

Monday, December 11

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E1), at 4.30 p.m.—Professor R. L. Martin (University of Melbourne): "Some Recent Aspects of Inorganic Chemistry".*

INSTITUTION OF MECHANICAL ENGINERS, HYDRAULIC PLANT AND MACHINERY GROUP—FLUID MACHINERY SECTION (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Glandless

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, WC2), at 6.30 p.m.—Sir Stanley Brown: Presidential Address.

Monday, December 11-Saturday, December 16

UNIVERSITY OF CAMBRIDGE, SCHOOL OF CLINICAL RESEARCH AND POST-GRADUATE MEDICAL TEACHING (at Churchill College, Cambridge)—Introductory Course on "The Biology of Skin".

Tuesday, December 12

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 1.15 p.m.—Professor R. King: "Faraday's Contribution to Electrochemistry".*

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, NW1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 5.30 p.m.—Professor H. H. Rosenbrock: "The Gap Between Multivariable Theory and Practice".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London. SW1), at 6 p.m.—Mr W. A. Crago: "Problems Associated with the Use of Skirts on Hovercraft".

INSTITUTION OF ELECTRICAL ENGINEERS, JOINT I.E.E./I.E.R.E. COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, WCI), at 6 p.m.—Mr P. Cox, Mr V. J. McMillan and Mrs D. Crook: "The Rvaluation and Prediction of Equipment Reliability".

Wednesday, December 13

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W1), at 5 p.m.—Mr G. Vallance and Mr R. A. Fortey: "A New Ordovician Fauna from Spitsbergen" (Demonstration): Dr R. Casey: "The Type-Section of the Volgian Stage (Upper Jurassic)" (Demonstration): Professor A. W. Skempton: "The Consolidation of Clays by Gravitational Compackion" (Paper).

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1), at 5.15 p.m.—Mr A. R. Thatcher: "The Distribution of Earnings of Employees in Great Britain".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Breakdown of Liquids" opened by Professor T. J. Lewis.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr A. J. Haselfoot and Mr A. Armstrong: "Design Features of a 240 MVA 275/132 kV Site-Assembled Autotransformer".

SOCIETY OF ENVIRONMENTAL Engineers (in the Mechanical Engineering epartment, Imperial College, London, SW7), at 6 p.m.—Discussion meeting "Environmental Engineers or Witch Doctors" opened by Mr J. Salter nd Mr H. Goldberg.

INSTITUTE OF INFORMATION SCIENTISTS (at Knightway House, 20 Soho Square, London, W1), at 6.15 p.m.—Mr R. E. Fry: "The GLC Research and ntelligence Unit".

Society of Chemical Industry, Food Group—Nutrition Panel (at 4 Belgrave Square, London, SW1), at 6.15 p.m.—Dr E. Kodicer and Dr P. Iwood: "Absorption of Nutrients".

Wednesday, December 13-Thursday, December 14

BRITISH CERAMIC SOCIETY, BASIC SCIENCE SECTION; and the MINERA-GGICAL SOCIETY, CLAY MINERALS GROUP (at the Royal Aeronautical Society, Hamilton Place, London, W1), at 10 a.m. daily—Meeting on "Clays and ther Colloidal Systems in Ceramica".

Thursday, December 14

CHEMICAL SOCIETY (at the Royal Institution, 21 Albemarle Street, London, 71), at 5 p.m.—Professor C. A. Coulson, FRS: "Symmetry" (Faraday ecture).*

INSTITUTE OF FUEL (in Lecture Room 220, Imperial College, Exhibition oad, London, SW7), at 5.30 p.m.—Professor Sir Owen Saunders: Melchett

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 ew Cavendish Street, London, W1). at 5.30 p.m.,—Mr D. A. Hammett: "The overcraft as a Special Purpose Vehicle".

Institution of Electrical Engineers (at Savoy Place, London, WC2), 5.30 p.m.—Mr A. Stark: "Computer Methods for Electrostatic Field etermination and Ray Tracing in Image Intensifiers".

Institution of Mechanical Engineers, Nuclear Energy Group (at Birdcage Walk, Westminster, London, SW1), at 6 p.m.— Discussion Meetgon "The Choice of Blower Drives for Nuclear Reactors".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS ROUP (at 14 Belgrave Square, London, SW1), at 6 p.m.—Mr J. Weaver: ome Aspects of Materials for Concrete Roads".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, andon, WC1), at 7 p.m.—Mr G. R. A. Short: "Flavours and Colours in od and Pharmaey" (Harrison Memorial Lecture).

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W1), at 7.30 p.m.—Professor W. E. Kershaw, Dr D. J. Lewis, Dr T. R. Williams and Dr B. O. L. Duke: Symposium on "Onchocerciasis—Entomological Aspects". Chairman: Professor P. C. C. Garnham, CMG, FRS.

Thursday, December 14-Friday, December 15

BIOCHEMICAL SOCIETY: and the SOCIETY FOR GENERAL MICROBIOLOGY (at the University of Leicester)—Symposium on "Control of Gene Function in Micro-organisms"; Ordinary Meeting for the presentation of communications; Demonstration Meeting on "Anaerobic Techniques and Multiple Inoculation Methods" and a Symposium on "The Teaching of Microbiology in Schools".

Friday, December 15

BRITISH INSTITUTE OF RADIOLOGY (at 32 Welbeck Street, London, W1), at 2.30 p.m.—Meeting on "Malignant Disease in Children".

INSTITUTION OF ELECTRICAL ENGINEERS, JOINT I.E.E./I.E.R.B. MEDICAL AND BIOLOGICAL ELECTRONICS GROUP, at the Institution of Electrical Engineers, Savoy Place, London. WC2), at 2.30 pm.—Discussion Meeting on "Applications of Thin Films and Integrated Circuits in Medical and Biological Instrumentation".

Saturday, December 16

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3.80 p.m.—Dr Peter Ucko: "Art, Magic and Religion in the Old Stone Age".*

Monday, December 18

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Cabling for Difficult Industrial Environments" opened by Mr G. L. Leighton, Mr D. Balk, Mr D. J. Mills and Mr T. P. P. Balk T. B. Rolls

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Recent Developments of Inductive Ratio Standards", opened by Mr J. J. Hill.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the

APPLICATIONS are invited for the following appointments on or better dates mentioned:

LECTURER (with special interests in ecology including population ecology, or ecological methodology or physiological ecology) in Botany at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mail, London, SWI (New Zealand and London, December 15).

LECTURERS/ASSISTANT LECTURERS (2) in SOCIOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devongation (December 18).

shire (December 18).

shire (December 18).

SENIOR LECTURER or LECTURER (with a science degree specializing in geology, considerable research experience, and preferably with a higher degree) in GEOLOGY at the University of Queensland, Australia. The Association of Commonwealth Universities (Branch Office), Martheorough House, Pall Mall, Loudon, SW1 (London and Brisbaue, December 20).

LECTURERS (2) in the DEPARTMENT OF CHEMICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (December 23).

Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (December 23).

Lecturer of Assistant Lecturer in Sociology—The Secretary of the University Court, The University, Glasgow (December 30).

Lecturer (with research interests in cell biology, especially its more molecular aspects) in the Department of Biophysics, School of Biological Sciences—The Registrar, King's College (University of London), Strand, London, WC2 (December 31).

Scientific Officer of Senior Scientific Officer (with a good homours degree in mathematics with some training in statistics, or a degree in statistics, and preferably an interest in computer programming)—The Secretary, ARC Institute for Research on Animal Diseases, Compton, near Newbury, Berkshire (December 31).

Glassblower in the School of Physical Sciences—The Registers, The New University of Ulster, Coleraine, Northern Ireland (January 1).

Research Fellow (organic chemist, with a Ph.D. or equivalent research experience) in the Department of Medical Research, Institute of Advanced Studies, Australian National University, to work on the synthesis and reactions of nitrogen heterocycles—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SWI (Australia and London, January 1).

Senior Lecturer of Lecturer in the Department of History and Lecturer in the

Office), Marlborough House, Pall Mall, London, SW1 (Australia and Longou, January 1).

Senior Lecturer of Lecturer in the Department of History and Philosophy of Science (to be Head of the Department)—The Secretary. The University, Aberdeen (January 13).

Lecturers/Senior Lecturers (with a good university degree in mining engineering and experience in metalliferous mining of rock mechanics) in Mining Engineering, University of Queensland—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (London and Brisbane, January 15).

Lecturer (preferably with an interest and experience in thermodynamics applied to high temperature reactions) in Chemical Thermodynamics—The Staff Officer (598/6), The University of Aston in Birmingham, Gosta Green, Birmingham, 4 (January 16).

UNIVERSITY LECTURERS and/or UNIVERSITY ASSISTANT LECTURES in PURE MATHEMATICS—The Secretary of the Appointments Committee of the Faculty of Mathematics, The University, Silver Street, Cambridge (January 16).

Faculty of Mathematics, The University, Silver Street, Cambridge (January 16).

I.ECTURER or ASSISTANT LECTURER in Physical Chemistry—The Registrar and Secretary, The University, Leeds, 2, quoting Ref. 68/24/1 (January 31).

LECTURER/SENIOR LECTURER (preferably with research experience or special interest in (a) plant physiology; (h) physiological ecology; and/or (c) cytology and histochemistry) in BOTANY at Massey University. Palemerston North, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pali Mall, London, SW1, or the Registrar of the University (New Zealand and London, January 31).

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (with a degree or equivalent in a suitable subject, and training and experience in botany, agricultural botany or agricultural chemistry) for laboratory and glasshouse research to assess potentialities of new herbicides—The Secretary, ARC Weed Research Organization, Begbroke Hill, Kidlington, Oxford, quoting Ref. 17/67.

LECTURER/SENIOR LECTURER (graduate in metallurgy or physics) in PRYSICAL METALLURGY to give lecturer courses and pursue research in one or more of the following subjects: metal physics, modern physical metallurgy, crystallography—The Secretary, Sir John Cass College, Jewry Street, London, EC3.

lurgy, crystallography—The Secretary, Sir John Cass College, Jewry Street, Jondon, BC3.

Physicist or Engineer for research in high temperature plasmas and on supersonic combustion waves; work will involve advanced electronics and modern high speed optical recording—Professor A. R. Ubbelohde, FRS, Head of the Department of Chemical Engineering and Chemical Technology, Imperial College, London, SW7.

RESEARCH ASSISTANT (with a good honours degree in zoology and an interest in marine ecology) in Zoology—The Academic Registrar, Hatfield College of Technology, Hatfield, Hertfordshire.

TECHNICAL ASSISTANT (preferably with experience in chromatographic techniques) for RESEARCH INTO BILE SALT CHEMISTRY AND METABOLISM—Professor G. A. D. Haslewood, Blochemistry Department, Guy's Hospital Medical School, London Bridge, SE1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Great Britain and Ireland

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National Hydatids Council. Seventh Annual Report and Statement of Accounts year ended 31st March, 1967. Pp. 24. (Wellington: National Hydatids Council, 1967.)

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NATURE Volume 216 DECEMBER 16, 1967

Slaughter or Vaccination?

THE most severe outbreak of foot and mouth disease ever to occur in Britain is at last being brought under some semblance of control. The Minister of Agriculture said in the early stages of the epidemic in October that the traditional slaughter policy—the slaughter of all livestock on infected farms and the prohibition of movement of livestock in infected areas—must be made to work. It seems as if it has. But in the process, three hundred thousand animals have been killedalmost one in every two hundred farms. The cost has been so great that the whole slaughter policy should be rigorously re-examined in the post-mortem that is bound to follow the final eradication of this epidemic. The British Government and the farmers must ask themselves if it is still realistic to try to fend off the disease by slaughtering and if it is not, what should be done instead.

Foot and mouth disease is not endemic in Britain. All infection comes from abroad. Obviously the best way to keep the country free from this disease would be to cut off the sources of infection. The source of the latest outbreak has yet to be identified, but the Gowers report in 1954 made it quite clear that contaminated meat imported from countries where the disease is endemic has been the chief source of infection. Migratory birds and tourists are blameless. Even so, successive British governments since 1954 have chosen to chance their luck and run the risk of foot and mouth disease rather than to ban meat imports from infected countries and suffer the commercial consequences that would follow. The decision which the Minister of Agriculture made last week to temporarily ban further imports of meat from South America and South Africa, which now has its own outbreak of foot and mouth disease, was a splendid illustration of how to shut the stable door after the horse has bolted. It certainly provoked an immediate reprisal from Argentina, which declined to buy racehorses at the Newmarket sales.

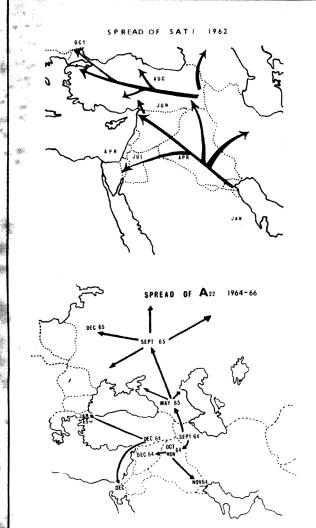
It is of course just possible that the British Government was being more subtle than usual. Is the temporary ban quietly going to become permanent? It is certainly difficult to believe the official reason for the ban, that veterinary resources could not stand up to another primary outbreak. Perhaps the government hopes that, once everybody is used to a temporary ban, this can be made permanent with the minimum of reprisals and this may be quite sensible. Even if

the latest outbreak did not originate in contaminated meat, it has shown just what havoc foot and mouth disease can cause and the folly of neglecting any measure which can reduce the risk of infection. To insist on a slaughter policy and do nothing to stop one of the sources of infection is to court disaster.

If it is decided that Britain simply cannot afford to lose cheap imported meat and perhaps export markets as well, better precautions must be taken to inspect imported meat. The existing arrangements are totally inadequate. There is also much to be said for increasing the penalties for the misuse of swill in the feeding of animals. Food waste like this often contains scraps of imported meat which should strictly be sterilized by boiling for at least an hour. The National Farmers' Union admits that some farmers may still feed unsterilized swill to pigs. If the penalties were increased, they would no doubt be more inclined to obey the law.

This week the National Farmers' Union began talks with the government about foot and mouth disease. Certainly if the slaughter policy is maintained, some form of compulsory national insurance to cover the consequential cost of foot and mouth disease, the loss of income which in the present outbreak the farmers may suffer for several months, should be considered. At present individual farmers can insure against the disease but few do so because of the high premiums and the fact that, in the past at least, it has seemed unlikely that a major outbreak would occur.

But the real question that has to be answered is whether or not Britain should use vaccination against foot and mouth disease. The current techniques of vaccination are admittedly not entirely satisfactory. This is hardly surprising. Countries such as the United States, Great Britain, Australia and New Zealand, which might be expected to finance large scale research into foot and mouth disease, use the slaughter policy. Countries where the disease is endemic are often less fully developed and lack the resources to devote to research. As it is, the Agricultural Research Council's Animal Virus Research Institute at Pirbright, with an annual budget of only about £0.5 m, has won a high international reputation for research into foot and mouth disease. The vaccines and vaccination techniques it has developed are used throughout the world except—ironically enough—in Britain, where the use and even the production of vaccine is illegal except



These two maps show the rapid spread of two sub-types of foot and mouth disease virus, SAT1 and A_{22} , in 1962 and 1964-66 respectively. In both epidemics the spread of the virus into Western Europe was prevented by vaccinating cattle at the Greek, Turkish and Bulgarian frontier region. A_{22} is, however, at the moment causing outbreaks of the disease in the Soviet Union and presents a great threat to the herds of Western European countries which, although vaccinated against the sub-type A_{3} , are susceptible to infection by A_{22} (ARC Animal Research Institute, Pirbright).

at Pirbright. British farmers are not free to import vaccine and use it, even if they are prepared to have their animals slaughtered. Vaccination is prohibited because, apart from the cost, there is the widespread belief, officially recognized by the Gowers report in 1954, that once vaccination is used the disease will become endemic.

But is this really so? It may have been the case in 1954, but since then better vaccines have been developed and workers at the Pirbright Institute, who should know more about foot and mouth diseases than most other people, believe that, given proper control, vaccination could be used without the disease becoming endemic. Dr Brooksby, the director of the Institute at Pirbright, for example, said last week that he did not believe foot and mouth disease was endemic in

France where vaccination is used. If the current epidemic had spread still further, vaccination would no doubt have been used to cordon off infected regions. This, after all, is why the government authorized the importation of five million doses of vaccine, costing £300,000.

A part of the argument against the use of vaccines is that animals carrying the disease would then be concealed in British herds. But carrier animals which have the disease but do not show clinical symptoms may appear in unvaccinated herds as well as in those which have been vaccinated. So the existence of carriers is really no argument against vaccination. Admittedly it is impossible to give pigs immunity by vaccination for any length of time with existing vaccines, but there is no reason why this should not be done if more effort and resources were put into research. Experiments in West Germany may well provide a means of doing this, but, even if they do not, there is no excuse for the belief that solutions are unattainable.

The argument that it is cheaper to slaughter all animals on infected farms than to vaccinate the national herd is true only so long as an outbreak is brought quickly under control. The Ministry of Agriculture claims that to vaccinate all cloven-hoofed animals in Britain would cost between £26-£50 million a year. This is likely to be an overestimate. £14 million has already been paid in compensation for livestock killed in this epidemic. But nobody, government or the farmers, has the slightest idea how much the so-called consequential costs have been. They are likely, in the long run, to exceed the amount paid in compensation. It is difficult to understand how the government can be certain vaccination is more expensive than the present policy when nobody knows just how much the real costs of the slaughter policy are. Some attempts should be made to devise a costing system to assess the cost of the slaughter policy in outbreaks of varying degrees of severity.

The most obvious losers and therefore the firmest opponents of vaccination are the breeders of pedigree livestock. They fear that, once vaccination is started, traditional markets in North America and Australasia will be lost. Breeding and exporting pedigree livestock seems to be involved with national pride, but the value of these exports is relatively trivial, running to between £2 million and £5 million a year and including a good deal of business with countries where foot and mouth diseases are endemic or where vaccination is used. But this is a seller's market, and countries which need British pedigree livestock to maintain the quality of their natural herds would quickly learn to accept vaccinated cattle. Even the spectre of the occasional carrier could be eliminated by the simple expedient of the Proband test.

In the circumstances, it is plain that the case against vaccination needs urgently to be re-examined. It is by no means as strong as received doctrine would suggest. The question of whether the national herd should be vaccinated is not the only one to ask. It is

also important to develop some feeling for the strategy of how vaccination could be used to contain outbreaks of foot and mouth disease. Much will depend on the improvement of vaccines and of techniques which the years ahead are certain to bring. Already there is a vaccine against three strains of the virus which costs no more than the univalent preparations available a decade ago. The authorities should seek more deliberately than in the past to exploit the new opportunities which are arising. There is also a strong case for believing that progress would be faster than it is if research were prosecuted more urgently. The epidemiology of foot and mouth disease cries out for more serious attention than it has so far received, and this is only one of the directions in which efforts should be encouraged.

There is also a strong case for a better co-ordination of national policies towards foot and mouth disease, especially in tightly knit and intensively farmed regions such as Europe, for foot and mouth virus has no respect for national frontiers. Above all, it is necessary somehow to throw off catatonic acceptance that foot and mouth disease can only be countered by slaughtering. There is too much similarity for comfort between the funeral pyres on British farms in recent weeks and the sacrificial flames with which the ancients sought to keep away evil spirits.

Hemispheric Queue

THERE is rough justice in the fact that British people wanting to emigrate to America in the next three years will find themselves at the end of the queue. What Congress intended when it liberalized the American immigration laws in 1965 was to end the system of choosing aliens on a basis of their country of origin, northern Europeans preferred, that had given offence around the world since 1921. The new law says simply, in President Johnson's words, "that those wishing to emigrate to America shall be admitted on the basis of their skills and their close relationship to those already here". The old British quota-65,361 a vear—the largest allotted to any country—was based on the fact that the British were the original settlers whose descendants formed the dominant ethnic group in isolationist post-world War I America. Not only did this generous quota mean that the British could emigrate to America on the spur of the moment, but also that about 35,000 visas a year were left unused.

But good intentions can backfire. The first big shock came when Congress realized that its new immigration law was a positive invitation to skilled and professional people from underdeveloped countries to join the brain drain. In the transition period between October 1965 when the bill was passed and next July, when it comes into full effect, unused portions of national quotas were transferred to countries with long waiting lists. Immediately the flow of loctors, scientists and engineers from Asia, Africa and South America jumped dramatically (the number of mmigrant visas given to Asian professionals nearly ripled between 1965 and 1966).

Now July 1968 is approaching; that is the date on which national quotas expire completely and Britain

loses its special privilege of easy entry for ever. Stiddenly people have taken a look at the waiting ist which is accumulating in the State Department files in Washington and have discovered that the category of so-called "third preference" immigrants (processional, as distinct from technically skilled) is filled for the next three years. And there are few, if any. British, German, Irish or Scandinavian names among the 48,000 on the list.

Mr William Douglas of Careers Incorporated thinks that the new restrictions could put such recruiters for American industry as himself out of business. Dr F. E. Jones of the Working Group on Migration prefers to wait and see. So does the consular section of the American Embassy in London. The law, like env other, has its loopholes. It is certainly possible to go to work in America without getting an immigrant visa: there are various forms of permits allowing for exchanges, training programmes and special exceptions in the national interest. At this point it would be rush to say that American universities or industries could not find a visa for the British apple of their eye. What it is safe to promise, however, is that idle daydreams of "perhaps I'll emigrate" will have to stop. Profes sional men will have to take a firm decision well in advance to go to America or sit back and wait for the exceptional job offer from the company that is willing and able (no one is sure if and how this will be done) to pull strings to get round the new law.

As it stands, the Immigration Act allows 17.000 visas a year for emigrants in the professional class from the eastern hemisphere. (Canadians and Latin Americans are allowed in more freely.) Visas will be awarded on a first-come, first-served basis, with members of each preference class competing with equality for the limited number of places, regardless of their courtry of origin. The actual numbers of professionals taken may be smaller still, as wives and children will be given visas from this list. There is, moreover, a ceiling of 20,000 on emigration of all kinds, other than

relatives, from any one country.

The American Embassy's advice to any intending British emigrants is to apply before the end of next June. After that, really desperate measures might be necessary: emigrating to Canada first, or marrying an American. Helping Britain to stem its brain drain was the last thing Congress had in mind when it tackled this controversial problem in 1965. Yet Bithought might be consolation to any Britons who are unable to move across the Atlantic in the next few years. They might also be glad to be relieved of the Anglo-Saxon's guilt of lolling in and out of American will while countless southern Europeans and Orientals waited outside in vain.

Conceivably Congress could amend the law. Some American companies—those along Route 128 near Boston, for example—are worried about losing their British recruits. And some congressmen are worried about the unexpectedly weak demands for visas in the close-relative categories. The House of Representatives Judiciary Committee may soon hold hearings about the problem. But if any amendment very to be made, priority would probably be given to stomming the drain from underdeveloped countries and attempt then to bend the law to allow more British in would smack of the old favouritism, quite apart from the outcry it would start in this country.

Nuclear Merger

In contrast with the United States and West Germany, Britain has not been a successful exporter of nuclear instruments and equipment. The Parliamentary Select Committee on Science and Technology has explained the poor export performance of British nucleonic firms by the fact that most of them were small, and centred scientifically as well as geographically about Atomic Energy Authority establishments, so that some of them have no customers apart from the authority, and many have no research departments.

The recent merger of three of the largest nucleonics firms, Nuclear Enterprises (GB), and the nucleonics subsidiaries of EMI and Elliott-Automation, so as to create a £2m company, is an important event for the industry. The Industrial Reorganization Corporation supplied Nuclear Enterprises with £600,000 for the take over, all the more willingly, no doubt, because Elliott-Automation had been negotiating the sale of its subsidiary with the American company, Teledyne. The factories of the enlarged Nuclear Enterprises will be at Edinburgh and near Aldermaston, the EMI staff moving from their present sites at Hayes and Wells.

Nuclear Enterprises itself concentrated on the manufacture of instruments such as radiation detectors, gamma-ray cameras, and automatic radioactive sample changers used in research in hospitals, universities and industry. To this range will now be added the health physics equipment, notably hand and foot monitors, and data processing machinery produced by EMI, as well as the extensive contribution of industrial analytic and gauging equipment from Elliott-Automation. Most important, the new Nuclear Enterprises will be able to offer two British made multi-channel analysers, where it previously only acted as import agent for Italian machines.

It is claimed that the new firm will be the biggest of its kind in Europe, but, as so often in the industry of advanced technology, this only means that the company compares with a medium-sized American firm. Nuclear Chicago, to give an indication of American scale, sells \$23m worth of instruments each year, exporting 20 per cent of its output—more than \$750,000—to Britain alone; the top six British firms have a combined turnover of about three million pounds.

The amalgamation of three of Britain's biggest manufacturers of nucleonics may encourage similar association among some of the other three or four bigger firms and subsidiaries. But if and when the big can be persuaded to become bigger, there will still be the problem of how to encourage export and research activity among the forty or so smaller nucleonic firms within the satrapies of the various AEA establishments.

Trade in Technology

WITHIN the next month or so, the Ministry of Technology is hoping to sign a new technological agreement with the Soviet Union. Mr Anthony Wedgwood Benn, the minister, is particularly keen about the agreement. "It will pave the way for closer contact between Britain and the Soviet Union in the future. Each has a great deal to gain from an exchange of information in the fields of forward research, industrial technology and

planning techniques." But Mr Benn thinks that the greatest gains will come from the development of international systems for the exchange of scientific and technological information by means of a world information retrieval and dissemination system.

Similar agreements have already been signed by the United Kingdom with Poland and Hungary and, if they are anything to go by, the Anglo-Soviet agreement is likely to be a pretty innocuous document. But this need not matter very much. The first reaction from industrialists was that with agreements of this sort, what they say is often less important than the cordial relations they foster. The Confederation of British Industry is enthusiastic about the agreement. "The important thing is not the pious statements of intent, but the fact that there is a determination to make the agreements work," one CBI official said. The CBI sent a mission to the Soviet Union recently, and has been surprised how quickly the benefits have been showing themselves. It has also been surprised at the wide range of projects on which the Russians were eager to collaborate—instead of searching around for points of contact, the mission found it was easier to add up the number of things in which the Russians were not interested. Eight particular areas of interest emerged from the CBI mission-electricity supply and generation, heavy generating plant, the motor industry, the metallurgical industry, industrial pollution, patents, the railway system with particular emphasis on containers, and standards. Some of these subjects may be mentioned in the agreement when it is signed; other possible subjects are coal mining machinery, computers and electronics, and chemicals and chemical plants. It is also thought that the Russians may be interested in civil aviation technology. This is a field where Russian designs would be unlikely to sell on a world market, even if there were no political barriers. For one thing, the period between engine overhauls in Russian civil aircraft has tended to be much too short for airline economics.

Some of the bigger British firms already have a foot in the door. Imperial Chemical Industries, for instance, has negotiated its own agreement with Russia, after Sir Paul Chambers met Mr A. Kosygin in 1964. The agreement, signed in October 1966, provides for collaboration in plastics, petrochemicals and synthetic fibres. And British Motor Holdings, while admitting that the new agreement would "obviously be very useful", says that it has already submitted plans for a complete car plant to build two BMC models, the 1100 and 1800, in the Soviet Union. A team of BMC engineers will be going to Russia next month to hold discussions, principally about engines, automatic transmissions and suspension systems. Leyland and Courtaulds are two other British companies which have shown interest in the Russian market, and Leyland has put forward plans for a complete factory to build commercial vehicles in Russia. English Electric has sold a number of computers to Eastern Europe, most recently a System 4 computer to Russia.

But what of Mr Benn's ambitious schemes for an international data storage and retrieval system? So far the Soviet Union has shown interest in only one such proposal, put forward by the Internationa Atomic Energy Agency. This is a development of the scheme now in operation for sharing nuclear cross sections internationally, and would provide an internationally.

national nuclear information service based on Vienna. The proposal has been widely discussed over the past few years, but has not yet been finally agreed. The Soviet Union does have its own information retrieval system, called Viniti, and has done some fundamental studies of language and mechanization. But it is not clear exactly what stage the Russian system has reached. Mr John Grey, of the British Office of Scientific and Technical Information, says that to produce an effective international system it would be necessary to produce standard formats and procedures, as well as compatible indexing systems and programmes. With the third generation of computers, he hopes that this will be very much easier, but he stresses that all systems are still experimental and that the next two or three years are likely to be of crucial international collaboration. "There can't be too much discussion on it," he said.

Culham Divided

It now seems probable that the Science Research Council will be taking over responsibility for at least part of Culham Laboratory. Dr Robert Wilson, who is director of the group at Culham working on astrophysics, says that "agreement has been reached in principle" for the phased take-over of his group by the SRC. But he emphasizes that consultations are by no means complete, and there are a number of questions connected with the staff which still have to be settled. If the transfer can be made, it will do something to restore optimism at Culham—since the Minister of Technology, Mr Anthony Wedgwood Benn, announced in July the decision to cut the laboratory's expenditure by 10 per cent a year for five years, there has been something of an air of gloom. Because the astrophysical work was supported from the fusion budget, it was as vulnerable as any other project at the laboratory.

If the discussions go well, the astrophysical group will be converted to an SRC unit based at Culham. This process should be complete by April 1969, and the group will remain at Culham at least until 1971. The group consists of 18 professionals and 10 others, and the annual budget has been running at something like £0.25 million a year. (This is not a hard and fast figure, because it includes overheads and some laboratory plasma work which the SRC may not take over. The exact financial commitment of the SRC will not be clear until the negotiations are complete.) As well as studying the solar spectrum with stabilized Skylark cockets, the group has been responsible for the measurement of new spectral lines and of atomic cross-sections. t now hopes to move on to stellar spectroscopy, with rockets stabilized on stars.

So far, nothing has been decided about the team which is working on the design of a large astronomical elescope for ESRO. This is a different team, consisting of 25–30 professionals working part time. As yet, the lecision is not urgent, because ESRO has not decided whether to proceed with the project. If the LAS project does proceed, Dr Wilson says, the position of he team working on the design will have to be conidered.

There is little doubt that the move to the SRC will nake for better co-ordination of the British space esearch programme, but there is no particular reason to believe that money will then be easier to come by. The programme will have to find its own level within the competitive atmosphere of the SRC. As a number of commentators—Nature among them—have suggested, a move to the SRC might be the answer for the whole of the Culham establishment. But there has so far been no hint of that.

Facing the Future with SI

A conference of editors of British scientific journals adopted on December 11 a recommendation that editors should collectively encourage the use in scientific journals of the system of metric units known as SI (which is an abbreviation for Système International d'Unités). A document prepared during the past six months by a working party under Professor James Lighthill has produced a detailed list of definitions and suggestions including, in particular, the view that "the journals devoted to science and engineering should seize the opportunity of playing a crucial role in helping to end the confusion and wastefulness (both mental and material) resulting from the present multiplicity of units". This decision has been given the encouragement and the blessing of the Ministry of Technology, which is now hoping for more or less complete metrification of the British system of weights and measures by the mid-seventies.

The basic units of the SI system are the metre (m), kilogramme (kg), second (s), ampere (A), degree Kelvin (°K) and candela (cd). Allowable derivatives include the Joule (J) but not the calorie, the weber (Wb) but not the Gauss, the hertz (Hz) but not the cycle per second and the degree Celsius (°C) but not the degree Centigrade (which is said to be one of the points on which French delegates to the international conferences have placed a great deal of emphasis). One of the characteristics of the SI system is that fractions and multiples of units should wherever possible be quoted to the nearest integral or fractional multiple of a thousand, which means that the Angstrom (10-10 m) is banned and even the centimetre (10-2) is frowned Needless to say, the foot, the pound, the gallon and the Btu have nothing said in their defence.

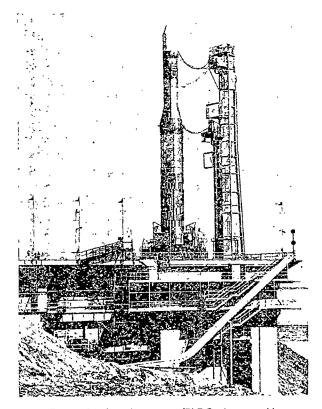
By all accounts, a great many British journals have already agreed to regard 1968 as a period of experiment and of transition. Although the document produced by the working party (see *Nature* next week for further details) seems to have obtained a sympathetic reception, it remains to be seen how many journals will follow Professor Lighthill's suggestion that they should, after a suitable warning period, automatically return manuscripts in units other than SI.

ELDO Fails Again

from Angela Croome

Although the political crises for co-operative European space enterprises took place in 1966, the technological failures which give a gloss of justification to the political wrangling have occurred this year. There were the pre-launch troubles with the first ESRO (European Space Research Organization) satellite in the spring which have made necessary a year's postponement in the launching of the backup vehicle and considerable extra cost. Now the two ELDO shots of the

year, designated Flight 6/1 and 6/2, have both failed. The second of these flight tests from Woomera took place last week on December 5. ELDO, the European Launcher Development Organization, is concerned with space tools and not research. The two launches were stages in the development of a large carrier rocket based on the British Blue Streak for European purposes.



Europa I in launch position (ELDO photograph)

The failure of the second launch has marked similarities with that of F6/1 on September 2. In both cases the programme sequencer was implicated. This is mounted on the French second stage and both its manufacture and its checkout are French responsibilities. The cause of last week's vehicle failure was very simple: the programme-sequencer did not start at take-off and did not work at all during the few minutes' flight. Consequently the second stage (Coralie) did not separate and because it did not separate it did not fire. The second stage electronics failure on September's F6/1 test was more complex. Then the sequencer malfunctioned briefly—possibly for as short a time as 200 milliseconds—during the first/second stage separation phase as Blue Streak boost ended. The consequence was that although the second stage separated it did not fire and a few seconds later was automatically destroyed. On both the F6 flights the Blue Streak booster stage functioned "normally", as it has done on the preceding five flight tests.

Apart from giving the first "live" test to the second stage, the objective of F6/2 was an operational run of the complete ground network for control and tracking. This was partially achieved. The upper parts of both this year's flight vehicles, representing the German

third stage and the Italian test satellite, were dummie though of correct weight and aerodynamic characteristics. Despite the setbacks with the second stage there is apparently to be no change in the firing schedul for which 12 flight tests of the Europa I configuration extending through to 1970 are laid down. The nex flight (F7), due next summer, will have all three rocke stages live and will be the first potential orbital flighthough not carrying a fully instrumented satellite Clearly the French will have to do some intensive homework to avoid frustrating this trial and it successors. The F6 booster has already completed it tethered test firings at Spadeadam (Cumberland) and is being made ready for shipment from Hawker Sidde ley's Hatfield works.

The still distant F11 and F12 orbital tests may carr components of the perigee/apogee system (PAS)—th key element in converting Europa I into Europa IIor a low polar orbit into the high equatorial typ favoured for communications satellites. France head the list of intending buyers of the Europa II launche on which she is dependent for her ambitious plans o direct French communications to link metropolitax France with French-speaking territories oversea (SAFRAN and SAROS). Enhanced reality was giverto the PAS project last week by the formation oan industrial management company representing th 11 European companies involved in its developmen-Called SETIS (for Société Européenn for ELDO. pour l'Etude et l'Intégration des Systèmes Spaciaux with a full-time technical staff drawn from these firms a French president and a Paris headquarters, its chie task will be the coordination and integration of work on PAS. The firms involved are: Hawker Siddeley Rolls Royce (Britain); SEREB (France); Bölkow Erno (Germany); C.I.A. (Italy); A.C.E.C., M.B.L.E and B.T.M.C. (Belgium); Philips and Fokker o Holland.

No Joy for Euratom

EURATOM struggles from crisis to crisis. The lates misfortune to assail the organization is a drastic budge cut, enforced last Friday at the Council of Ministers in Brussels. Although the Euratom commission has proposed a budget of \$82 million, the ministers cut this down to \$40.695 million after discussions which lasted all day. Even the commission's proposal was modest in comparison with earlier years, when the budget has run at about \$90 million.

The budget cuts are only the visible symptoms of ε malaise which now threatens the whole future of the organization. As is reported elsewhere in this issue, the Dragon reactor project, by general agreement a success ful example of co-operation, is threatened by the in ability of Euratom to determine its research programme for 1968. This kind of project, in which Euraton contributes but is not wholly in control, seems to have been a main topic for argument when the ministers men last Friday. All that could be agreed was that these projects would be further considered in the next fewmonths, but no provision was made for them for 1968 At the meeting, France and Italy were both attempting to cut back the budget, while West Germany and Holland supported the commission and Belgium and Luxembourg remained neutral. With the refusal to sanction work on association projects, the only work

that can go ahead in 1968 is the work in Euratom's own laboratories.

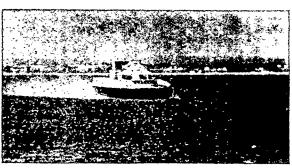
The French view of the association contracts is that, if the work is worth doing, the money will eventually be forthcoming, but that the contracts must be left in suspension until the new arrangements for co-operation can be worked out. The French argue that it is no longer sensible to produce a grand plan detailing all that needs to be done; instead, each individual decision should be agreed by the Euratom members. This formula, the argument goes, need not be applied rigidly, and if any member of Euratom wanted to opt out of any particular project it could do so, leaving five, four or even three countries collaborating. With this as a basis, it would be impossible for any single country to veto a project which the others wanted to support.

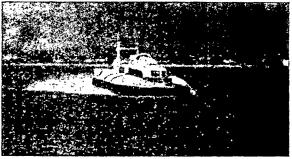
Developing Hovercraft

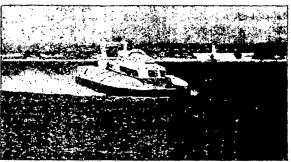
WHEN the first hovercraft flew nearly nine years ago, it did so without the aid of flexible skirts. The air cushion beneath the craft was kept in place by jets of air around the periphery, which produced a hover height of ten inches. But, as Mr W. C. Crago, Chief Research Engineer for the British Hovercraft Corporation, explained to the Institution of Mechanical Engineers on December 12, this was an unsatisfactory solution. It used far too much power, so much that, without enormous power units, hovercraft designed then would have had a clearance of only about 1 ft., not enough to clear even quite small obstacles. The solution was the invention of the flexible skirt—which offered increases in hard structure clearance by as much as eight times. But the arrival of skirts brought further development problems, and these formed the substance of Mr Crago's talk.

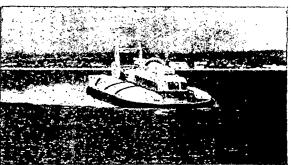
The modern hovercraft skirt, Mr Crago explained, is a sophisticated inflated structure, consisting of an upper bag portion which is more or less self supporting and acts as a shock absorber in heavy waves—and a series of discrete fingers which hang from it. One of the critical parameters is the pressure inside the skirt. There are two opposing demands—to form a stable structure, skirt pressure should be high, but, to minimize drag and improve hover performance, a low skirt pressure is better-and a compromise, based on model testing, is usually adopted. Hovercraft also demonstrate a form of behaviour known as "plough-in", for which there is no real counterpart in other marine craft. The pictures show this process happening to an SRN 6 hovercraft during trials. As can be seen, the process is started by the contact of the skirt with the water; the drag then causes the nose to dip even further, and if unchecked the process can lead to overturning of the craft. Fortunately, the process is well understood, and Mr Crago said that skirts of low hydrodynamic drag should be used; for this reason, skirts with fingers are particularly suitable. Another possibility under active investigation is the lubrication of the skirts by air, which is blown down the outside of the skirt and reduces drag at the point where the skirt comes into contact with the water. This seems likely to be a very useful development.

As well as the danger of overturning at high speed after plough-in, low speed overturning can sometimes be experienced. This happens at a fixed forward speed, when the length of the wave generated by the air cushion is the same as the length of the craft. Again cushion pressure is an important criterion. Finally, Mr Crago discussed the problem of skirt wear. One of the problems in investigating wear, he said, was that it was difficult to reproduce in the laboratory the type of









An SRN 6 hovercraft "ploughing in". Photograph by permission of the Institution of Mechanical Engineers.

wear—delamination—shown by the cushions in operation. Ultimately it was found that a specimen mounted in the outlet of an air blower could be made to flap like a flag, and produce delamination of the material much like that observed in practice. As a result the skirts, which consist of a nylon or terylene core coated on both sides with rubber material, have been markedly improved.

Commonwealth Foundation

THE Commonwealth Foundation has produced its first progress report after 18 months of official activity as a charitable organization providing funds for professional bodies on a supra-national basis. The Foundation aims to encourage and support contacts and exchanges between members of the professions in the Commonwealth countries, through conferences, exchanges of personnel and information, and the setting up of Commonwealth-wide associations not centred on Britain. So far grants for nearly £200,000 have been allocated for sixty different projects throughout the Commonwealth. Accountancy, architecture, education, engineering, law and various aspects of medicine and science have been supported, with amounts varying from £35 for publishing an accountancy bulletin in Zambia, to £24,000 for professional centres with basic reference libraries in Kampala and Port of Spain.

A meeting of representatives of the professions, arranged by the British Commonwealth Office in 1964, produced the idea for the Foundation. The proposal was announced at the Commonwealth Prime Ministers' Conference in June 1964; terms of reference were agreed in 1965 and, after appointment of a permanent staff of three, the Foundation became operational in March 1966. Twenty-four of the twenty-five Commonwealth countries contribute a quarter of a million pounds a year to the Foundation, at rates agreed to by the Prime Ministers concerned, to correspond with the size and affluence of each country.

Each contributing country is represented on the Board of Trustees, which has so far met five times, under the chairmanship of Sir Macfarlane Burnet. Applications for grants are accepted from individuals or professional bodies throughout the Commonwealth, for first consideration by the full-time director, Mr John Chadwick. Provided the applicant can produce good reasons for his application, and it falls within the terms of reference of the Foundation, it is passed to the Board of Trustees with the director's report. Chadwick thinks the board will have to meet three times a year at least, and a sub-committee has been set up to consider applications which are urgent or for small sums of money. The main task of the staff in getting the Foundation has been to make its existence known, for individuals and professional bodies can only apply for grants if they know the facilities exist.

The Foundation, which describes its private but international set-up as "without precedent", is not stating its policy too firmly at this stage, and is prepared to experiment. A pilot project is under way in the field of journal distribution. The London Bureau of Hygiene and Tropical Diseases is collaborating with the Foundation to provide copies of the *Tropical Diseases Bulletin* for those in underdeveloped countries who cannot afford the subscription. The London bureau is pleased how quickly the Foundation accepted the scheme.

Dragon Reprieved

THE Dragon high temperature reactor project will be kept in being for one more year at least. During 1968, the costs of the project will be borne by the United Kingdom, Austria, Denmark, Norway, Sweden and Switzerland. The Euratom countries, the other supporters of the project, still have no firm policy for

1968 and thereafter. As a result, they have so far refused to extend the project until 1970. But they have at least been able to sanction an arrangement by which Austria, Denmark, Norway, Sweden and Switzerland pay their normal contributions to the 1968 budget of £2·1 million, with Britain making up the rest. It has also been agreed that Euratom will finally make up its mind by the end of July 1968 and, if approval to continue the project is reached, then the contributions made for 1968 will be taken into account. But if no agreement is reached, Euratom will waive its rights to the written down value of the fixed assets of the project. Meanwhile, Euratom will continue to receive the same benefits, in the form of information and secondment of personnel to the project, as if it were paying its full share.

This generous settlement indicates a good measure of enthusiasm for the project among the non-Euratom countries. Some observers see the high temperature reactors as a good possibility for industrial use, able to produce electricity and high grade steam in reactor sizes smaller than would be economical with other reactor types. The news of progress with Dragon comes at the same time as the first experimental results from another high temperature reactor, the AVR, built in Julich by Brown Boveri/Krupp Reactorbau GmbH for a group of German utilities. The reactor has so far run for about three months, most of the time well below its full design capability of about 50 MW (thermal). The work has established that the system is safe, and approval has been given for full power operation. Powers of 18 MW (th) and helium outlet temperatures of 590° C have been reached.

Living at High Altitude

Some 30 million people throughout the world live above 10,000 feet—more than the total population of 85 per cent of individual nations. They live under perpetual physiological stress, that of hypoxia—oxygen shortage—often compounded by cold stress. Yet there is little systematic knowledge of the effect these conditions have on the high-altitude dwellers and the biological adaptations that are developed to cope—a situation thrown into relief by next year's Olympic Games in Mexico City, which lies at 7,349 feet.

To counter this lack and promote useful research programmes the regional office of the World Health Organization and the US International Biological Programme Committee jointly sponsored an international conference on "Man at High Altitude" in Washington last month. The study of high altitude peoples is of great interest to the Human Adaptability Section of IBP. The conference hardly threw a glance at the problems of Olympic athletes, but a couple of papers were relevant to this practical problem as they discussed the relation of oxygen uptake and work capacity.

Dr R. F. Grover of the University of Colorado has compared lung function and working capacity on athletes from two groups; one, a community settled for several generations at Leadville 2 miles up in the Rockies, the other a group from lowland Kentucky It was found that maximum capacity for consuming oxygen at Leadville was reduced as much in the Leadville native athlete as in the Kentucky athlete Dr Grover wondered whether the long-adapted Andeas

native (Peruvians have lived at about 10,000 ft. for thousands of years) would be physically superior (at altitude) to the well conditioned athlete from the lowlands. Among Leadville non-athletes, hypoventilation (shallow breathing) was common.

This seems a regular characteristic of those highland communities which have been most thoroughly studied. Dr John W. Severinghaus of the University of California San Francisco Medical Center described work on Peruvian Indians which indicated that the native who has always lived at high altitude does not breathe more deeply in a diminished oxygen atmosphere whereas a sea-level native who goes to the highlands does. Dr Severinghaus suggests that the highlander has lost sensitivity in the chemo-receptors governing this reflex. This may have very far-reaching consequences: longevity, intelligence, learning, fertility, infant survival, susceptibility to respiratory diseases may all be implicated. This reflex loss appears to tie in with the characteristic health hazard of high altitude, pulmonary oedema. This was by far the most common affliction of the several thousand Indian troops who have been stationed on the Himalayan border between 11,000 and 18,000 ft. since the 1962 emergency. Dr Inder Singh, in discussing the clinical problems encountered by the Indian forces medical services in the area, reported that diuresis induced by drugs has been found to relieve the condition.

A number of recommendations for both research projects and for practical action were adopted by the meeting. One was the preparation of a short, concise book on high altitude medicine to cover high altitude diseases, modification of common sea-level diseases at high altitude, as well as the modification of drug and anaesthetics action. It was also agreed to found a research centre of high altitude studies to act as a clearing house for information on the subject and improve scientific communication in this field.

Manpower for Electronics

A CONTINUING shortage of scientific and technological manpower is envisaged in the electronics industry, together with a growing demand for engineering skills. These and other findings of the enquiry undertaken by the Manpower Research Unit, in association with the Electronics Economic Development Committee, are presented in *Electronics*—the fifth in a series of reports by the Manpower Research Unit (HMSO, 6s. 3d.). The purpose of the enquiry was to gain a clearer picture of likely manpower developments within the industry up to and beyond 1970, and forty-six firms participated.

According to estimates made by the Ministry of Labour, 371,000 people were employed in the electronics industry in June 1965—288,000 were in electronics and 83,000 were in telecommunications. Shortages of labour were common throughout the industry. The over-riding factor in determining the size of the labour force—not surprisingly, perhaps—was the level of demand for products. The development of microcircuits, the report says, calls for more support at technician level, while the development of printed circuits and semi-conductors would probably involve less mechanical assembly and a move from male to female assessors.

Most firms, it is reported, thought that technological

change, including the use of computers for planning and managerial control, would be the principal means of increasing productivity. Incentive schemes were favoured by some firms but criticized by others and other important contributions to productivity were expected from work and methods study, training and standardization of production.

Estimates were made of changes in the occupational structure of the industry between 1965 and 1970 and an increase in the employment of scientists and technicians is expected in the fields of radio and other electronic apparatus and in telecommunications. Shortage of production engineers, systems and circuit engineers and computer staff was particularly evident at the time of the enquiry, and it was suggested that, although in general the basic cause of shortages was the rapid expansion of the industry and the rate of technical change within the individual firm, the most important fact was the continuing transfer of scientists and technologists to managerial posts. Furthermore, the report suggests that there is a need to encourage more young people to take up science and engineering and the "one-way traffic" of graduates into the academic field is criticized.

Parliament in Britain

Nuclear Ships

MR A. Wedgwood Benn, Minister of Technology, has agreed to consider the recommendation of the Select Committee on Science and Technology that a departmental committee be convened to examine the possibilities of nuclear marine propulsion. He pointed out that the responsibility for the Atomic Energy Authority, the nuclear industry, the manufacture of marine engines and shipbuilding had all been placed on his department. It was in touch with all the interests concerned and he intended to re-examine the position to ensure, as far as possible, that Britain was in a position to embark on a major project as soon as it appeared that commercial demand for such ships justified the considerable cost involved. (Written answer, December 5.)

Patents

In a debate in the House of Commons on November 30, Mr C. Fletcher-Cooke and Sir Lionel Heald challenged the idea that the Patent Office and the Trade Mark Registry should be self-supporting. Mr Fletcher-Cooke maintained that Parliament had never said that they should pay their way and argued that the increase in fees proposed for filing patent applications, complete specifications, trade marks and their renewals was unjustified and constituted a discouragement to invention. Sir Lionel Heald questioned the organization and efficiency of the Patent Office. Had any real effort been made to produce a more modern and efficient system? The Minister of State at the Board of Trade, Mr G. Darling, who replied for the Government, did not think that the increased fees would discourage applications, and pointed out that on an average the Patent Office spent £55 on each application against the £15 which would now be received and that 70 per cent of all applications came from overseas. He paid tribute to the efficiency of the Patent Office and its staff. He would be glad, he said, to see it moved from its present premises, but he did not think it should be run at a loss. (Debate, November 30.)

NEWS AND VIEWS

What Price Uniformity?

THE decision by the conference of editors which the Royal Society has sponsored (see page 1061) that British journals should quickly persuade their contributors to adopt the SI system of units is a reasonable step, on the face of things at least. There is a good deal in the argument that life would be simpler, and possibly a good deal simpler, if all scientists used more or less the same system of units. It goes without saying, of course, that scientists, who do most to create new units of measurement, are less hampered than most other kinds of people-architects, for example-by the coexistence of different systems of units, but even in science it is sometimes hard to tell where inconvenience stops and confusion begins. It is also, perhaps, quite proper openly to acknowledge that the devoted people who seek uniformity are also often much addicted to the invention of new units. The passion for uniformity has, for example, produced as one of its side-effects the strange confusion which now abounds about the units of magnetic flux. There may be quite unexpected benefits if the energies of the unit-mongers can now all be harnessed to a single and probably a lasting cause. Whether, in these circumstances, it is reasonable for by-standers to hope that metrification of the scientific journals will somehow help the rest of the world to abandon its devotion to the inch and even centimetre is another matter. Mr Wedgwood Benn may be determined to show that in weights and measures, if not in monetary systems, Britain is capable of satisfying the most exacting demands of the President of France, but the Ministry of Technology must know by now that its real battles will come with the butchers, bakers and candlestickmakers, not with laboratory scientists.

That said, it remains to be seen how quickly the SI system will sweep everything in front of it. In matters like this, editors of journals may be influential but their influence cannot be decisive. In the last resort, there are limits to the extent to which contributors can be made to toe a common line. The problem is not merely that quantities such as 1.3 Å must be replaced by 130 pm (for picometre). Sometimes there are no exact equivalents for the units which authors choose to use. Who but an author can translate 1,500 feet,for example? In the circumstances, the journals can only provide encouragement and, sometimes, discouragement. Most of them, no doubt, will be guided not so much by the convenience of contributors as by that of readers—this at least is how the balance should be struck. This is where consistency is a real asset, and where it will also be extremely helpful if the practices of all the journals can be brought more closely into concert. But flexibility also matters, which implies

that authors should not shrink from lapsing even into e.g.s. units if this seems to serve the convenience of readers. Familiarity is also important, which means that those who have spent lifetimes using words such as Ångstrom and calorie should be allowed a generous interval in which to change their ways.

The question then arises of how Nature will respond to the call for a uniform adoption of SI units. The first thing to be said is that the problems of an international journal are in many ways exceptional, and at present there is only a hope that journals in the United States will follow the British. The scope of this journal is also a complication. It may not be entirely unkind to biologists to suggest, for example, that some of them may not know what a Joule is, let alone how to use it instead of a calorie. In the circumstances, it would seem that the best course will be to use 1968 as a year of experiment biased in favour of SI units, and there are a number of ways in which this can be done. One way in which this can be done is to allow SI units to be abbreviated but to require that others should be spelled out in full. Thus an author will be allowed to say Hz, but abbreviations such as c/s and c.p.s. will be replaced by "cycles per second". The dyne (which is 10-5 Newtons) will be spelled out in full. For the time being, the atmosphere will remain the atmosphere, instead of being converted to 101-325 Newtons per square metre. But it is clear that the British thermal unit, beloved of engineers, is on the way outin the new system, it works out at 1055.06 Joules. And if people insist on using the nautical mile, a unit wholly contrary to the spirit of the SI system, it is hard to see how they can be out-manoeuvred, because the nautical mile is wellnigh unconvertible. In fact, it is equal to 1.85318 km, on the European side of the Atlantic at least. To start with, at the beginning of 1968, there will be nothing to be done about "1,000 feet" but to complain. It will also seem entirely sensible that units such as the Angstrom, centimetre and gram should be allowed to flourish (if authors wish) without much hindrance for the time being. For the rest, it will be interesting to see what happens. This is not a crucial issue, but SI deservesa fair wind.

Count More Galaxies

Some of the first results of the new sky survey carried out at the Mullard Radio-astronomy Observatory at Cambridge were described last week to the Roya. Astronomical Society by Sir Martin Ryle. From hispreliminary paper with Mr G. G. Pooley, it seems that the Fifth Cambridge Catalogue of radio sources with

contain something like 100,000 sources per steradian, compared with 600 per steradian for the fourth catalogue and a mere 45 for the third, which was based on measurements made in 1958. One of the most striking of the points made by Sir Martin Ryle last week was that the region of the universe now accessible to the instruments at Cambridge is so great that the outer limits of observation probably correspond to times in the history of an evolving universe at which the formation of galaxies was commonplace.

The instrument being used in the new survey is the one mile telescope. A large tracking array 5,000 feet by 60 feet is used in conjunction with movable instruments in such a way that the combination of the records of successive scans of the same part of the sky can simulate the sensitivity of a much larger telescope. In the parts of the survey so far carried out, the aerial system has been used to track chosen parts of the sky for 12 hours a day in a period of 2 months. The result, at a frequency of 408 MHz, is a survey of a patch of sky 4 degrees in diameter. So far, three such patches have been covered with a limit of sensitivity of 0.01×10^{-26} watts m⁻² sec⁻¹. With plausible assumptions about the intensity distribution of the radio frequencies, this corresponds to a limiting flux density twice as great as at the frequency of 178 MHz used in earlier surveys. Each of the three patches of sky so far surveyed appears to include some 200 radio

One of the principal objectives of the work so far has been to determine the variation of the numbers of sources with varying brightness. In practice, this means constructing the relationship between log N and $\log S$ where S indicates radio flux and N the number of sources with radio flux greater than S. It has been necessary to make comparatively rapid surveys of the whole sky so as to pick out comparatively intense radio sources which can be used as a means of calibrating the new survey. Sir Martin Ryle said last week that the initial slope of the curve—corresponding to comparatively intense sources—is essentially the same as the value of -1.8 obtained in earlier surveys, but that the slope progressively decreases in magnitude to about -0.8. Compared with the predictions of a static Euclidean model of the universe with uniformly distributed radio sources, the new survey shows an excess of sources of intermediate radio flux. Sir Martin Ryle said that the differences between observation and the predictions of an Einstein-de Sitter model and of the steady-state theory are considerable, and great enough to show "that important evolutionary effects are taking place". He explained that "The observations show a distribution in which there is an apparent ncrease in the space density of sources followed by a narked decrease. The gradient of the decrease may pe determined by the spread in intrinsic luminosities and, if this is as great as that found for the intense ources, the cut-off must be very rapid." This cut-off probably corresponds to the "epoch of galaxy ormation".

Binocular Vision in Cats

from our Neurophysiology Correspondent ULESZ has recently shown that depth perception in uman vision does not depend on the existence of monocular cues but that it can be based simply on disparities between the two retinal images (see Nature, 215, 1225; 1967). Barlow, Blakemore and Pettigrew (J. Physiol., 193, 327; 1967) have now set out to discover the mechanisms involved in depth perception in anaesthetized cats. The nub of their conclusion is that in cats, as in men, the recognition of structure usually precedes the investigation of depth but that this sequence of events is not essential.

The technique is to record from single cells in the primary visual cortex, stimulating them either with moving black and white bars or with the edges between black and white areas. Having found the gross receptive field for a single cell, the receptive field is determined for each retina separately. The next step is to determine the disparity between the responses to two identical stimuli, presented binocularly, which produce the greatest excitation of the cortical unit.

For each cat they found a range of disparity of about 6° in the horizontal plane and 2° in the vertical. This is interesting, because it is similar to Julesz's result. He found that man could maintain fusion of a stereoscopic pair of images as they were pulled apart on the retinae for a distance in the horizontal three times greater than that in the vertical direction. Precise measurements of retinal disparity are extremely difficult, even in an anaesthetized preparation, because of residual eye movements, and the impossibility of determining the direction of each optic axis accurately. Barlow et al., however, reduced possible movements by paralysing the preparation with 'Flaxedil' and attaching the two eyes to firmly fixed rings. Ophthalmoscopic observation during the experiments showed no perceptible changes in eye position. In any case, the most important feature of their results is the range of disparities observed for each cat, coupled with the horizontal-vertical anisotropy. They suggest that their results indicate that the cat observes depth over a wide range, but at low accuracy (because of poor depth of focus in the visual field), unlike man, who perceives depth accurately over a short range. This seems rather unlikely, both on behavioural grounds and because there would seem to be little advantage in seeing depth in out-of-focus objects. Their results could equally well be taken to show that cats, like humans, can accommodate a large range of depths, or retinal disparities at the same distance and that each part of this range is served by a particular population of cortical neurones. It would be interesting to know whether cats could recognize structures in Julesz patterns: in other words, it would be interesting to know whether cats need to recognize structure monocularly before they can determine the retinal disparities leading to depth percepts. At the moment there is no evidence against this, although Barlow and his colleagues say that their results suggest that the cat recognizes primitive structure first and then decides on the appropriate pairing of parts from the fields of vision of the two eyes.

One of the most important by-products of these experiments is the renewed emphasis on the need for very precise control of eye movements, refractive correction and stimulus presentation in studies on the visual system. Indeed, this is one of the few reports in this field where adequate care has obviously been taken.

No Easy Cure for Migraine

from a Correspondent

"I have my own remedy," said Sir George Godber, in his address to the second Migraine Symposium, held at the National Hospital, Queen Square, on November 24. With this remark, Sir George, who is chief medical officer at the Ministry of Health, associated himself with the many migraine sufferers who have learnt to manage their headaches and the associated symptoms after years of trial and error. For them and for others whose migraine is still unmanageable, the second symposium was a sort of progress report on current research and therapy. Patients who are treated with mono amine oxidase inhibitors and who include certain foods—particularly chocolate and cheese—in their diet tend to suffer from headache and hypertension; the similarity to the dietary factors in migraine was noted. Certain amines, in particular tyramine and dopamine, have been implicated as the causal agents when they are contained in the diet, and gain access to the general circulation. It has now been demonstrated that 100 mg of tyramine taken orally will induce migraine in patients with a convincing dietary history, and who normally exclude foods containing these amines from their diet. On the other hand, there does not appear to be any deficiency of mono amine oxidase in the temporal artery of migrainous subjects when a small biopsy of this artery is removed in an acute attack and stained histochemically. There is increasing interest in the action of amines on blood vessels in vivo and in vitro, and the relevance of these findings to migraine may be significant.

On the therapeutic side, Sir Derrick Dunlop, Extra Physician to the Queen, and chairman of the Ministry of Health's committee on the safety of drugs, reviewed the current methods of treatment. Ergotamine, either orally, by inhalation, injection or in the form of a suppository, remains the most effective form of treatment in an attack, if it is taken sufficiently early. Methysergide is a useful prophylactic, but when taken in adequate doses may lead to inflammatory fibrosis. There have been reports of the development of retroperitoneal fibrosis, valvular heart disease and pleuropulmonary fibrosis during methysergide therapy. Prompt reduction in the dose of the drug does lead to

regression of the side effects.

The development of newer drugs and the pharmacological investigation of other compounds of ergot was the subject of one of the papers. Of the compounds investigated, at least one, 1-methyl-ergotamine. deserves clinical trials. It is difficult to design such trials and equally difficult to assess the effectiveness of a particular drug or regime in a subjective condition such as a headache. An added difficulty, described in one of the contributions, is that patients often do not take the drugs as prescribed. This is demonstrated by the amount of the drug returned by the patients on their next visit to the doctor. Despite the many pitfalls, clinical trials are obviously essential in establishing the value of any new treatment, and conflicting results may be explained by the nature of the trial or the selection of patients admitted to it. The use of hormones in migraine was the subject of another contribution, which described the trial of a progestagen as a prophylactic in migraine. While results are not vet complete, the initial response is not encouraging.

Viruses and Cancer

A LARGE audience attended a review lecture on virus and cancer, given by Professor J. F. Enders at the Royal Society on December 8. Professor Enders was concerned with the problems of the relationship of the virus to the transformed cell, as illustrated by experiments with the oncogenic agent simian virus 40. He posed two questions. Does the virus persist in transformed cells and, if so, in what state? Does the virus specifically condition the shift from normal to cancerous state? The fact that certain viruses are capable of inducing solid tumours or leukaemias when introduced into a susceptible host under the appropriate conditions has been known since the beginning of the century. As an introduction to his lecture, Professor Enders outlined some of the features of oncogenic viruses; they occur in four of the six classes of animal viruses and, in general, do not seem to differ biologically or biochemically from non-oncogenic viruses. They do, however, have two outstanding features. Referring to oncogenic members of the adeno and papova viruses, he pointed out that they replicate in the nucleus and that the expression of the viral genome is partly suppressed so that production of new particles is stopped short of Attempts to isolate these infectious completion. viruses from tumours which they induce have failed, and this has formed the subject of much investigation. In addition, because they replicate in the nucleus, these viruses can react directly with cellular DNA which, as might be expected, is reflected in unusual changes in the normal functions of both the virus and cell. Professor Enders described how, for simian virus 40, both direct and indirect evidence has been produced to show that the viral genome can be expressed in at least two different ways in the transformed cell. Interesting indirect evidence which has come from immunological procedures has led to the discovery of two new virusassociated antigens in the transformed cell: the S antigen which only occurs in the cell nucleus, and the T or transplantation antigen. Professor Enders and his associates have shown that S antigen can appear in the absence of T antigen in cells which have been exposed to SV40. Whether or not such cells become oncogenic as a result of exposure to the virus has not yet been established.

Professor Enders related how he and his associate: had investigated the phenomenon of tumour progres sion and had shown that, when hamsters are inocu lated intravenously with one million transformed hear cells, oncogenicity increased with animal passage and * striking increase in the rate of growth of tumour was observed. Furthermore, metastases—a sign o high malignancy—were associated with tumours which have been induced by animal passaged cells. It ha been concluded that "oncogenic potential of cells transformed in vitro by SV40 may vary widely and this variation is independent of the presence of the viragenome".

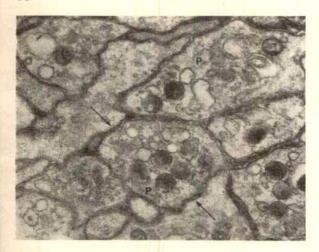
Progress in Thrombosis

from a Correspondent

A conference on thrombosis was held in Washingtoin November to consider the effectiveness of currer trends in investigation and how improvements can b made. The conference was sponsored by a task force

on thrombosis of the Division of Medical Sciences of the National Research Council in association with the International Committee on Haemostasis and Thrombosis, and was attended by more than 150 people from 15 countries.

Dr D. G. Frieman of the Beth Israel Hospital, Boston, working on venous thromboembolic disease, has found the incidence of pulmonary embolism in routine post-mortem examinations to be more than 40 per cent. He has found a high incidence in patients with gastro-intestinal bleeding and a low incidence in patients with cirrhosis. Dr R. E. Horowitz of the University of Southern California School of Medicine confirmed the low rate of clinical diagnosis of pulmonary embolism when this is compared with the autopsy findings. Dr Sevitt of Birmingham described his findings in post-mortem examinations of older injured patients: 65 per cent had deep vein thrombosis of the legs without embolism and 20 per cent of the patients had a major pulmorary embolism. It appeared that bed rest and not the injury was the precipitating factor. Dr E. M. Devitt of Cornell University considered that suppression of lactation increased the incidence of



Part of an aggregate of tightly packed platelets (P) in an "artificial thrombus" produced by Chandler's method. Dense material is seen between adjacent platelet membranes (arrows) after treatment with ruthenium red × 20,000. This dye is thought to stain acid mucopolysaccharides external to cell membranes (Luft, J. H., Fed. Proc., 25, 1773; 1967).

post-partum venous thrombosis. Dr L. Dexter of the Peter Bent Brigham Hospital, Boston, provided valuable data on the prognosis of massive but not immediately fatal pulmonary embolism. Approximately one half of such patients die in half an hour and three-quarters in two hours. The pulmonary circulation has to be reduced by about 50 per cent before circulatory disturbance arises. The alterations in the haemodynamics of the venous system of the lower limb following thrombosis were described by Dr J. A. Haller of the Johns Hopkins Hospital, Baltimore. The clinical outcome may well depend on whether the circumflex femoral veins become occluded.

Speakers on epidemiology of thrombosis attempted to estimate the magnitude of the problems of cerebral thrombosis and acute myocardial infarction. It was concluded that there were few reliable data on cerebral occlusion but that the Framingham heart epidemiological study provided data on coronary disease. Dr F. H. Epstein of Michigan described how the epidemio-

logy of coronary heart disease is complicated by the difficulty of epidemiological studies among the living so long as this is confined to manifest disease rather

than the underlying lesion.

The anatomy of a thrombus was described by Dr Chandler of Georgia, who again emphasized the importance of platelets and leucocytes in the structure of the thrombus, as well as the episodic course of its development. An account was given of the electron microscopic appearance of experimental thrombi by Dr J. E. French of Oxford; platelets in an experimental thrombus are not fused together initially and there is a narrow gap between them. A coating material, possibly mucopolysaccharide, has been

observed on the surface of the platelets.

Dr T. Ashford of Boston described observations on the discontinuous nature of the endothelial cells in special areas. Contraction of endothelial cells may be responsible. Simple relaxation would then be sufficient to reconstitute the surface. The role of fibrin in surrounding platelet aggregates and in preventing their disintegration was described by Dr N. F. Rodman of Chapel Hill. The aggregation of platelets under the influence of adenosine diphosphate has a biphasic pattern; the secondary aggregation can be prevented by the ingestion of aspirin (Dr Sucker, New York). Dr Mustard of Hamilton, Ontario, has demonstrated that, in certain circumstances in vivo, platelet aggregation can be reversed by compounds such as aspirin and phenylbutazone.

Origin of Man

from our Cell Biology Correspondent

Comparison of the structure of protein molecules—chemical palaeogenetics as Zuckerkandl calls it—is clearly going to provide important evidence of genetic and hence evolutionary relationships between different species. Zuckerkandl has already produced a convincing picture of the evolution of the haemoglobin—myoglobin molecule in mammals, and Sarich and Wilson (Science, 158, 1200; 1967) now suggest, on the basis of a comparison of the antigenic properties of serum albumins of the anthropoids, that human beings and African apes shared a common ancestor as recently as five million years ago. Most palaeontologists have inferred from the fragmentary fossil record that this divergence occurred at a considerably earlier point in time.

Earlier this year Sarich and Wilson (Proc. US Nat. Acad. Sci., 58, 142; 1967) found that the serum albumins of man, apes, old and new world monkeys all react to about the same extent with antibody against the albumin of Galago crassicaudatus. (Galago, the bush baby, is a "living fossil"; it is a lemur-like animal with many of the skeletal characteristics of the fossil insectivores from which the primates evolved.) They argued from this that the serum albumins of all the anthropoid lines have changed to about the same extent as they have evolved from the common ancestor. In other words, the albumin molecules in the anthro-

poid lines have evolved at a steady rate.

In their latest experiments they find that human, chimpanzee, gorilla and orang-utan albumins all give virtually identical reactions with antiserum against human albumin. To make these serological tests quantitative, they have measured an index of

dissimilarity, that is the factor by which the concentration of antiserum against a particular species of albumin has to be raised, in complement fixation experiments, to give a reaction with a heterologous albumin equal to that given with the homologous albumin. For example, the index of dissimilarity between human and chimpanzee albumins is 1-14.

The very similar antigenic properties of albumins of humans and African apes imply that these molecules have very similar structures. If Sarich and Wilson's previous conclusion—that albumin molecules of all the anthropoid lines have evolved at steady rates since they diverged from a common ancestor—is accepted, it follows that humans and African apes shared a common ancestor much more recently than is usually supposed.

But how can the index of dissimilarity be related to real time? The fossil record of primate evolution is too fragmentary to make a direct correlation, but Reichlin (1966) and Salthe and Kaplan (1966) have related the index of dissimilarity of various dehydrogenases of fish, amphibians, reptiles and birds with the fossil record which shows when these classes diverged. The logarithm of the index of dissimilarity, measured in immunological experiments, is approximately proportional to the time of divergence. Assuming this relationship holds for the serum albumins of primates, Sarich and Wilson calculate that humans and African apes had a common ancestor only five million years ago. Not every anthropologist who has studied the fossil record would argue against this date, but it will remain tentative until it has been proved that serum albumins evolve at much the same rates as other proteins. Sarich and Wilson suggest that an immunological study of the serum albumins of ungulates, for which there is an extensive fossil record, might settle this point.

Nucleases

from our Molecular Biology Correspondent

One of the most beguiling visions on the horizons of enzymology is the prospect of being able to compare the catalytic mechanisms of a range of endonucleases. The three-dimensional structure of one of these is already largely known, and others are being studied; sequences have been determined, and an enormous mass of enzymology and protein chemistry has accumulated. Some interesting new work on T1 ribonuclease and on staphylococcal nuclease has now been described.

Tl ribonuclease is remarkable because, although it binds to a highly negatively charged substrate, it is nonetheless a very acidic protein, with only five basic groups (three of them histidines). As a first step to defining the active centre, Takahashi, Stein and Moore (J. Biol. Chem., 242, 4682; 1967) have studied the inactivation of T1 ribonuclease by iodoacetate, a reagent which inactivates pancreatic ribonuclease by reacting with the histidine residues at the active centre. It is startling to find that in T1 the reaction occurs at a glutamic acid residue—an amino-acid not previously imagined to be capable of reacting with iodoacetate. The carboxyl group, identified as glu-58, is esterified to —COOCH₂COO-. This involves a displacement of the charge by 4 Å, which is evidently sufficient to cause inactivation. That this unique reactivity is conferred upon glu-58 by its specific environment is shown by the loss of reactivity with iodoacetate on denaturation of the protein.

Staphylococcal nuclease has been completely sequenced by Anfinsen and his associates (Taniuchi et al., ibid., 4759). It has 149 residues, is basic and has no disulphide bridges. Its most striking feature lies in the physical changes which occur when it interacts with competitive inhibitors. Cuatrecasas et al. (ibid., 4759) have found that binding of a nucleoside diphosphate in the presence of calcium ions (which are also required for activity) generates a sizable ultraviolet difference spectrum, arising from perturbation of tyrosine residues; moreover, spectrophotometric titrations show that their ionization is inhibited by the diphosphate, and finally a spectrophotometric method (solvent perturbation) shows that three or four of the seven tyrosines at the same time become effectively inaccessible. Although one cannot entirely rule out a direct interaction of all these tyrosines with the substrate, the observations are altogether consistent with a considerable convulsion in the conformation of the enzyme when the substrate binds.

Whether such a conformational change occurs, and its nature and extent, are, of course, general issues in enzymology. It is worth noting that the most direct evidence of such an effect emerges from preliminary X-ray data, just reported by Lipscomb's group on carboxypeptidase (Steitz et al., ibid., 4662). binding of a dipeptide generates quite considerable changes in electron density around the active site region. In lysozyme Phillips and his colleagues observed very small changes, whereas in pancreatic ribonuclease Richards' group found no significant changes at all in electron density of the enzyme when specific inhibitors were introduced. Their technique of diffusing substrates into the crystal in the diffractometer lends itself particularly well to precise determinations of small differences, and it must be concluded that in this case the conformation remains undisturbed. Evidently, therefore, generalizations about "induced fit" effects are not warranted at this stage.

Genetic Polarity

from our Cell Biology Correspondent

EARLIER this year, Imamato and Yanofsky found evidence that polar mutations in the lactose operon of *E. coli* cause a failure in transcription of the DNA beyond the mutation (see *Nature*, **215**, 1327; 1967). At the same time, there is strong evidence that polarity involves a failure in transcription. It is easy enough to reconcile these two sets of evidence because translation can control transcription by regulating the release of mRNA from the DNA template (see *Nature*, **214**, 228; 1967). So at least in organisms which have DNA as the genetic material, polarity seems to affect both transcription and translation.

Engelhardt, Webster and Zinder (J. Mol. Biol., 29, 45; 1967) and Capecchi (J. Mol. Biol., 30, 213; 1967) have now detected polarity in vitro with RNA from amber mutants of RNA bacteriophage. This result, together with Fink and Martin's work on polarity in the histidine operon of S. typhimurium (J. Mol. Biol., 30, 97; 1967), suggests that polarity is fundamentally a translational phenomenon which only incidentally involves transcription.

The RNA bacteriophages f2 and R17 are virtually identical. Both Zinder's group at the Rockefeller University and Watson's group at Harvard have

isolated amber mutants of f2 and R17, respectively, which have the amber mutation at the position of the codon specifying the sixth amino-acid in the phage coat protein. These mutants are polar in vivo. When E. coli is infected with the mutant, neither phage coat protein nor phage RNA replicase is made even though the RNA replicase cistron does not itself contain any mutation. This in vivo polarity could involve either transcription or translation because, of course, phage RNA is both replicated and translated in the cell (see Nature, 216, 639; 1967).

Both Engelhardt et al. and Capecchi programmed a cell free protein synthesizing system with RNA isolated from the polar mutants of f2 and R17 and asked, in the complete absence of any transcription, does the mutant RNA show polarity? Both groups found that with the correct ionic conditions in the cell free system the polar mutant RNA is indeed polar in vitro. Neither phage coat protein nor the other phage proteins are made. This clear cut result means that at least for the RNA phages polarity can be explained purely as a failure of translation. Spiegelman's recent report that phage RNA is replicated in the 3' to the 5' direction (see Nature, 216, 639: 1967) also supports this conclusion.

Fink and Martin have studied polar mutants in the histidine operon of S. typhimurium. This operon con-

tains six cistrons, and polar mutants in the first of the cistrons show a steep gradient of polarity. Those at the operator proximal, N terminal end of the cistron are strongly polar; those near the C terminal end are weakly polar and there is a fairly sharp transition from strong to weak polarity somewhere in the centre of the cistron. In all the subsequent cistrons, however, there is either no gradient of polarity or else a very shallow gradient. Polar mutants irrespective of their position in the cistrons show about the same degree of polarity.

This absence of a sharp gradient of polarity in all but the first cistron is the chief reason why Fink and Martin question simple transcriptional-translational models of polarity. They propose that once transcription has proceeded beyond a critical point in the first cistron it is uncoupled from translation. Any polar mutant beyond the critical region would not block further transcription and the polarity shown by such mutants would depend entirely on translational

processes.

There will be no satisfactory model to account for all the polarity phenomena which have already been observed in bacteria until more is known about transcription and translation. Polarity in the RNA phages, however, can now be explained solely by a translational model.

Magnetohydrodynamics and Power Generation

by
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Recent reports of the plans of the Central Electricity Generating Board have caused some confusion.

MANY readers will be aware that for several years a very considerable research and development effort has been devoted by the Central Electricity Generating Board to studies of the generation of electricity by magnetohydrodynamic methods. Recently a decision was taken to reduce the magnitude of this work very substantially and it is of interest therefore to record some of the history of the project so that the reasons for beginning the work and now for its reduction can be noted and judged.

Work began in 1959, when the CEGB set up a small study group to examine the possible application of alternative methods of electrical generation to central power station use. In particular, preliminary and small scale experiments were begun on fuel cells, thermionic and thermoelectric devices as well as on magnetohydrodynamics. Obviously, a number of small scale studies such as this could not all be taken through to large scale development, and thermionic and thermoelectric devices were abandoned first, but both fuel cells and MHD were continued and the laboratory experiments increased in scale to involve installations costing a few tens of thousands of pounds.

Technically, the work on fuel cells proved to be of great interest and led to a very clear indication of the points where progress was needed, but with the effort available we were not convinced that these problems could be solved. It seemed unlikely, too, that costs could be achieved which would provide a competitive source of electrical generation, and the work finally stopped.

Studies were continued on MHD and by 1964 it was clear that many of the research problems were identified and that further progress would require large scale experimental work. An outline design of an MHD power station indicated that it would be competitive with nuclear stations (on the price assumptions current at that time) for several years provided that it could be brought into commission in the period 1975–1979. The difficulties of achieving this were recognized, but, in spite of the risk, the profit to be gained from a successful outcome was sufficient to persuade us to make the effort. The manufacturers of heavy electrical equipment (AEI, C. A. Parsons, English Electric, GEC), the Water Tube Boilermakers' Association and the National Coal Board generously joined in a collaborative pro-

gramme of research which has been constantly reviewed by a co-operative group. A very remarkable spirit of unity has prevailed since the first coming together of the collaborating groups all of whom have made substantial financial and technical contributions. It has been a constant stimulation and encouragement to me as chairman of the main committee to have the

support which has been so loyally provided.

The problems to be dealt with included the design of duct electrodes, air preheaters, combustion chambers for fossil fuels especially coal, seed recovery and recirculation, superconducting magnets and instrumentation and control problems. Much of the work could be carried out on small installations with burners of 2 to 40 MW ratings, but for some studies a larger rating of 200 MW was felt to be needed leading ultimately to a continuously running prototype. This latter part was the so-called Marchwood experiment since it was intended to be carried out at the CEGB laboratory at Marchwood.

Most of the small scale work has been highly successful and a good deal has already been published. Arrangements are being made to ensure that any further work will similarly be published as soon as possible. Satisfactory solutions can be seen to the problems of combustion and air preheaters although costs may be a little higher than had originally been contemplated. Also we have had a successful development of a superconducting magnet using a composite superconductor developed in collaboration with Imperial Metal Industries. This might be regarded as one piece of technical "fall-out" of some importance.

Work is incomplete, however, on seed recovery and on the development of proved long life electrodes (in this field there is a joint research programme with Electricité de France) although reasonable solutions seem to be in sight. This we shall establish within the next year by which time we believe that the research stage of the project can be regarded as completed with a not unreasonable measure of success. Whether any work will be continued after that time is an open question, but it is not likely to be substantial, since we cannot see the economic justification any longer for the large expenditure which the development stage would require.

The main reason for this is that the cost of nuclear power stations of the AGR type is still tending to fall while costs of fossil fuel fired plant are now tending to rise. This has brought closer the date at which an MHD station must be commissioned, and has reduced the period of time when it would be competitive with a nuclear station practically to zero. This and the delays in commissioning the Marchwood experiment have reduced the prospect of an economic return on the expenditure to a level so low that it is sensible to divert the resources to more promising activities.

It is easy to assess the costs of the work done in this joint study and the total must be about £2.5 m. It is more difficult to assess the benefits. There is some technical fall-out and there has been some stimulation of academic studies, but most important, perhaps, British manufacturers now have knowledge and skills derived from the joint study which will be of benefit if MHD development occurs elsewhere in the world.

GTP-stimulated Binding of Initiator-tRNA to Ribosomes directed by f2 Bacteriophage RNA

by JOHN S. ANDERSON* JAMES E. DAHLBERGT MARK S. BRETSCHER MICHEL REVELT BRIAN F. C. CLARK

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Binding of formyl methionyl-tRNA to ribosomes, directed by RNA extracted from the f2 bacteriophage, is dependent upon initiation factors, GTP and the presence of the formyl group. Binding of f2 RNA to ribosomes is, however, independent of initiating factors, amino acyl-tRNA and GTP.

THERE is much evidence that formylmethionyl-tRNA§ (F-Met-tRNA)1,2 participates in the initiation of protein synthesis in cell free systems of E. coli³⁻⁶. Messenger RNA containing the appropriate codon directs the binding of F-Met-tRNA to ribosomes. Such messenger activity

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§ Abbreviations used in this paper include: GTP, 5'-guanosine triphosphate; GMP-PCP, 5'-guanylyldiphosphonate; ApUpG, adenylyl (3',5') uridylyl (3',5') guanosine; GpUpG, guanylyl (3',5') uridylyl (3',5') guanosine; F-Met-, formylmethionyl-; &RNA, transfer ribonucleic acid.

can be stimulated by the trinucleoside diphosphates ApUpG and GpUpG and by short defined oligonucleotides which contain these triplet sequences4.7-10. A natural messenger RNA such as that extracted from the f2 bacteriophage^{11,12} will also promote the formation of the initiation complex of F-Met-tRNA and ribosomes. Initiation factors¹³⁻¹⁵, which promote the *in vitro* incorporation of amino-acids into polypeptide products, enhance the ApupG-directed binding of F-Met-tRNA to This stimulated binding is greatest at a ribosomes16. magnesium ion concentration of about 5 mmolar¹⁷⁻²⁰, and is dependent on the presence of the formyl group 17,19 and

GTP¹⁸⁻²⁰. Initiation factors and GTP also stimulate the polyuridylate-directed binding of acetylphenylalanyltRNA^{21,22}.

The requirement for GTP in protein synthesis was first described by Keller and Zamecnik²³. There seems to be a stoichiometric relationship between the number of peptide bonds which are formed and the number of moles of GTP which are hydrolysed to GDP and orthophosphate²⁴. The β,γ-methylene analogue of GTP, 5'guanylyldiphosphonate (GMP-PCP), cannot be hydrolysed in this manner and is a competitive inhibitor of the GTP effect in protein synthesis²⁵. Formation of F-Met-puromycin and F-Met-phenylalanine by washed ribosomes supplemented with initiation factors and directed by ApUpG and ApUpGpUpUpU, respectively, required GTP26,27

In this paper we report that binding of F-Met-tRNA to ribosomes, directed by the natural RNA extracted from the f2 bacteriophage, is dependent on initiation factors, GTP and the presence of the formyl group at a magnesium ion concentration of 5 mmolar. Reaction of F-Met-tRNA with puromycin at a concentration of 5 mmolar magnesium ion is also dependent on the initiation factors and GTP. Although GMP-PCP can effectively substitute for GTP in binding F-Met-tRNA to ribosomes, it does not substitute for GTP in the reaction of F-Met-tRNA with puromycin. Binding of f2 RNA to ribosomes is independent of initiation factors, amino acyl-tRNA and GTP.

The preparation of ribosomes, initiation factors, 35S-F-Met-tRNA and 35S-Met-tRNA have been described before¹⁹. For separation of the two initiation factors, the crude initiation factors prepared through ammonium sulphate precipitation as previously described19 were dialysed against 0.01 molar tris-hydrochloric acid, pH 7.4, 0.002 molar magnesium chloride, 0.06 molar ammonium chloride and 0.006 molar β-mercaptoethanol (buffer A) and were applied to a DEAE-cellulose column (Whatman DE-52) which had been equilibrated with buffer A. One of the factors (F₁) was not adsorbed. A small amount of inactive material was eluted when the concentration of ammonium chloride in buffer A was increased stepwise to 0.10 molar. The second factor (F₂) was eluted when the concentration of ammonium chloride of buffer A was increased to 0.18 molar. The protein in these fractions was precipitated with ammonium sulphate (52 g/100 ml.). After centrifugation the precipitates were dissolved in buffer A and dialysed for 4 h at 4° C against three changes of buffer A. These dialysed preparations rapidly lost activity at 0° C. The preparations could be stabilized by storage either as suspensions in ammonium sulphate at 0° C or as aliquots of the dialysed fractions which were kept frozen in liquid

Growth and isolation of the f2 bacteriophage were carried out according to a slight modification by Lodish (personal communication) of a method described by Leberman²⁸. Purified f2 bacteriophage (55 mg) in 6 ml. of 0.2 molar potassium acetate, pH 5, was extracted with an equal volume of phenol saturated with 0.2 molar potassium acetate, pH 5. The aqueous phase was re-extracted with saturated phenol. The f2 RNA was precipitated from the aqueous phase by addition of three volumes of ethanol. The precipitate was recovered by centrifugation; it was redissolved in 1 ml. 0.2 molar potassium acetate, pH 5, and reprecipitated with three volumes of ethanol. The precipitate was collected by centrifugation. It was well drained and the remaining ethanol was removed at reduced pressure. The f2 RNA (17 mg) was dissolved in water to give a concentration of 4 mg/ml. and was stored in aliquots at -20° C until it was used.

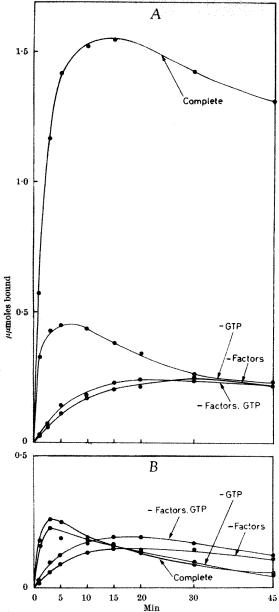
32P-labelled f2 bacteriophage was isolated according to the procedure of Lodish et al. 29 except that centrifugation at 40,000 r.p.m. for 2 h was substituted for 'Sephadex' chromatography. The RNA was extracted as described here. The specific activity of the RNA ($\sim 5 \times 10^5$ c.p.m. per ug) was reduced by the addition of unlabelled f2 RNA.

Assays for the binding of F-Met-tRNA and 22P-f2 RNA to ribosomes were performed according to established procedures 30,31 with only slight modifications. Reaction mixtures contained 0.1 molar tris-hydrochloric acid, pH 7.2, 0.05 molar potassium chloride, 0.01 molar ammonium chloride, and 0.005 molar magnesium acetate. amounts of ribosomes, initiation factors, f2 RNA and F-Met-tRNA are described in the legends of the figures and table. After incubation at 37° C, the reaction mixtures were diluted 50- to 100-fold with cold 0-1 molar trishydrochloric acid, pH 7·2, 0·05 molar potassium chloride. 0.005 molar magnesium acetate and were immediately filtered on 'Millipore' cellulose nitrate filters. The reaction tubes and filters were washed three times with 3 ml. portions of the same buffer. When dry, the filters were inserted into vials containing a scintillation solution of 0.4 per cent 2,5-diphenyloxazole (PPO) and 0.03 per cent 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in toluene and were counted by liquid scintillation.

The RNA which can be extracted from f2 bacteriophage directs the binding of F-Met-tRNA to ribosomes13. At a concentration of 5 mmolar magnesium ion, both the rate and extent of this binding are significantly increased by the addition of initiation factors and GTP to the binding reaction mixture (Fig. 1A). The rate of binding of F-Met-tRNA to ribosomes is ten times greater in the presence of initiation factors and GTP than in their absence. Furthermore, the total amount which is bound is more than six times greater. The rate of binding of F-Met-tRNA in the presence of initiation factors but in the absence of GTP (Fig. 1A without GTP) initially approaches that observed in the presence of both components, but binding under these conditions rapidly reaches a maximum value which is considerably less than that of the complete system. The observed binding was dependent on the f2 RNA. Control curves (see legend to Fig. 1) for the four sets of conditions but without f2 RNA The nonformylatable showed essentially no binding. species, Met-tRNA_M, does not bind to ribosomes in the presence or absence of initiation factors and GTP at the low concentration of magnesium ions used in these experiments.

The results of similar experiments in which nonformylated 35S-Met-tRNA replaced the 35S-F-Met-tRNA are shown in Fig. 1B. It is evident that the nonformylated Met-tRNA binds much less than F-Met-tRNA in the complete system (compare Fig. 1A and B). Omission of GTP had little effect. Binding curves for 35S-Met-IRNA in the absence of initiation factors or in the absence of initiation factors and GTP were very similar to those obtained using F-Met-tRNA. The limited amount of binding of 35S-Met-tRNA was also dependent on the f2 These results show very clearly that maximal binding of the initiator tRNA at a concentration of 5 mmolar magnesium ion requires initiation factors, GTP, the formyl group, and messenger RNA, in this instance f2 RNA. Earlier we reported similar requirements for ApUpG-directed binding of the initiator tRNA19

The above results show that GTP plays a significant part in the formation of the binding complex of F-MettRNA with the ribosome. Because the GTP which is utilized in protein synthesis is hydrolysed at the B.ypyrophosphate linkage, and because the β,γ-methylene analogue of GTP is an inhibitor of protein synthesis, the ability of GMP-PCP to substitute for GTP in the binding reaction was investigated. The effect of the concentrations of GTP and of GMP-PCP on the amount of complex which is formed is shown in Fig. 2. These curves show that a final concentration of 0.2 mmolar GTP or GMP-PCP gives rise to a near maximal stimulation. It should be noted that the amount of F-Met-tRNA which is bound in the presence of GMP-PCP is frequently somewhat less than that bound with an equivalent amount of GTP. The curves of Fig. 2 and the time course for binding shown in Fig. 3A show that GMP-PCP does indeed support the



Min

Fig. 1. Requirement for the formyl group, initiation factors and GTP in the binding of Met-tRNA to ribosomes under the direction of f2 RNA. Components of the complete reaction mixtures (250 μl.) included 4.7 A₂₈₀ units of E. coli MRE 600 ribosomes, 4.7 A₂₈₀ units of F2 RNA, 50 μg of initiation factors, 0.23 mmolar GTP, and either 0.53 A₂₈₀ units of ²⁸S-F.Met-tRNA (38.2 μμmoles ²⁸S-methionine; 70 per cent formylated) (A) or 0.46 A₂₈₀ units of ²⁸S-Met-tRNA (33.5 μμmoles ²⁸S-methionine; less than 2 per cent formylated) (B). Incubation was at 37° C. At the times indicated 25 μl. samples were diluted with buffer and assayed as described earlier. The curves show the effect of omitting components. Examples of the amount of binding in μμmoles taken at the 15 min incubation point in control experiments which lacked f2 RNA for A and B were as follows: +factors+GTP, 0.06, 0.02; +factors-GTP, 0.02, 0.02; -factors+GTP, 0.04, 0.04; -factors-GTP, 0.03, 0.03.

formation of the F-Met-tRNA-f2 RNA-ribosome complex nearly as well as does GTP.

The components which are required for reaction of F-Met-tRNA with puromycin at a concentration of 5 mmolar magnesium ion were investigated. Fig. 3B shows that a rapid formation of F-Met-puromycin occurred if the reaction system contained F-Met-tRNA, ribosomes, 12 RNA, initiation factors, GTP and puromycin. Omission of any one of these components essentially abolished F-Met-puromycin formation. Whereas GMP-PCP is effective as a substitute for GTP in the binding assay (Fig. 3A), it is not an effective substitute for GTP in the reaction with puromycin. The rate of F-Met-puromycin formation

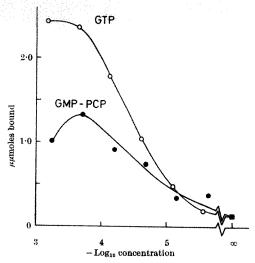


Fig. 2. Dependence of F-Met-tRNA binding on the concentrations of GTP and GMP-PCP. Each reaction mixture (50 μ L) contained 0-90 A_{260} units of ribosomes, 0-98 A_{260} units of f2 RNA, 17 μ g initiation factors and 0-14 A_{260} units of 38 S-F-Met-tRNA. GTP and GMP-PCP were varied to give the indicated molar concentrations. Incubation was for 10 min at 37° C.

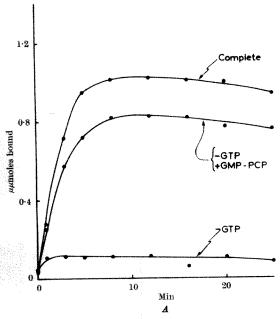
in the presence of GTP (0.32 $\mu\mu$ mole/min) is forty-five times greater than that observed with GMP-PCP (0.007 $\mu\mu$ mole/min).

Because messenger RNA is necessary for the binding of ²⁵S-F-Met-tRNA to ribosomes, an investigation was made of the requirements for the binding of 32P-labelled f2 RNA to ribosomes. Preliminary experiments indicated that crude initiation factors alone caused high levels of binding of 32P-f2 RNA to 'Millipore' filters even in the absence of ribosomes. In order to reduce this background, partially purified initiation factors obtained by chromatography on DEAE-cellulose were employed. Even the partially purified fractions F1 and F2 caused considerable background binding of ³²P-f2 RNA to 'Millipore' filters. Consequently, the amount of the purified factors was reduced as much as possible in an effort to minimize the background without significant alteration of the capacity of the factor preparation to stimulate f2 RNA-directed binding of 35S-F-Met-tRNA to ribosomes. The effect of initiation factors, GTP, and 35S-F-Met-tRNA on the binding of ³²P-f2 RNA to ribosomes is shown in Table 1. Comparison of the amounts of 32P-f2 RNA bound in the presence or absence of 35S-F-Met-tRNA (unfractionated tRNAs charged with 35S-methionine and nineteen 12Camino-acids) clearly shows that the amino acyl-tRNA has no effect on the binding of messenger RNA to ribosomes either in the presence or absence of initiation factors and GTP. Nor is the non-specific binding of 32P-f2 RNA to 'Millipore' filters by the initiation factors influenced by amino acyl-tRNA. The amount of ³²P-f2 RNA which is bound to ribosomes in the presence of initiation factors

Table 1. Effect of initiation factors, gtp and amino acyl-trna on the binding of \$2p-f2 rna to ribosomes

Components of reaction mixtures						
Ribosomes	$(\mathbf{F_1} + \mathbf{F_2})$ plus GTP	RNA	⁸⁶ S-F- Met-tRNA	Net c.p.m.	bound	
+	+	+	+	3,380	2.200	
+	+	- 1 -	Man	3,310	L	
en-a		+	+	2.400	45	
www	+	+	www.	2,590	entre.	
+		+	+-	1.050	52	
+	***			1,220		
+	+	-	+	*****	240	

The complete reaction mixture (50 μ l.) contained 0.90 A_{250} units of ribosomes, 0.47 A_{240} units of **P-f2 RNA (36,100 c.p.m.), 2 μ g of the F₁ initiation factor, 3 μ g of the F₂ initiation factor, 0.14 A_{240} units of **S-F-Met-IRNA (30,600 c.p.m., also includes all other amino acyl-IRNA species and 0.22 mmolar GTP. Incubation was for 10 min at 37° C. Reactions were diluted and filtered as earlier described. All **P values were corrected by the amount of **P-f2 RNA bound to the filters in the absence of both ribosomes and initiation factors (430 c.p.m.). All **S values were corrected for the energy spectrum overlap by **P.



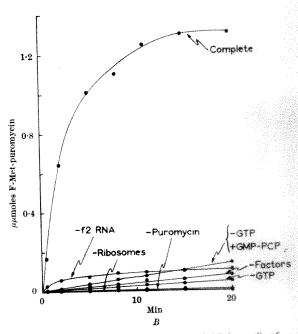


Fig. 3. A, Binding of F-Met-tRNA to ribosomes with either GTP or GMP-PCP. Reaction mixtures (250 μl.) contained 4-5 A₂₀₀ units of ribosomes, 4-7 A₂₀₀ units of f2 RNA, 100 μg of initiation factors and 1-72 A₂₀₀ units of ⁵⁵S-F-Met-tRNA. When present the GTP was 0-23 mmolar and the GMP-PCP was 0-22 mmolar. Incubation was at 37° C. At the intervals indicated 25 μl. aliquots were diluted with buffer and filtered. B, Requirements for reaction of F-Met-tRNA with puromycin. The complete reaction mixture (200 μl.) contained 0-1 molar and filtered. B, Requirements for reaction of F-Met-tRNA with puromycin. The complete reaction mixture (200 μl.) contained 0-1 molar tris-hydrochloric acid, pH 7-2, 0-05 molar potassium chloride, 0-01 molar ammonium chloride, 0-005 molar magnesium acetate, 0-22 mmolar tris-hydrochloric acid, pH 7-2, 0-05 molar principle. 0-01 molar ammonium chloride, 0-005 molar magnesium acetate, 0-22 mmolar tris-hydrochloric acid, pH 7-2, 0-05 molar principle. 0-01 molar ammonium chloride, 0-005 molar principle. 0-023 mmolar graph of initiation factors, and 0-86 A₂₀₀ units of ⁵⁴S-F-Met-tRNA charged with 19 μμmoles of ⁵⁴S-methionine of which 70 per cent was formylated. Incubation was at 37° C. At the indicated intervals 25 μl. aliquots were withdrawn and incubated with 50 μl. of 0-25 molar ammonium lacubation was at 37° C for 10 min. The alkaline hydrolysates were quantitatively applied to Whatman 3 MM filter paper and electrophoresed hydroxide at 37° C for 10 min. The alkaline hydrolysates were quantitatively applied to Whatman 3 Mm filter paper and electrophoresed for 50 min at pH 3-5 in 0-09 molar pyridinium acetate—acetic acid buffer with a potential of 60 V/cm. F-Met-puromycin migrated 6 cm towards the cathode and was located by autoradiography. All areas of the electropherograms with a mobility corresponding to F-Met-puromycin were cut out and counted by liquid scintillation.

and GTP is approximately the sum of that bound by the ribosomes alone plus that bound by the initiation factors and GTP without ribosomes (the background). These results suggest that binding of f2 RNA to ribosomes is independent of initiation factors, GTP, and amino acyltRNA. Clearly ³⁵S-F-Met-tRNA was bound when the system contained ribosomes, the initiation factors, GTP and f2 RNA, but not when any of these components were omitted. It is difficult to convert the data accurately from counts/min into µµmoles because the ³²P-f2 RNA was not necessarily intact (that is, in the 27S form). One molecule of F-Met-tRNA, however, was bound to the ribosomes/molecule of f2 RNA under these conditions. This is to be expected because the amount of f2 RNA was in excess of the amount of ribosomes²².

The high background of binding of f2 RNA in the presence of the initiation factors is of interest because a similar background was not observed for tRNA. This background could be reduced by the addition of unlabelled f2 RNA but not by the addition of unlabelled ribosomal RNA (J. E. Dahlberg, unpublished results). It is not clear if the interaction is specific for messenger RNA or if it just depends on the secondary structure of the RNA involved.

The initiation of protein synthesis involves several events occurring in sequence. One of the first events is the binding of messenger RNA to ribosomes. Results presented here indicate that the binding of messenger RNA to ribosomes is not dependent on the binding of **F.Met**-tRNA. Similarly, the initiation factors F_1 and F_2 and GTP seem to have no effect on the binding of messenger RNA. This agrees with the recent work of Revel and his colleagues³³. They have isolated a third protein factor which stimulates the binding of bacteriophage RNA to 30S ribosomal sub-units. Whether the messenger RNA binds directly at the codon for chain initiation and only at that codon is unknown. The results of Dahlberg and Haselkorn^{31,32} and Takanami et al.³⁴ show that such

specific messenger–RNA–ribosome interaction does occur. The suggestion has been made, however, that F-Met-tRNA is also involved in this specificity³⁵.

The GTP requirement for the F-Met-tRNA binding was not observed in much of the original work because the magnesium ion concentrations which were used were in the range 10-20 mmolar. At these concentrations F-Met-tRNA and Met-tRNA are bound to the oligonucleotide-ribosome complex without addition of GTP. Several groups have shown that accuracy in translation of messenger RNA is increased by using magnesium ion concentrations in the range 4-8 mmolar. Whether such concentrations approach physiological conditions more closely than do the higher concentrations which we used before for in vitro translation is not easily resolved. Nevertheless formation of the initiator–tRNÅ–ribosome complex occurs with f2 RNA at an optimum concentration of 5 mmolar magnesium ion, both in the absence and presence of initiation factors¹¹. The GTP requirement which is observed in the presence of initiation factors at 5 mmolar magnesium ion endows a stringent specificity for the formyl group. A similar requirement and specificity have already been reported where the trinucleoside diphosphates ApUpG and GpUpG were used instead of f2 RNA¹⁸. Recently other laboratories have also shown the requirement for initiation factors and GTP in the binding of F-Met-tRNA to an oligonucleotide-ribosomal complex18,20,36,37. It is generally agreed that at magnesium ion concentrations above 10 mmolar the enhancement of the binding reaction by factors and GTP becomes diminished or lost altogether.

The reaction of F-Met-tRNA with puromycin is analogous to normal peptide bond formation. Because both initiation factors and GTP are required to bind F-Met-tRNA, it follows that both the reagents must be present for the formation of F-Met-puromycin at 5 mmolar magnesium ion. The ability of puromycin to react with an acyl-tRNA has been taken as an indication that the

tRNA occupies the peptidyl-tRNA binding site on a ribosome³⁸. Although the F-Met-tRNA which is bound in the presence of GMP-PCP does not react readily with puromycin, the binding shows features which can be attributed to binding in the peptidyl-tRNA site. These include (1) a low magnesium ion requirement^{4,10}, (2) the dual coding of ApUpG and GpUpG for this tRNA4,10,10,10,10, and (3) the requirement for initiation factors which apparently do not influence the binding of amino acyl-tRNAs¹⁶. In spite of these considerations we cannot discard the single entry site model in which F-Met-tRNA first enters the amino-acid site and is subsequently shifted into the peptide site with the concomitant hydrolysis of We cannot explain why F-Met-tRNA, which, although bound in the presence of GMP-PCP, reacts very slowly with puromycin. It is unlikely, however, that hydrolysis of GTP is involved in the actual step of peptide bond formation because the F-Met-hexanucleotide fragment of F-Met-tRNA produced by the action of T₁ ribonuclease reacts with puromycin on the 50S ribosomal sub-unit without any requirement for GTP39. Furthermore, the puromycin reaction occurs at higher magnesium ion concentrations, that is, 10 mmolar, without any apparent requirement for GTP³⁸. If GTP were a source of energy for driving peptide bond formation, such a subtle change in the concentration of magnesium ions would not be expected to abolish the requirement.

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Fitting Cosmological Models to the Radio Source Counts

by

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Inspection of a properly formulated distribution for radio sources of local luminosity shows that the distribution is not as wide as has been thought. The distribution fits a Gaussian curve, with a standard deviation of one, to a good approximation.

No completely satisfactory or unique interpretation of the radio source counts by cosmological models has yet been achieved, but sufficient progress has been made to justify the optimism that it is now only a matter of time before this objective is reached. Because the counting of radio sources has demonstrated more clearly than any other test that the large scale features of the universe have evolved from an earlier denser state, it seems highly probable that, with an accepted explanation of the distribution of radio sources in depth, many questions in $cosmole\mathbf{g}y$ will be answered. In this article I shall discuss some important features of the problem which have emerged from recent investigations.

In view of the sustained evidence of a large scale isotropy in the distribution of the sources1,2, my early conclusion³ still holds. This is that in the past radio sources must have had a greater mean power than they have now, or they had a higher space density relative to

ordinary galaxies than is observed now, or (very plausibly) both these characteristics applied in some degree to the earlier radio sources. The problem is to determine the extent to which these evolutionary trends developed, and if possible to establish at the same time the geometry and kinematics of the underlying cosmological model.

It is now clear from the work already carried out4-7 that both "power evolving" and "density evolving" models can reproduce many features of the counts. In particular, the $\log N(S)$ against $\log S$ graph can be fitted quite closely on either hypothesis, provided an appropriate limiting epoch t^* and thus a limiting red-shift z^* for any given cosmolegical model is specified. The evidence acquired by Davies and myself from a representative range of models^{4,5,7} is that, if all classes of radio sources are allowed to evolve, then density evolving models (having no power evolution) require a significantly lower limiting red-shift z* than models evolving in power only. For

mixed models it became clear that the more density evolution there is relative to power evolution the lower z^* becomes, to such an extent that even in a model retaining substantial power evolution z^* had to be less than the red-shift of some recently identified quasars.

A significant discovery made by Davies and myself^{4,5} is that, in any model giving a satisfactory fit, the dispersion of power among the sources dominating the counts must have been much less in the past than that established from complete samples at high flux density S. This result has now been confirmed by Longairs, who found it impossible to fit the counts with the dispersion found at high S. Longair has taken the line that this indicates that only the high powered quasars have evolved, in either power or density, and that statistically the less powerful radio galaxies have retained the same mean power and space density relative to ordinary galaxies which they possessed at their first appearance in the universe. This particular hypothesis seems unlikely to be substantiated for at least two reasons. One is that the power distributions predicted for sources having $S \ge 20$ flux units in Longair's models (see his Fig. 7(c)) are at pronounced variance with the observed distribution for $S \ge 20$ flux units. The latter is shown in Longair's Fig. 5(a), and from wider statistical samples in Longair and Scott's* Fig. 7. As might be expected from Longair's hypothesis. there is a definite peak in the predicted distributions at $P_{178} = 10^{27}$ or 10^{28} W c/s⁻¹ ster⁻¹ which does not correspond to reality. It also seems that not enough power evolution has been allowed to take place in the range $10^{24} < P_{178} <$ 1026 W c/s-1 ster-1. Another argument against the hypothesis is that the recent work by Ryle and Longair, suggesting that the quasars, radio galaxies and normal galaxies are part of an evolutionary chain at a given epoch, may be interpreted as evidence against the possibility that statistical evolution with epoch is confined to quasars alone.

It seems more probable that, while the discovery of Davies and myself certainly implies a differential secular evolution among the different classes of radio source—presumably because intergalactic conditions affect the power, numbers and lifetimes of the sources—this evolution should be in the sense of narrowing down the power dispersion about the median power class observed at successively lower values of S. Because it seems certain from the earlier trials, and also from more recent evidence^{10,11}, that a successful model will incorporate at least some power evolution, this means that while the median power, in a given range of S, increases as S decreases, the numbers of sources on either side of the median must relatively diminish, whatever the rate of increase of their absolute numbers.

Such a trend does not exclude evolution in power at the higher end of the luminosity distribution. On the contrary, assuming that the quasars are at cosmological distances, because they all have large red-shifts those actually observed should properly be regarded as being chiefly outside the local luminosity distribution of radio sources, but may be interpreted in terms of a luminosity distribution the median of which moves upwards, although its dispersion is narrowing, as S decreases. A small contribution by high powered quasar type sources to the local luminosity distribution may, of course, be inferred if many local sources have passed through a quasar stage, even if none is observed locally at the present time.

It is therefore clear that what is taken to be the local distribution of radio sources is very important to the whole construction of evolving models designed to interpret the counts. Fortunately, it seems that recent systematic investigations into the distribution of power at high S contain the answer to this problem. Here I wish to make some observations which are relevant to model fitting, and to draw some conclusions from recent data which are not immediately apparent from the form in which they have been presented.

Adopting the terminology of Longairs, I shall distinguish between the local "luminosity distribution" and the local "luminosity function". In theory the former is the (arbitrarily scaled) frequency distribution, $n_0(P)$, of sources observed with S values exceeding some sufficiently high value S_0 . Thus $n_0(P)$ dP is proportional to the number of sources in the power range P to P+dP (at 178 Mc/s, say) with $S > S_0$, where \tilde{S}_0 is chosen as high as possible consistent with obtaining a local luminosity distribution from statistically adequate samples. local "luminosity function" is $\rho_0(P)$, where $\rho_0(P)$ dP is the number of sources in the range P to P+dPin unit volume of space at the present epoch. As has been shown in detail3,4, in any theoretical model which is spatially isotropic and in which an ideally smooth distribution of sources is assumed, there is the limiting relation as $S \to \infty$

$$n_0(P) \propto \rho_0 P^{3/2}$$
 (1)

In any such theoretical model, this relation will in fact be realized as $S \to \infty$, and so it is of basic importance to choose the correct scaling of $\rho_0(P)$ so that the theoretical log N against log S curve shall match correctly the observed data when evolutionary and statistical effects have been allowed for in the latter.

The relation (1), however, will correspond to, and can be matched with, reality in this way only if $\rho_0(P)$ can be determined from a tolerably small region of our neighbourhood, so that the static Euclidean approximations. implied in relation (1), can apply and will not be affected by the very evolutionary characteristics which we are trying to analyse. This means restricting the data as far as possible to radio sources identified within regions of small red-shift. In particular, the light-time must not exceed the average of individual radio sources by too many orders of magnitude. For the majority of sources a lifetime of between 106 and 107 years seems to be appropriate, and so an upper limit to the time lapse for suitable identifications will be about 5×108 years, corresponding to red-shifts z < 0.05 approximately. At this range the kinematic effects of red-shift are still small and cosmological densities are increased by a factor of only 1.16, which is probably a tolerable deviation from static uniform conditions.

At z=0.05, radio galaxies of absolute photographic magnitude, $M_{\rm pg}=-20.5$, have apparent photographic magnitude, $m_{\rm pg}\sim16$, and fortunately this is the limiting brightness above which complete catalogues of galaxies are available, for example, those of Vorontsov-Velyaminov and Zwicky. In a recent paper, Caswell and Wills¹² made use of these catalogues and a complete sample of radio sources of the 4C survey, having S>2 flux units, to establish the local $\rho_0(P)$ distribution in the range $3\times10^{22} \le P_{178} \le 10^{25}$ W c/s⁻¹ ster⁻¹. Below this, the $\rho_0(P)$ distribution in the range $10^{21} \le P_{178} \le 3\times10^{22}$ was given previously by Heeschen and Wade¹³. Thus in the range $10^{21} \le P_{178} \le 10^{25}$ the luminosity function $\rho_0(P)$ has been established from complete samples of galaxies having z<0.05, and therefore fully justifies the description of "local" in the sense required for model fitting. For $P_{178}>10^{25}$, W c/s⁻¹ ster⁻¹ identifications have to

For $P_{178} > 10^{35}$, W c/s⁻¹ ster⁻¹ identifications have to be made at higher flux densities if the sources are not to be located too far away to be considered local. It may, however, be calculated retrospectively that within a red-shift z=0.05 there are statistically only about 0.08 radio sources in one steradian, or about one in the whole sky, which have powers in the range $3 \times 10^{25} \le P_{178} \le 3 \times 10^{26}$ W c/s⁻¹ ster⁻¹. To obtain any statistics at all, identifications have therefore had to be used of galaxies having $m_{\rm pg} > 16$ and z considerably in excess of 0.05. In practice, the $\rho_0(P)$ for $P_{178} > 10^{25}$ W c/s⁻¹ ster⁻¹ has been calculated by Longair⁶ from the n(P) distributions⁸ in successive ranges of S down to S=15 flux units. For example, Longair⁶ presents a certain complete sample of forty-two sources having $S \ge 20$ flux units at 178 Mc/s,

thirty-eight of which have now been identified. Assuming that radio galaxies have $M_{\rm pg}=-20.5$ and that such sources having $m_{\rm pg}>20$ cannot usually be identified, it follows that identifications of radio galaxies are possible only if z<0.25. In this case, all sources of the sample having $P_{178}<1.2\times10^{26}~{\rm W}~{\rm c/s^{-1}}~{\rm ster^{-1}}$ should be identified, but of those having $P_{178}>1.2\times10^{26}~{\rm W}~{\rm c/s^{-1}}$ ster-1 some will have z>0.25 and lie outside the range of identifications. All those sources which have $P_{178}>1.2\times10^{26}~{\rm W}~{\rm c/s^{-1}}$ ster-1 and z<0.25 should be identified, but even within this red-shift there is statistically still only about one source in the whole sky of power $2\times10^{26}<P_{178}<3\times10^{27}$. As it happens, ten of the thirty-eight identified sources of the specified sample are quasars, having $M_{\rm pg}\sim-25$, with P_{178} ranging from 10^{26} to $2\times10^{28}~{\rm W}~{\rm c/s^{-1}}$ ster-1 and z values of order unity.

It is therefore to be concluded that because even at a red-shift z=0.25 the light-time is already about 2×10^9 yr, the $\rho_0(P)$ distribution for $P_{178}>10^{26}$ W c/s⁻¹ ster⁻¹ is precariously defined. This has been recognized by Longair, who for the purpose of model fitting simply guessed by extrapolation the local "luminosity function" for $P_{178}>10^{26}$ W c/s⁻¹ ster⁻¹, the plausibility of which was to be tested by comparing the predicted n(P) distributions with those observed at successive values of S. Evidently this procedure will be somewhat circular, because the necessary "evolution" to fit the observed distribution will depend on what has been postulated for $\rho_0(P)$. Unfortunately, it is just the doubtful range of $\rho_0(P)$ that Longair has chosen to evolve in his models. This error is likely to be considerably reduced if, in accordance with the view presented here, the median value of the local "luminosity distribution" is the dominant zero-point of departure for evolutionary trends.

A point I now wish to make is that it is questionable whether a correctly formulated "local luminosity distribution" is as broad as recently claimed by Longair⁶, or has as high a median power as can be inferred from the earlier work by Longair and Scott⁸. By the "local luminosity distribution" I mean the $n_0(P)$ distribution in the sense of satisfying relation (1). Already it seems that the latest information provided by Caswell and Wills¹² leads to a distribution in this sense which is significantly different from the observed n(P) distribution for $S \geq 15$ or 20 flux units. Fig. 1 reproduces the $\rho_0(P)$ distribution suggested by Caswell and Wills for the complete range $10^{20} \leq P_{178} \leq 3 \times 10^{27}$. Clearly backward extrapolation from high red-shift regions has had to be effected

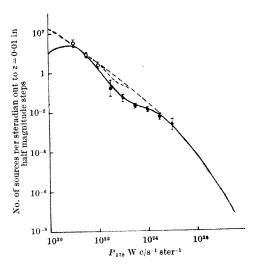


Fig. 1. The local luminosity function (full line) for the radio sources according to Caswell and Wills¹². This gives effectively the local source density $\varrho_0(P)$, expressed here as the number of sources in half magnitude steps per steradian out to a red-shift z=0.01. The new function corrects an earlier version by Longairs (broken curve). Full circles indicate data obtained by Caswell and Wills, open circles by Heeschen and Wade¹³.

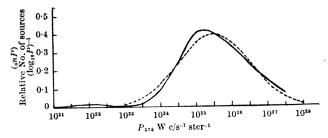


Fig. 2. The local luminosity distribution $n_0(P) \propto \varrho_0(P) P^{a/s}$ at 178 Mc/s as it would appear in a given range of high S in the Euclidean limit as $S \to \infty$, converted from the local luminosity function of Caswell and Wills¹². The ordinate is normalized so that it gives the relative number of sources per unit of $l(l=\log_0 P)$, that is, the ordinate is $\varrho_0(l)P^{a/s}$ where $\varrho_0(l)$ is number of sources $m^{-3}l^{-1}$, and $j\varrho_0(l)P^{a/s} dl=1$. The mode of the distribution is at $P_{17s}=1.5\times 10^{2s} \text{ W c/s}^{-1}$ ster⁻¹ and the median at $3.16\times 10^{2s} \text{ W c/s}^{-1}$ ster⁻¹. The Gaussian distribution (broken curve) shown for comparison has ordinate $(\sqrt{(2\pi)}\sigma)^{-1} \exp{-l^2/2\sigma^2}$ where σ has been chosen as unity and l=0 is taken at $P_{17s}=3.16\times 10^{2s} \text{ W c/s}^{-1}$ ster⁻¹.

to obtain $\rho_0(P)$ for $P > 10^{26}$ W c/s⁻¹ ster⁻¹, but the shape of the associated $n_0(P)$ curve is only marginally sensitive, within the likely error, to these approximations at high P (see further comment later). In Fig. 2, I have plotted the $n_0(P)$ distribution, calculated in the sense of relation (1) from Caswell and Wills's $\rho_0(P)$ function. The ordinate is normalized to give the relative number (fraction) of sources per unit of $\log_{10}P$ at each value of P. Thus, if the distance along the horizontal axis is measured in units of $l = \log_{10}P$, the total area under the graph is unity. For comparison I have also shown a log-Gaussian curve to the same scale, its equation being

$$n(l) = \frac{1}{\sqrt{(2\pi)}\sigma} e^{-l^2/2\sigma^2}$$
 (2)

where σ has been chosen equal to unity.

It is seen that a Gaussian curve with standard deviation unity is still, for theoretical purposes, an adequately close approximation to the local "luminosity distribution", contrary to Longair's claim that "the better estimates of the luminosity distribution indicate that it is much broader and flatter than a Gaussian". Moreover, it is to be observed that the median \bar{P}_{178} of the Gaussian curve of best fit is at about $3\cdot 2\times 10^{25}$ W c/s⁻¹ ster⁻¹, while the mode of the actual $n_0(P)$ distribution is even less at about $1\cdot 5\times 10^{25}$ W c/s⁻¹ ster⁻¹. In Fig. 3 the $n_0(P)$ distribution (broken curve) is contrasted with the observed n(P) distribution given by Longair and Scott* for $S\geq 15$ flux units. The latter is drawn with the same normalization for $n_0(P)$. The Long ir–Scott curve has a mode-median of about $P_{178} = 8\times 10^{25}$ W c/s⁻¹ ster⁻¹.

It is to be concluded therefore that the local luminosity distribution on which we must regard past-directed evolution to operate is neither as broad nor as high powered as has been thought in the past. On the contrary, the evidence is that already at $S \geq 15$ flux units the n(P) distribution has evolved away from the $n_0(P)$ distribution. Furthermore, a lower value for \bar{P} than previously assumed implies a somewhat smaller distance scale for the unidentified sources.

Even with the $n_0(P)$ curve given in Fig. 2 there is evidence that a degree of cosmological error in estimation is still present at higher powers. Calculation shows that for the Caswell and Wills $\rho_0(P)$ function, given in Fig. 1, the integral $\int \rho_0(l) P^{3/2} dl$ in the range $10^{20} < P_{178} < 3 \times 10^{27}$ W c/s⁻¹ ster⁻¹ is about 2.8×10^{-36} , $\rho_0(l)$ being expressed in sources $m^{-3}l^{-1}$. If the limiting relation (1) applied at high S, then the corresponding N(S) would be

$$\frac{1}{3} \, \times \, 2 {\cdot} 8 \, \times \, 10^{-36} \, S^{-3/2}$$

At S=35 flux units this would yield $N\sim 4.5$ ster⁻¹. The observed count down to S=35 flux units is $N=4\pm0.8$ ster⁻¹. Because the total sky count for this range includes

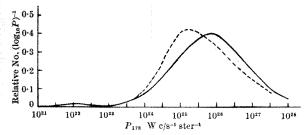


Fig. 3. The luminosity distribution according to Longair and Scott*, as observed among radio sources having flux density in the range $S_{178} \ge 15$ flux units and emission power $10^{21} < P_{178} < 10^{28}$ W c/s^{-1} ster $^{-1}$. The median and mode of the distribution are both at about $P_{178} = 8 \times 10^{28}$ W c/s^{-1} ster $^{-1}$. The local distribution $n_b(P)$ of Fig. 2 is also shown for comparison (broken curve).

sources of considerable red-shift, for example, 3C 295 and 3C 298, which have flux densities S_{178} equal to 73 flux units and 44 flux units with red-shifts 0.46 and 1.44, respectively, it is indicated that a value of 2.8×10^{-36} for $A = \int \rho_0 P^{3/2} dl$ is still probably a little too high. Indeed, an extrapolation of Gower's curve of the complete counts to date, for example, down to $S \sim 100$ flux units, can produce a value for A as low as 2×10^{-36} . At S = 35 flux units this would yield a Euclidean count of about 3.2 ster-1, which is still not outside the error range for the observed count at 35 flux units.

Attempts at fitting cosmological models to the counts of radio sources indicate that the dispersion of power must narrow into the past, presumably because intergalactic conditions affect the powers, numbers and life-

times of the sources. Inspection of a properly formulated "local luminosity distribution" for radio sources shows that it is not as wide as has been thought and that a Gaussian curve having $\sigma=1$ is still a good approximation to it. Moreover, the median power P_{178} is no more than about $3\cdot2\times10^{25}$ W c/s⁻¹ ster⁻¹, while the mode is at $1\cdot5\times10^{25}$ W c/s⁻¹ ster⁻¹, and there is evidence that at $S_{178} = 15$ flux units the sources have already "evolved". It seems certain that to explain the counts "power evolution" into the past will be necessary, although there may also be some limited "number evolution". Although the former effect gives a relatively greater distance scale for the unidentified sources, the value of \bar{P} now suggested may well require a limiting red-shift z^* for the sources of no more than 3. It is indicated that whatever the evolution has been, it is not confined to quasars.

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Correlation between Marine and Terrestrial Pleistocene Successions

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Using evidence from cores from Marks Tey, Essex, it is concluded that the glacial cycles suggested by Emiliani give a much truer picture of the chronology of glacial events in Europe than those suggested by Ericson et al.

NOBODY today would seriously attempt to review the methods available for dating the glacial events of the Pleistocene without considering the evidence which has been obtained from the study of deep-sea cores. The reason is that it is only in the relatively stable environment of the ocean bed, far from land, that continuous successions are to be found. It is therefore most unfortunate that two of the most widely quoted interpretations^{1,2} of the deep-sea evidence should differ so enormously; the two interpretations are compared in Fig. 1. In connexion with this figure, two points must be clarified.

First, the discrepancy in interpretation is in no part caused by differences in the absolute dating of the events recorded in the cores; over the upper 400,000 yr the two interpretations have in part been derived from identical material3. Second, the shorter chronology proposed by Emiliani is in no way incompatible with dates of well over 106 years which have been obtained for glacial events in several parts of the world, or with the dating of the base of the Pleistocene at about 2×10^6 years (ref. 5), because he has not yet examined in detail material relating to the earliest periods.

If we accept the fact that cross-correlation between deep-sea cores is a valid procedure, the deep-sea record becomes a yard-stick against which Pleistocene events may be dated. The difficulty lies in the recognition of terrestrial climatic events in the marine record. For this purpose, the definition of the terms "glacial" and "interglacial" must be framed in such a manner that the events which they define may be recognized in a sequence of marine deposits. For example, evidence for a custatic fall in sea level of 100 m or more could be regarded as a suitable criterion by which to recognize a glacial episode.

Unfortunately, in deep-sea deposits the direct evidence for change in sea level is masked by other effects, while stratigraphically long sequences of undisturbed sediment are not generally available from shallower water. It has recently been shown, however, that the abstraction of large quantities of isotopically light water from the oceans to build up continental ice sheets results in an easily detectable change in the isotopic composition of the oceans. Thus isotopic analysis provides a convenient means for identifying glacial events in a marine sequence.

The definition of the term "interglacial" is less straight-Suggate has tackled the problem, and has provided a definition which many workers find useful. By his definition, "interglacial" implies both the melting of ice sheets to about their present level, and the maintenance of a warm climate for a sufficient length of time to permit certain vegetational changes to take place. According to this definition, the length of Postglacial time has been sufficient to characterize the present as "interglacial" rather than "interstadial".

It is clear that, according to this definition, zone x of Ericson *et al.*, or core stage 5 of Emiliani, represents an interglacial, because isotopically it appears similar to the present, implying the melting of ice sheets, while its duration was about 30,000 yr (ref. 10). Presumably it may appropriately be identified with the Last Interglacial.

The interglacial before last is identified in the Ericson chronology with zone t, which had a duration of about 600,000 yr (ref. 5). If we consider as an alternative that zone x represents the last interglacial, as already suggested, then zone v, with a duration of about 250,000 yr, must represent the previous interglacial. According to Emiliani's scheme, his core stage 7, with a duration of about 50,000 yr, should be identified with the penultimate interglacial. It seems that research into the Pleistocene could be seriously hampered by the acceptance of an estimate for the duration of an interglacial which could be in error by a factor of ten.

For this reason, an estimate of the absolute time span of the interglacial before last would be of critical importance, though its absolute age may not be known. In this connexion the evidence which has been obtained from the complete sequence through the Hoxnian interglacial at Marks Tey would seem to be crucial.

At Marks Tey, Essex, a series of Pleistocene lacustrine deposits infill a narrow trough, floored with boulder clay but cut into the subglacial surface. Pollen analysis has shown that continuous deposition took place in this basin from Lowestoftian Late-glacial to Gipping Early-glacial times, that is to say throughout the Hoxnian¹¹.

A borehole at the Marks Tey brickworks, put down near the deepest part of the lacustrine basin, yielded a particularly interesting stratigraphy. Beneath a thickness of Gipping Early-glacial clays there occurred finely but evenly laminated organic clay-muds, which were shown

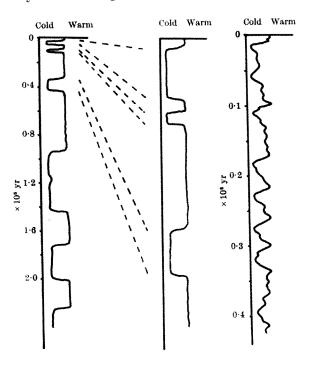


Fig. 1. Left: Pleistocene record in deep-sea cores, according to Ericson et al.¹ (as recently modified*). Centre: Upper part of left-hand curve, expanded time scale. Right: Generalized temperature curve according to Emilian¹, time scale as centre curve. The left and centre curves are based on the abundance of the foraminiferal species Globorotatia menardii, according to the method described by Ericson and Wollin². The right-hand curve is based on the relative abundance of the oxygen isotopes in the tests of planktonic foraminifera. The time unit is 10° years for all curves; the zonations are those of the workers cited (see ref. in text).

by pollen analysis to have been deposited during the Pre-temperate and Temperate zones of the Hoxnian interglacial. Unfortunately, the uppermost portion of these laminated clay-muds had been disturbed by slumping and consequent brecciation in this boring, although undisturbed deposits, covering the complete latter part of the Hoxnian interglacial, are well preserved elsewhere in the basin.

The general stratigraphy at this point, from borehole and brickpit sections, is

F about	22 m	Grey silty clay, generally	Early-Gipping
E	5·3 m	laminated Brecciated, finely lamin-	Hoxnian Zones III a-b, ? IV
D	6·5 m	ated organic clay-mud Finely laminated organic	111 a=0, . 1 ·
		clay-mud The upper 4·5 m evenly and distinctly	Hoxnian Zones II c and III a
		laminated Below this more finely and so less distinctly	
		laminated Becoming more poorly laminated towards	Hoxnian Zone I
C	1.3 m	the base Grey clay, sometimes shelly	Late-Lowestof- tian
B A		Sand and fine gravel Poulder clay (not proved in this boring)	

The upper, Gipping grey clay (F) shows clay-silt lamination akin to glacial varves. The rhythmic lamination structures of the underlying clay-mud are of an entirely different nature. Fine, silty, organic laminae alternate distinctly and regularly with grey, buff-yellow or white laminae, each lamination pair being generally less than 1 mm thick.

These structures have been examined in thin section. Where well developed, two features emphasize the lamination of the clay-mud: (1) the regular deposition of distinct layers of the crowded frustules of the diatom Stephanodiscus astraea var. minutula (the light laminae); (2) the gradually increasing deposition of organic material following the period of diatom deposition, leading to the production of darker intervening laminae. It is difficult to demonstrate fluctuations in the precipitation of calcium carbonate during this sedimentation cycle, because the matrix of the entire sediment consists of that material. Nevertheless, a period of high calcium carbonate precipitation seems to have taken place immediately after the formation of the diatomaceous layer, because this is generally overlain by a thin, almost pure layer of calcite crystals also contributing to the light laminae.

Both calcium carbonate deposition and diatom flushes are known to have a seasonal rhythm in modern lakes. Calcium carbonate deposition is at a maximum in spring and early summer as an indirect effect of rapid plant growth, photosynthesis and rising temperature on the carbon dioxide balance in lake waters¹². Lamination structures formed by the precipitation of annual laminae of calcium carbonate in this way have been described from sediments of the Faulenseemoos near Spiez, Switzerland¹³, and from lakes in Ontario¹⁴.

In North German lakes today, Stephanodiscus astraea is known to produce flushes in late winter and early spring, although it is present in the plankton throughout the year. Although usually annual, these flushes are probably controlled by nutrient conditions rather than by temperature.

Further work, possibly finer pollen analyses, will be necessary to give definite indications of the annual or non-annual origin of the lamination structures at Marks Tey. On balance their apparent cycle of deposition

corresponds to known annual sequences of depositional events in modern lakes. It would certainly be difficult to invoke longer cycles of, say, 5, 10 or even 50 yr to

produce such regular sedimentary features.

A total of 4,486 lamination pairs have been counted from cores from the top 4.46 m of the undisturbed claymud (D), where lamination was best developed (possible counting and sampling error under 10 per cent). This stratum covers a sizable part of the temperate period of the Hoxnian interglacial (pollen zones II b and most of III a, see Fig. 2). In the clay-mud below this the lamination structures are finer and cannot be counted accurately, but a reasonable estimate is that between 5,000 and 10,000 lamination pairs are represented. The composite pollen diagram, Fig. 2, shows the extent of these laminated portions of the interglacial deposits within the context of the vegetational succession of the whole interglacial. The vertical scale of this diagram is based on thickness of deposition and suggests that the latter part of the Hoxnian interglacial, from which we unfortunately have only brecciated or unlaminated deposits, occupied a similar period of time to the earlier part already discussed.

If the lamination features prove to be annual, then both the nature and time-scale of the vegetational changes which took place during the early part of this interglacial are closely paralleled by those of the Flandrian Postglacial period. An estimate for the total length of the Hoxnian interglacial period would be of 30,000–50,000 yr. The only other direct assessment of the duration of an interglacial period is that of 27,000–35,000 yr from the possibly Cromerian deposits at Bilshausen, Germany¹⁸, again a figure based on lamination structures.

It should be emphasized, at this point, that there are no signs of any important unrecorded vegetational or sedimentary hiatus within the Hoxnian deposits at Marks Tey. Furthermore, clear stratigraphic evidence exists there for truly glacial conditions preceding the Hoxnian interglacial, and likewise for severely arctic conditions succeeding it, although the site was never actually reglaciated. The vegetational evidence from Marks Tey provides a conclusive correlation of the Hoxnian with the Holstein interglacial on the Continent. Nowhere in North-western Europe does the vegetational record of this interglacial support such conjectures as a two-phase

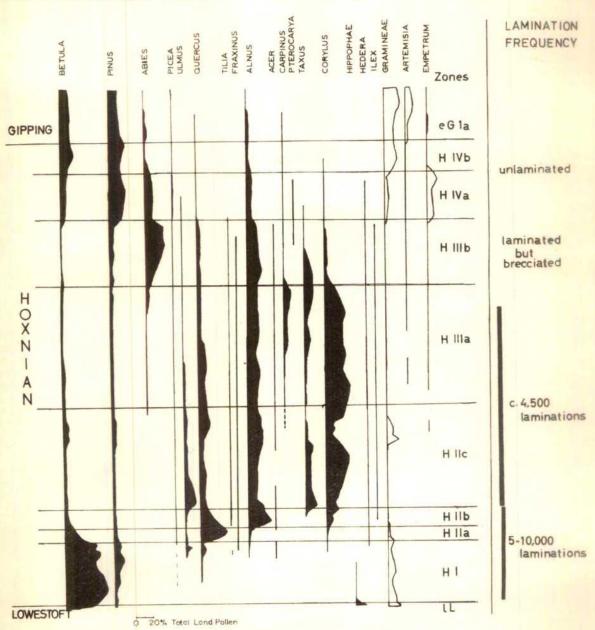


Fig. 2. Hoxnian vegetational succession at Marks Tey, Essex.

penultimate interglacial interrupted by a cool interval. or the more complex subdivisions sometimes interpreted from the study of river terraces¹⁶. There is no evidence to support the application of the old term "Great Inter-glacial" to the penultimate interglacial as it is now

defined and recognized.

It is quite clear that the evidence for the duration of the Hoxnian interglacial, as recognized here, is compatible in scale with the oxygen isotope chronology proposed by Emiliani, but not in any sense with that proposed by Ericson et al. and modified by Glass et al.5. The same may be said for the evidence obtained from Bilshausen 15. The fact that the record obtained by Emiliani is more complex than the classical picture of the timing of the Ice Ages cannot any longer be taken to imply that it is in error. On the other hand, it does imply that identifying named terrestrial events with recognized points in the marine sequence can only be done with the aid of accurate absolute dates, as Emiliani has pointed out2. It is not sufficient to identify the largest events with the best known names and leave it at that, as seemed tempting when marine sequences first became available; this is especially true in view of the fact that the glacial successions are not definitively established even in the Alps and Northern Europe.

We are therefore forced to the conclusion that although Glass et al.5 have obtained a complete and reproducible sequence through the two million or so years of the Pleistocene, the micropalaeontological record which they have obtained bears little relation to the chronology of glacial events in Europe. In contrast, although Emiliani's oxygen isotope chronology may only span about one-fifth of the duration of the Pleistocene, it records glacial cycles on the scale at which they occurred in Europe.

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Shatter Cone Orientation at Gosses Bluff Astrobleme

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Shatter cones caused by shock fracturing are widely developed at the Gosses Bluff cryptoexplosion ring structure in central Australia. The force field can be reconstructed whereby the applied shock arrived centrally and from above, which is consistent with a cosmic impact. For this and other reasons, Gosses Bluff is an astrobleme.

Gosses Bluff is an isolated ring structure of upturned Lower Palaeozoic rock nearly 3 miles across, situated in the Amadeus Basin of central Australia, about 200 km west of Alice Springs. It rises about 700 ft. above the surrounding plain and is breached by an intermittent stream, on the north-east side. The term "bluff" is something of a misnomer, or at least is an inadequate description.

The interior bowl shaped basin might well be misinterpreted as a modern meteorite crater (Fig. 1). It is, however, at the same level of erosion as the surrounding plain and is not depressed, and there are no remnants of lake beds which would indicate that a former closed basin existed here. Furthermore, geological inspection shows that it is the upturned ring of a great dome, the soft centre (Larapinta) of which has been erosionally gutted, forming the central bowl. Lining the bowl, the cliff-forming Meerenie sandstone now stands etched out in high bold relief and is in turn surrounded by the Pertnjara formation.

If Gosses Bluff is to be interpreted as a meteorite structure, then it can only be regarded as an astrobleme-an ancient cosmic impact scar, with the bluff per se being the uplifted central dome of a much larger circular structure. Although the strata are largely hidden from view outside the bluff, this interpretation is quite permissible. Gravity and magnetic surveys at the Bureau of Mineral Resources show that the bluff marks the centre of a circular deformation 12 miles wide. A structure of this size is also apparent

as a double "ghost ring" on a Gemini IV photograph from near space (Fig. 2).

On the basis of much evidence of explosive force, Crook and Cook1 rejected an earlier view which was widely held, that Gosses Bluff was a salt diapir: while agreeing that it was of cryptoexplosive origin, Crook regarded it as an astrobleme and Cook thought it was cryptovolcanic.



Fig. 1. Aerial oblique view of Gosses Bluff looking south, showing the ring of upturned strata,



Fig. 2. Gemini photo of Gosses Bluff from near space. Dark circle in centre marks the upturned ring of rocks 2.5 miles across. Note "ghost ring" surrounding Gosses Bluff which identifies the full extent of the structure, 12 miles across.

Cook² has since revised his opinion and concurs in the astrobleme interpretation. Daniel Milton and Robin Brett, who are at present mapping this structure in detail as a joint project of the US Geological Survey and Australian Bureau of Mineral Resources, also regard it as an astrobleme. Their results will soon be published. This article is limited to a discussion of shatter coning at Gosses Bluff which further supports this interpretation.

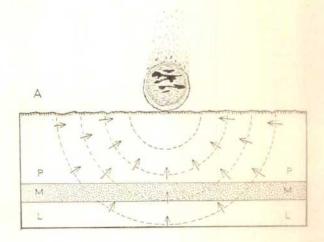
Shatter cones (Fig. 3) were first noted by Crook and Cook, who, however, regarded them merely as an incidental aspect of the structure with the orientation of the shatter cone being random. Actually, Gosses Bluff is the most intensely shatter coned of the eighteen sites I know around the world⁴ and the cones show a high degree of preferred orientation which reveals the shock force field impressed on the structure. Crook and Cook also doubted the validity of shatter cones as a criterion for astroblemes, but I have attempted to answer their objections elsewhere⁵.

In order to study the shatter cone orientation and distribution at Gosses Bluff, I made several spoke-like traverses of the structure commencing at the centre (ground zero) and working radially outward. Shatter coning was found to be extensively developed throughout the structure on a scale which I have never before observed,



Fig. 3. A group of shatter cones from the Pertnjar a formation showing orientation oblique to bedding.

with the possible exception of the Vredefort Ring of South The coning is least developed in the southern quadrant and most strongly developed in the northern quadrant where, in places, nearly all rock talus revealed shatter coned faces. There is also a strongly preferred orientation of the cone apexes. In the vertically dipping Larapinta formation of the central basin and in the Meerenie sandstone marking the interior "lining" of the uplifted ring, the shatter cones are oriented radially outward—that is, normal and upward with respect to bedding. Further out from ground zero (4,000-7,000 ft.) in the Pertnjara formation, the shatter cones point generally outward but only at angles of about 60° upward with respect to bedding. Still further out, at two sites, shatter coning is oriented upward and parallel to bedding. The first of these two sites occurs close to the spot where the road track enters the breach in the bluff ring at a position about 10,000 ft. north-east of ground zero. The other site is a hogback, fully detached from the ring proper and lying about 13,000 ft. north-west of ground



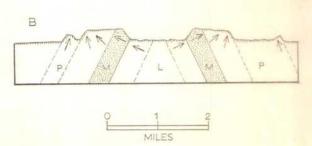


Fig. 4. Diagrammatic representation of shatter cone orientations at Gosses Bluff revealing the shock wave force field which was impressed on the structure. A. Impact of bolide and radial spreading of shock wave instantaneously prior to upheaval. L. Larapinta group; M. Meerenie sandstone; P. Pertnjara formation. B. Presently observed orientation of shatter cones (arrows) in upturned strata. Vertical exaggeration, 2:1.

If one can envision a return of the rocks to their presumed pre-event, essentially horizontal position, we observe a pattern of shatter coning which reconstructs the shock wave force field which acted on the structure (Fig. 4). Before upheaval the shatter cones in the central region pointed directly skyward, those farther out pointed inward and upward, while those farthest out pointed radially inward. Because the apices of shatter cones point toward the impressed shock wave front, this indicates a shock originating from above as, most likely, the impact of a cosmic body with an apparent or effective diameter (that is, for purpose of impressing a parallel shock front) of about 7,000 ft. This derived diameter may well be beyond the limits of resolution of the

method and the object could have been much smaller because we are now examining an inverted mass of rock. The strata now at ground zero were originally positioned several thousands of feet deeper. This effect tends to enhance the normal and upward-to-bedding aspect of shatter cones. Nevertheless, the apparent diameter of the bolide would seem to have been at least a few thousand feet.

As elsewhere, there are exceptions to the preferred orientation noted here, but these are sufficiently few to be regarded as uncommon. Most often, fully inverted cone orientations are found; more rarely the cones point randomly. It is necessary, of course, to distinguish positive cone faces from negative ones and to measure the apical direction of a full 360° cone rather than the directions of various cone segments; otherwise confusion results, giving a false appearance of randomness. According to the impact interpretation, random and inverted orientations may be ascribed to the reflexion of shock waves which were still sufficiently intense to cause shatter

Outcrops in the outer annulus of the structure, and peripheral to Gosses Bluff proper, are few and far between. Those outcrops which were found were searched; no shatter cones were found. Possibly at this range of from 2 to 6 miles from ground zero, shock pressure had already dropped below the level required to produce shatter coning (20 to 80 kbars). It would not be surprising, however, if a further search revealed some shatter coning on a reduced scale. This places Gosses Bluff in an intermediate position among known shatter coned structures, in most of which shatter coning is confined to the central eye (for example, Serpent Mound, Ohio) and the very large astroblemes where shatter coning extends outward, 15 miles or more (Sudbury, Vredefort Ring).

At seven sites around the bluff, I found nests of breccia containing shatter coned fragments. In no case does the shatter coning extend into the breccia matrix. This is expected for an astrobleme because, in a cosmic impact, the target is first engulfed by the shock wave, followed almost instantaneously, but clearly separated in time, by brecciation and upheaval.

The bluff is circular in its external limits: the interior plan, however, as outlined by the Larapinta and Meerenie formation, has the form of an equilateral triangle with truncated basal angles and with a sharp apical angle to the north, giving the internal structure an overall pentagonal form. An imaginary line running from north to south through the apex provides an axis of bilateral symmetry. Such bilateral symmetry is a well known aspect of some other astroblemes3 and it could reflect the arrival of the bolide at an oblique angle from the south and towards the north. Shatter coning is clearly more intensely developed at the northern apex of the structure than elsewhere.

While I remain convinced of the astrobleme interpretation, numerous questions remain unanswered. what was the original form of the crater? Second, why is the central dome so large (about 3 miles) and uplift so great (about 10,000 ft.) with respect to the overall 12 mile diameter of the structure? One suggestion is that the cosmic bolide was a comet head combining sufficient excessive hypervelocity and low density to provide for a surface or very shallow detonation which enhances the central uplift effect6. Third, what is the age of the structure? As Crook and Cook point out, Gosses Bluff appears to have been exhumed after early Tertiary peneplanation so that a Mesozoic age seems reasonable.

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Molecular Shape and Odour: Pattern Analysis by PAPA

by

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The PAPA pattern recognition machine, consisting of an image dissector and computer, can rapidly and accurately make comparative measurements of molecular model silhouettes. This will be most useful for research on the stereochemical specificity of the sense of smell.

THERE is evidence that the specificity of the human sense of smell depends very largely on the molecular shapes of odorous compounds1-3. A successful but tedious manual method of measuring molecular shape was worked out by Amoore³. On each of three silhouette photographs of a scale molecular model, the molecular shape was estimated from measurements of thirty-six radii of the molecule (Fig. 1). This may be called the "manual radius method". It measured the molecular shape of the test compound in terms of its overall similarity to the molecular shape of a standard compound.

Professor A. Borsellino suggested to us that the molecular silhouettes could be scanned and computed automatically by the PAPA pattern recognition machine evolved by Palmieri et al.⁴⁻⁸ (PAPA, Probabilistic Automatic Pattern Analyser). The instrument scans the molecular silhouette with a reproducible collection of 4,096 random lines, many of which will intersect the silhouette (Fig. 2). This may be called the "PAPA intersection method". It can express the similarity in molecular shape between a test compound and the chosen standard. We have found that 1,600 comparisons of molecular silhouettes, which had previously taken about 3 months by hand, may be accurately accomplished by the PAPA machine in 3 hours.

The pattern recognition machine consists of three sections: an input, a computer and an output. In the input the patterns are surveyed to extract the required information, by measuring the most prominent features of the pattern. During the instruction phase, when known examples are displayed, these data are stored in the computer. Conversely, during the recognition phase, the features of an unknown pattern are compared with the information previously memorized, and the similarity between the unknown pattern and the learned examples is computed. At the output the results of the calculation are printed as a probability value, or converted to a classificatory decision.

In the PAPA machine the patterns are analysed by means of random lines called associative units (A units). There are various ways of generating and storing these random lines. They may be obtained from a special pseudo random noise generator. Its output arises from a digital to analogue transformation (operational summing and passive integration) of the random square wave signal of several feedback-shift-registers. Special gates prevent the lines from going beyond the frame border. Two generators are used for the X-Y scanning, which is reproducible, because the circuits are essentially digital and therefore resettable.

Each random line intersects the pattern a variable number of times, and a threshold circuit generates a binary signal according to one of several alternative criteria: counting the number of intersections (intersection method), or the numerical parity (even or odd); measuring the widths of the intersections (area method), or the time intervals between them. The whole set of binary signals is stored for every A unit, while all the examples of one class are being shown to the machine, and a description of that class is formulated with increasing accuracy. Different descriptions are subsequently obtained for each of the other classes of pattern presented. It should be understood that each and every pattern, known and unknown, is scanned with the same set of random lines, which allows direct comparisons to take place in the computer.

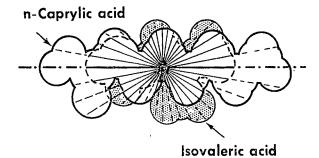


Fig. 1. The old manual radius method for measuring molecular shape. Top silhouette photograph of the elongated molecule of n-caprylic acid superimposed on that of isovaleric acid (the standard molecule for the sweaty primary odour). The centres of gravity and the main axes of the silhouettes were co-aligned. The differences in the lengths of the thirty-six corresponding radii (broken lines) measured the difference in molecular size and shape between these compounds. If the average radial difference was \vec{L} , the molecular similarity was expressed by the reciprocal form $1/(\vec{L}+1)$.

The use of a greater number of A units gives a higher percentage of correct recognition, but this advantage should be balanced against the longer time required and the limiting memory capacity of the machine. The principle of scanning with random lines permits the solution of entirely different experimental problems from a highly generalized point of view, thus avoiding possible bias

through the use of preconceived scanning shapes. On the other hand, the accumulation of information by a threshold circuit allows the classification to be executed by a linearization process, and at the same time makes the features extracted in this way adequately invariant. Thus in experiments with alphanumeric characters, patterns of varying size or position are easily recognized, although the machine was instructed with patterns of fixed size and position.

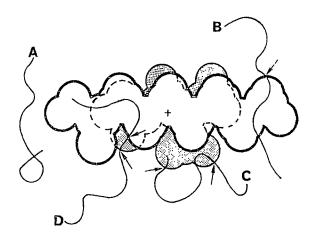


Fig. 2. The new PAPA intersection method. The machine scans each silhouette successively with the same collection of random lines, and counts intersections. For example, lines A and D each carry a similarity contribution, because A intersects neither silhouette and D intersects both silhouettes. Lines B and C each carry a dissimilarity contribution, for B intersects only one silhouette and C intersects only the other silhouette. Using 4096 random lines (A units) the machine computes probabilistically the molecular similarity in logarithmic form.

Although several different PAPA machines have been built with a variety of characteristics of performance, this experiment was performed with the most advanced design, now operating at the University of Genoa¹⁰. It may be thought of as a television camera linked to an electronic computer. It is a high-speed machine with a maximum recognition time of a few seconds, and can be arranged to view opaque patterns or transparencies. Its image-dissector camera, because of the non-storage properties of the tube, is very suitable for fast random scanning, while a computer unit with ferrite core memory rapidly accumulates the information. The answers are promptly printed out in the decimal number system, on a logarithmic probability scale with base 2.

Earlier PAPA machines have been successfully applied, for example, to recognize the rook and knight moves on a chessboard or to distinguish musical scores of J. S. Bach from those of an electronic composer¹¹. In recent months, to exploit the uncommon classificatory ability of the newest machine, some more practical problems have been presented, where the human visual system is quite unable to discern any pattern amidst a very large amount of data. For example, some preliminary experiments on weather prediction, directly from meteorological maps, have turned out to be very satisfactory.¹⁰

have turned out to be very satisfactory¹⁰.

The molecular silhouettes required by the present stereochemical problem, for displaying to the PAPA scanning camera, were prepared as follows. The scale molecular model (space filling type) was arranged in its most probable configuration and photographed in silhouette from three directions mutually at right angles². Rules of orientation were applied to ensure that the corresponding "top", "front" and "right" silhouettes of different molecular models represent comparable aspects. The positive prints were retouched with indian ink to fill any inter-atomic light chinks. The centre of gravity of the silhouette was found³ and marked by a small cross. The print was photographed again under dual electronic

flash with fixed geometry equipment and high contrast 35 mm film^2 . The negative film was mounted in 2×2 in cardboard projection slides, using a system of markers and templates to ensure accurate alignment. All numerals and markers were opaqued out on the film, except for the central cross, which is required for centring in the camera field of PAPA. The finished negative slides bear a single transparent molecular silhouette surrounded by a black background and have a scale of 0.14 cm/Å.

The selection of the optimal PAPA machine adjustments, for this particular problem, was approached by successive approximation, using a short series of fifteen molecular silhouettes obtained from a recent study of the odours of the aliphatic carboxylic acids. These compounds had earlier been shown to exhibit in varying degrees the primary odour of "sweatiness", depending on their resemblance in molecular size and shape to the standard primary odorant, isovaleric acid¹². The primary nature of this odour had been established by means of a group of persons specifically anosmic to (unable to smell) the sweaty odour. The degree of odour primacy of a given compound was estimated from the difference in its minimum detectable concentration between normal observers and specific anosmics. The molecular shapes had previously been measured by the manual radius method. The correlation coefficient then found between the manual measurements of molecular shape and the degree of odour primacy had been 0.75 (Fig. 3).

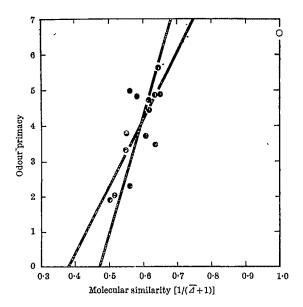


Fig. 3. Correlation between sweaty odour primacy among fifteen carboxylic acids and molecular shape similarity to isovaleric acid, measured by the manual radius method. The odour primacy scale is the log, of the threshold concentration ratio between specific anosmics and normal observers. (Two regression lines are shown because both variables are subject to experimental error; the open circle represents the isovaleric acid standard, which was omitted from the statistical analysis.)

Various settings of the PAPA machine were now tried, until the instrument became quantitatively responsive to the degrees of similarity between the molecular silhouettes. This was judged to have been accomplished when reasonably close agreement was reached between the instrumental measurements of molecular similarity and those obtained by the manual method. The top silhouettes only were used at this stage. Furthermore, only a single example silhouette was employed, that of isovaleric acid. In practice, the optimum performance by PAPA was achieved by appropriate adjustment of the average random line length, line curvature, threshold position and choice of comparative criterion.

The three values for the top, front and right silhouettes of each test compound were averaged to obtain the overall

molecular similarity between that compound and the standard compound, assessed by the PAPA intersection method. These molecular similarities were plotted against the same set of odour primacy values, previously obtained from the specific anosmia procedure. A correlation coefficient of 0.80 was achieved using the PAPA procedure (Fig. 4). This correlation coefficient by the PAPA intersection method was slightly better than by the manual radius method. It is interesting to note that the two scatter diagrams (Figs. 3 and 4) are almost identical; the correlation coefficient is 0.96 between the two measures of molecular similarity, manual and PAPA. The anomalous off-line position of the isovaleric acid standard itself in the radius method (introduced by the reciprocal term in the calculation) is avoided in the PAPA method.

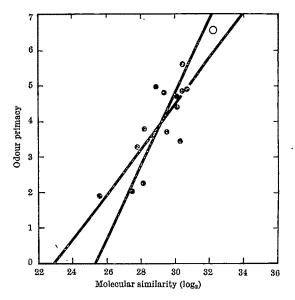


Fig. 4. The same odour primacy values as in Fig. 3, but plotted against molecular shape similarity measured by the PAPA intersection method. With slight adjustment of the horizontal scales, the points in Figs. 3 and 4 become almost superimposable. (The molecular similarity scale in \log_2 units gives relative values, not absolute.)

The same optimal machine settings which were effective for scanning the short series of fifteen carboxylic acids were now applied to review a long series of 107 compounds which also had been studied earlier¹³. In that survey not just one but five standard compounds were These were 1,2-dichloroethane; 1,8-cineole; employed. 15-hydroxypentadecanoic acid lactone; d, l- β -phenylethylmethylethyl carbinol; and d,l-menthone. represent, respectively, the ethereal, camphoraceous, musky, floral and minty classes of odour. (Note that these are merely classes of odour, and not proved primaries.) The similarities in odour quality between each of the 107 test odorants and each of the five standard odorants had previously been measured by means of a panel of normal observers, using a matching standards procedure¹⁴. In this method the judges compare the unknown with the standard odour, and rate the degree of similarity on an intuitive scale from 0 to 8.

The PAPA instrument can handle up to sixteen example classes simultaneously, and so the machine was instructed with the top silhouettes of all five standard compounds (one example only for each class). To avoid over-filling the machine's memory, the number of A units was reduced from 4,096 to 1,024. In response to each test top silhouette, the instrument printed out five values, representing the individual similarities of the test silhouette to each of the five standard silhouettes. Then all the memory circuits of PAPA were cleared, and the process was repeated for the front and right silhouettes.

Correlation coefficients were calculated between these molecular similarities obtained from the PAPA intersection method and the corresponding odour similarities measured by the matching standards procedure¹⁴. The results for the five classes of odour are shown in Table 1, together with the known correlation coefficients¹³ between the same odour values and the manual radius method of measuring molecular shape. In the camphoraceous and floral classes there was a small improvement in the correlation coefficient with the new PAPA method for assessing molecular shape. The ethereal class was unaffected, but the musky and minty classes showed a rather less satisfactory correlation, largely because of a slight bias in favour of the manual radius method contributed by the reciprocal term. Nevertheless, the changes do not materially affect the earlier conclusion 13 that there is a highly significant correlation between odour quality and molecular shape ($P < 10^{-9}$ for each class of odour).

Table 1. COMPARISON OF THE MANUAL RADIUS AND PAPA INTERSECTION METHODS FOR MEASURING THE MOLECULAR SILHOUETTES OF 107 ODOROUS COMPOUNDS

	Correlatio	n coefficient
Odour	Manual	PAPA
Ethereal	0.66	0.66
Camphoraceous	0.56	0.58
Musky	0.62	0.53
Floral	0.54	0.58
Minty	0.52	0.45

The two sets of molecular shape measurements were correlated with the same set of odour similarity measurements¹³. Results are given for five classes of odour.

Evidently, the PAPA machine can be considered to supersede the manual method for comparing molecular silhouettes, whenever more than a few compounds are to be studied, on account of its hundred times greater speed. Extensive tests have so far been made with only one method of scanning the silhouettes, the intersection method. Preliminary trials with the area method on the fifteen carboxylic acids gave an even higher correlation coefficient of 0.84. It is encouraging to find that more than 70 per cent of the variance in the sweaty odours of these acids can be explained simply with three photographs of a conventional molecular model.

The PAPA pattern recognition machine has much greater associative and inductive power than has yet been

applied to the problem of the molecular silhouettes. For example, instead of exhibiting just one standard compound with a particular odour, one could show to the machine many examples of compounds having the same odour, and let the machine develop its own criteria for distinguishing compounds possessing the odour. definitive information becomes available from the specific anosmia procedure on the identities and stereochemical characteristics of the primary odours12, the PAPA machine is expected to become increasingly useful for estimating theoretically the contribution towards each primary that can be expected from a newly identified odorant, or even from a projected synthesis.

Although the present application of the PAPA pattern recognition machine to the molecular morphology of chemicals has been exclusively with regard to their odours, it seems reasonable to suppose that similar principles should apply for other chemical-constitution/biological activity relationships. The highest activities of drugs, pheromones and pesticides usually require, among other things, optimization of molecular shape. A PAPA machine with enlarged memory, capable of conducting a massive screening of known chemical compounds against desired biological activities, might help reveal many valuable products lying dormant in the literature.

We thank Professor A. Borsellino for suggesting this collaboration, Professor A. Gamba for helpful discussion and Paul Kayfetz for special photography. The PAPA machine used in this experiment was built with the support of Consiglio Nazionale delle Ricerche through the Gruppo Nazionale Cibernetica.

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Regulation of Cytochrome Oxidase in Human Cells in Culture

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The range of adaptation of the terminal respiratory apparatus in response to environmental variation is considerably less for human cells than for yeast cells. In addition, availability of oxygen in human cells regulates the content of cytochrome oxidase rather than its activity.

THE regulation of the formation of mitochondria and of mitochondrial enzyme synthesis has been extensively studied in lower organisms but not in the cells of higher organisms. In yeast, for example, the synthesis of cytochromes and the formation of mitochondria are markedly repressed by anaerobiosis, and also by a high concentration of glucose in the growth medium1-4. For human cells, cell

culture affords a unique opportunity to evaluate mechanisms of regulation of cellular respiratory enzymes. Such studies are interesting because of the possibility that the control mechanisms are basically different from those of lower organisms, and also because of the possible relevance of regulation of cellular respiratory enzymes to human pathophysiology.

We report here studies of the regulation of activity of the terminal component of the electron transport chain, cytochrome oxidase, in cultured human cells. These findings have been correlated with cytological observations of mitochondria in conditions in which cytochrome oxidase is, and is not, induced. Both similarities and differences between human cells and those of lower organisms with respect to regulation of cytochromes and of the development of mitochondria have been revealed.

Cultures of fibroblasts from the foreskins of newborn humans were initiated and maintained by methods which have been described before. The growth medium for all experiments was medium 199 with 15 per cent foetal bovine serum. Aerobically grown cultures were flushed with 97 per cent air and 3 per cent carbon dioxide and were then sealed and incubated at 37° C. Anaerobically grown cultures were treated similarly except that there was

nitrogen instead of air in the gas mixture.

To estimate enzyme activity, monolayer cultures were suspended by trypsinization and trypsin was removed by centrifugation. Cells were disrupted by adding to the pellet cold 1 per cent deoxycholate in 0.013 molar phosphate buffer, pH 7.4. Disruption of cells in this way occurred immediately on mild agitation and pipetting. Assay for cytochrome oxidase was performed by following spectrophotometrically the oxidation of reduced cytochrome cafter the addition of the cell lysate. In addition to the cell lysate, the reaction mixture contained at the final concentrations, 1 per cent (w/v) reduced cytochrome c, 3 per cent (w/v) deoxycholate and 0.013 molar phosphate buffer, pH 7.4. The specific activity was calculated using the

extinction coefficient $\Delta_{EmM\, red. - ox = 19\cdot 1}$ for cytochrome c^6 . For preparation of mitochondria, cultures of fibroblasts were collected by trypsinization. The cells were suspended in 0.013 molar phosphate buffer containing 0.25 molar sucrose and were sonicated for 4 min, which resulted in disruption of 80-95 per cent of cells as determined by microscopic examination. (Sonication for longer than 4 min resulted in disruption of 100 per cent of cells, but diminished the enzyme specific activity.) The sonicate was centrifuged at 1,000g for 10 min. The pellet (P₁) consisted of nuclei, cell debris and the remaining undisrupted cells. The supernatant (S_1) was centrifuged at 10,000g for 10 min, giving a mitochondrial pellet (P2) and a supernatant (S_2) .

Protein was measured by the method of Lowry et al.7. Glucose was assayed enzymatically with glucose oxidase, using orthotolidin as oxygen acceptor⁸.

Table 1. OYTOCHROME OXIDASE ACTIVITY OF HUMAN ORLLS GROWN IN THE PRESENCE AND ABSENCE OF OXYGEN

Experi-	Cell	Duration	Cytochrome oxidase activity* Aerobic			
ment	type	of growth (days)	Aerobic growth	Anaerobic growth	growth, then anaerobic growth	
1 2 3 4 5 6 1-8 (mean ± SEM)	Fibroblasts	6 9 10 20 10 13	5·8 3·8 4·7 3·1 7·2 9·0 5·6 ± 0·9	1.0 0.8 2.5 0.8 2.2 1.3 1.4 ± 0.3	4·8 4·5	
7 8	HeLa	6 10	11·0 12·0	4·7 3·0		

^{*} μmoles of substrate oxidized/mg protein/min.

Aerobically grown cultures were grown in 97 per cent air-3 per cent CO_* ; anaerobically grown cultures were grown in 97 per cent N_* -3 per cent CO_* . Following the growth periods indicated, cytochrome oxidase assays were performed on cells lysed with deoxycholate.

Studies of the effect of anaerobic growth for one or two passages (five to ten cell generations) on cytochrome oxidase activity revealed consistently less activity in these cells than on controls grown aerobically (Table 1). In six experiments, the activity of cytochrome oxidase in lysates of cells which were aerobically grown was $5.6 \pm 0.9 \mu \text{moles}$ of substrate oxidized/mg of protein/min, compared with a

mean of 1.4 ± 0.3 for cells grown anaerobically. difference between the two means is significant at the level of P < 0.01. Comparisons of enzyme activity in aerobically and anaerobically grown HeLa cells revealed a similar phenomenon. Although the level of enzyme activity in HeLa cultures grown aerobically was higher than that of fibroblasts, HeLa cultures grown anaerobically had significantly lower activity than aerobically grown HeLa cultures, with means of 3.8 and 11.5, respectively.

In addition to its effect on cytochrome oxidase activity, anaerobiosis in fibroblasts resulted in a reduction of approximately 40 per cent in the yield of cells and of protein compared with that of aerobically grown control cultures. This difference in growth was also evident from a comparison of average generation times of aerobically and anaerobically grown fibroblasts which were 29 h and 41 h, respectively. Fibroblasts could be grown anaerobically for at least five passages without further change in growth parameters; HeLa cells, on the other hand, failed to survive in anaerobic conditions for longer than 6-7 days.

One possible explanation of the reduced enzyme activity of anaerobically grown cells is that anaerobiosis selected variants within the cell population which were heritably deficient in enzyme. If this were the case, restoration of anaerobically grown cells to aerobic growth would not result in recovery of cytochrome oxidase activity to levels characteristic of aerobically grown cells. As can be seen from experiments 3 and 4 of Table 1, however, when anaerobically grown cells with reduced cytochrome oxidase activity (activities of 2.5 and 0.8, respectively) were grown for one passage in air, activities characteristic of aerobic growth (4.8 and 4.5) were regained. The fact that the anaerobic effect was reversible makes it unlikely that anaerobiosis selected for cells with low enzyme activity.

Another means by which anaerobic growth might lead to reduction in cytochrome oxidase activity is by inducing synthesis of an inhibitor of enzyme activity. The presence of an inhibitor was sought by determining whether the enzyme activity of mixtures of deoxycholate lysates of aerobically and anaerobically grown cells was significantly lower than the mean of the component activities assayed independently. In such a mixing experiment (Table 2), no evidence for the presence of an inhibitor was found. The specific activity of aerobically grown cells was 5.9, that of anaerobically grown cells 1.3, and that of a 1:1 mixture of the two was 3.5.

Table 2. Enzyme Activity of Mixed Lysates of Aerobically and Anaerobically grown cells

Cell extract Sp	ecific activity
Aerobic growth	5-9
Anaerobic growth	1.3
1:1 mixture of aerobic and anaerobic growth	3.5

* μ moles substrate oxidized/mg protein/min.

Deoxycholate lysates were prepared from aerobically and anaerobically grown fibroblast cultures. Cytochrome oxidase activity was assayed in each lysate and in a 1:1 mixture of the two lysates.

A further possible mechanism of inhibition of cytochrome oxidase activity in anaerobically grown cells is that lack of oxygen caused conformational changes in the enzyme molecule which rendered it inactive. If this were the case, enzyme inactivated by anaerobic growth might be reactivated by in vitro exposure to oxygen and, conversely, active enzyme from aerobically grown cells might be inactivated by deprivation of oxygen in vitro. investigate this possibility, mitochondria were isolated from aerobically and anaerobically grown cells, then incubated with oxygen and nitrogen and the effect of this incubation on the enzyme activity was determined.

The distribution of cytochrome oxidase activity in different fractions of an aerobically grown fibroblast preparation is shown in Table 3. The bulk of the activity was present in P_2 , the 10,000g pellet. The activity in P_1 (approximately 6 per cent) is believed to be accounted for by cells which were not disrupted by sonication but which were lysed by subsequent exposure to deoxycholate. Of the remaining activity, over 98 per cent was sedimented at 10,000g, a force known to sediment mitochondria. Mitochondria were isolated from fibroblasts grown in air or in nitrogen for 10 days. Cytochrome oxidase assay was carried out on a portion of each mitochondrial preparation. The remaining portion of mitochondrial preparation from the culture which had been grown in nitrogen was suspended in phosphate buffer, through which air had been vigorously bubbled for 10 min. After suspension in buffer, the supernatant gas was continuously flushed with air for 5 min and cytochrome oxidase activity was measured. A portion of the mitochondrial preparation from cells grown in oxygen was suspended in buffer through which nitrogen had been bubbled for 10 min; the supernatant gas was flushed with nitrogen for 5 min and enzyme assay was carried out.

Table 3. DISTRIBUTION OF CYTOCHROME OXIDASE ACTIVITY IN MITOCHON-DRIAL AND NON-MITOCHONDRIAL CELL FRACTIONS

Fraction	Total	Total	Percentage	Percentage
	protein (mg)	activity*	total protein	total activity
P ₁ P ₂ S ₂	7·22	39	42	6·3
	2·16	564	12·6	92·0
S.	7.7	10	45.4	1.7

* µmoles substrate oxidized/min. Fibroblasts were disrupted by sonication for 4 min, then centrifuged at 1,000g for 10 min. The 1,000g pellet (P_1) , which consisted of cell fragments, nuclei and undisrupted cells (approximately 15 per cent of the total cells initially present), was dissolved in 1 per cent deoxycholate. The 1,000g supernatant was centrifuged at 10,000g for 10 min, giving a pellet (P_1) and a supernatant (S_2) . The pellet was resuspended in 0-013 molar phosphate buffer containing 0-25 molar sucrose. Cytochrome oxidase assays were performed on P_1 , P_2 and S_2 .

The results of this experiment are shown in Table 4. For the mitochondrial preparations not treated in vitro, the preparation from aerobically grown cells was four times as active as that from anaerobically grown cells (specific activities of 150 and 35, respectively). In vitro incubation of mitochondria from anaerobically grown cells in buffer saturated with air did not affect a rise in activity (preincubation specific activity 28·0). Similarly, incubation of mitochondria from aerobically grown cultures in buffer saturated with nitrogen did not reduce cytochrome oxidase activity (preincubation specific activity 150, post-incubation specific

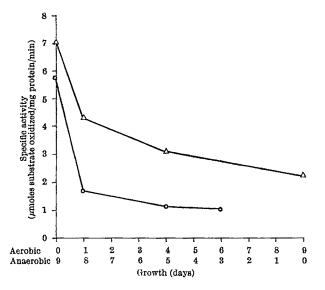


Fig. 1. Kinetics of loss of cytochrome oxidase activity of cultures transferred from aerobic to anaerobic growth. Groups of cultures were grown for 0, 3, 5, 8 or 9 days aerobically and were then transferred to anaerobic growth for the remainder of a 9 day growth period, at which time cytochrome oxidase assay was performed. The curves are from separate experiments.

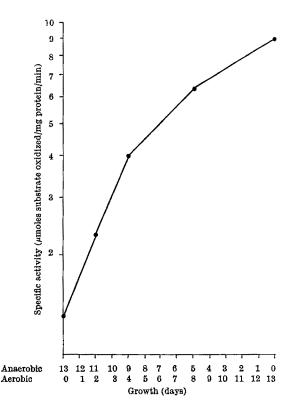


Fig. 2. Kinetics of gain of cytochrome oxidase activity of cultures transferred from anaerobic to aerobic growth. Groups of cultures were grown for 0, 4, 9, 11 or 13 days anaerobically, then transferred to aerobic growth for the remainder of a 13 day growth period, at which time cytochrome oxidase assay was performed.

activity 190). These data make it unlikely that growth in an altered gas environment resulted either in conformational or other alterations of enzyme molecule which rendered it inactive. Although in vitro incubation experiments cannot exclude conformation or other molecular changes dependent on an in vivo process, the implication of the in vitro experiments is that change in enzyme content, rather than alteration in pre-existing enzyme molecules, is involved in variation in enzyme activity resulting from variation in oxygen environment.

Table 4. EFFECT ON ACTIVITY OF BUBBLING OXYGEN AND NITROGEN THROUGH MITOCHONDRIAL PREPARATIONS

Growth condition	Treatment of mito- chondrial preparation	Activity of mito- chondrial preparation*
Aerobic	None Bubbled with nitrogen	150 190
Anaerobic	None Bubbled with oxygen	35 28

^{* \}mu moles substrate oxidized/mg protein/min.

Mitochondria were prepared from cells which had been grown aerobically and anaerobically as described in Table 1. Treatment consisted of bubbling the suspending buffer with the designated gas for 10 min, suspending the preparation in the bubbled buffer, and flushing the supernatant gas layer with the gas for 5 min. Control preparations were suspended in untreated buffer and were not flushed.

The mechanism of the anaerobic effect was further investigated by studies of the kinetics of loss of activity on transfer from aerobic to anaerobic growth, and of its regain when the transfer was reversed. It can be seen from Fig. 1 that activity decreased rapidly during the first 24 h after transfer from aerobic to anaerobic conditions. In two experiments, the loss in activity during the first 24 h was 40 per cent and 70 per cent of aerobic values, respectively. Based on an observed average generation time of 41 h for anaerobic cultures, the loss expected, if the mechanism of the anaerobic effect was to stop the synthesis of mitochondria and dilute pre-existing mitochondria by cell division, would be of the order of 30 per cent

 $\left(\frac{24}{41} \times 50 \text{ per cent}\right)$. It therefore seemed unlikely that the anaerobic effect on cytochrome oxidase was a function of cessation of synthesis of mitochondria. That the synthesis of mitochondria as such was not involved was also indicated by cytological studies.

When cells changed from anaerobic to aerobic growth the gain of enzyme activity proceeded exponentially during the first 4 days and then more slowly (Fig. 2). Within the limits of the experimental points obtained, there seemed to be no initial lag before the increase in activity following the transfer, suggesting that all components required for synthesis of enzymes were present at the time of transfer. This finding is under continued study.

activity in human cells was found to be reduced after anaerobic growth, genesis of mitochondria itself appeared unaffected. The more limited responses of human cells seem consistent with the more limited adaptive demands of cells the external environment of which is controlled (human) as opposed to uncontrolled (yeast). Human cells grown anaerobically did, however, have significantly lower cytochrome oxidase activity than aerobically grown These findings are consistent with those of controls. previous studies indicating decreased cellular respiration in anaerobically grown cells^{10,11}. That the availability of oxygen regulates the cellular content of cytochrome oxidase rather than its activity is indicated by the lack of effect on activity, of incubation of mitochondria in 100 per

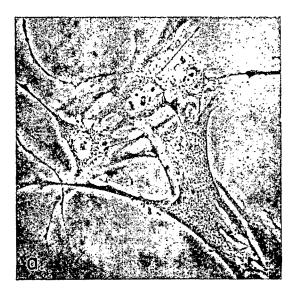




Fig. 3. Phase contrast photomicrographs of aerobically and anaerobically grown cells (×320). a, Aerobically grown cells; b, anaerobically grown cells. Abundant numbers of mitochondria are visible in each.

Cytological observations were made to determine whether anaerobically grown cells with reduced cytochrome oxidase activity had an associated reduction in the number of mitochondria in each cell. A comparison of aerobically and anaerobically grown cells by phase contrast microscopy (Fig. 3) did not reveal, however, any gross difference in the number of mitochondria which paralleled the differences in enzyme activity. This is in contrast to the findings for yeast, when cells grown anaerobically have fewer mitochondria or none at all3. It is of interest that a concentrated glucose medium, which strongly represses cytochrome synthesis in yeast4, had no effect on cytochrome oxidase of human cells. Cells were grown for 12 days in media containing a range of concentrations of glucose from 0.001 to 1.4 molar (normal growth medium contains 0.06 molar glucose). In the "high glucose" cultures, the glucose concentration was maintained by periodic medium changes. At the highest concentration of glucose used, growth was markedly reduced. At the lowest concentration of glucose used, glucose was not detectable in the medium at the time of assay. At no concentration of glucose, however, did the specific activity vary significantly from that of cells grown in the standard growth medium.

From the present studies, it seems that the range of adaptation of the terminal respiratory apparatus in response to environmental variation is considerably less for human cells than for yeast cells. Pertinent findings from the present studies in this respect are the insensitivity of human cells to repression of cytochrome synthesis by high glucose. In addition, although cytochrome oxidase cent oxygen or nitrogen in vitro (Table 4). Whether oxygen regulates the rate of cytochrome oxidase synthesis or of its degradation in vivo is not yet known. It will also be of interest to determine whether the regulation of cytochrome oxidase by oxygen plays a part in the pathophysiology of human hypoxic states; this depends, in part, on whether the level of cytochrome oxidase activity is a rate limiting step in respiration in human cells.

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LETTERS TO THE EDITOR

ASTRONOMY

Do Radio Stars Exist?

Wherick and Véron¹ have suggested that there is a new class of radio objects—namely, radio stars. This suggestion was based on a search in the positions of 3C (revised) radio sources having $|b^{\rm II}| > 30^{\circ}$ for which no identification with an optical object had been made. The search led to the discovery of apparent coincidences between the positions of seven radio sources and those of objects with normal stellar spectra and $m_v \leq 20$.

The positions of these sources have been determined with improved accuracy using the one-mile telescope² of the Mullard Radio Astronomy Observatory at a frequency of 1,407 MHz.

Table 1 gives the new positions together with their probable errors; it also gives the difference between these positions and the optical positions quoted by Wlerick and Véron, which have an accuracy of about ± 1 " are in each co-ordinate. Two of the sources are unresolved by the 23" are beam and only upper limits can be placed on their angular dimensions. Three of them are partially resolved, and the half-power widths of Gaussian source models, which fit the observations along particular position angles, are given in the table; the remaining two are well resolved double sources. This distribution of angular structure is very similar to that found in a large sample of 3C sources, most of which are identified with extragalactic Although it is only possible to guess at the physical mechanisms which might operate in a new type of galactic radio source of stellar dimensions, it would be remarkable if they gave rise to radio components with an angular structure so similar to that associated with extragalactic sources of similar flux density.

Sketch maps of the two double radio sources are shown in Fig. 1; the object suggested as being related to 3C 356 lies some $6'' \cdot 5 \pm 3'' \cdot 5$ off the radio axis³ while that suggested for 3C 435 is very close to the radio axis. It is well known that in the case of extensive double sources, it is difficult to establish conclusive identifications because of the large area in which a related object might lie; in the case of 3C 435, it is probable that, if the object mentioned by Wlerick and Véron had been a galaxy, previous authors would have regarded it as a good identification.

For the five compact sources, the positional discrepancies do not, in most cases, exceed the combined errors by a large factor. Nevertheless, it can be shown that the associations may probably be attributed to

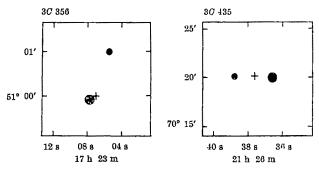


Fig. 1. Positions of the radio peaks (•) and the optical objects (+) mentioned in ref. 1, for the two well extended double sources. The larger spot in each region indicates the brighter component.

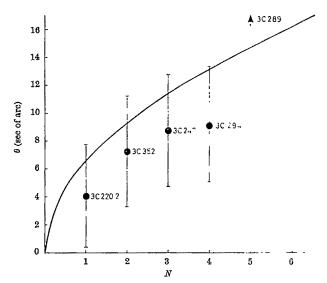


Fig. 2. N, The number of stars expected within an angular distance θ of the radio brightness peak over forty-six fields with $|\delta u| > 30^\circ$. The discrepancies between the radio positions and the optical positions given by Wlerick and Véron are also plotted for the five compact sources.

chance. As Wlerick and Véron have pointed out, it is difficult to make an estimate of the star density likely to occur in any given field. Data from another programme were, however, available which allowed the determination of the star density in areas covering the same ranges of galactic latitude and longitude as those examined by Wlerick and Véron. A count was made in each of fortyone areas $4'.5 \times 4'.5$ are in extent of the number of starlike objects with apparent photographic magnitude, $m_{\rm pg}$, ≤ 20 . An average of twelve stars was found in each region, with a standard deviation of six. If these areas can be regarded as having an average stellar population similar to those of the forty-six 3C fields with $|b^{\rm II}| > 30^{\circ}$ which were noted as "blank" by Wyndham⁴.

		Table 1	
	Radio position beginning of 1950	Position difference (radio minus optical)	Structure
3C 220·2	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{ccc} -0.3 \text{ s} & \pm 0.23 \text{ s} \\ 0.1'' & \pm 3'' \end{array}$	<17" at position angle 0 <10" at position angle 90
3C 247	10 h 56 m 08.41 s \pm 0.15 s 43° 17′ 20.0″ \pm 2″	$-0.52 \text{ s} \pm 0.25 \text{ s} \\ -6.4^{\prime\prime} \pm 3^{\prime\prime}$	~ 13.5" at position angle 70 <10" at position angle 160
3C 289	13 h 43 m 27.6 s ±0.2 s 50° 01′ 31.4″ ±2″	-1·2 s ±0·3 s 25·3" ±3"	~10" at position angle 110° <10" at position angle 20°
3C 294	14 h 04 m 33.95 s ± 0.15 s 34° 25′ 39.5″ ± 2″	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	~10" at position angle 20 ~10" at position angle 31" <10" at position angle 121°
3C 352	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccc} -0.25 & \pm & 0.3 & 8 \\ -0.7'' & \pm & 3'' \end{array}$	<14" at position angle 77°
3C 356	17 h 23 m 05·53 s ±0·3 s 51° 00′ 59·0″ ±3″ 17 h 23 m 07·87 s ±0·3 s 50° 59′ 54·1″ ±3″	$\begin{array}{cccc} -0.768 & \pm 0.48 \\ -0.168 & \pm 0.48 \\ 59.1'' & \pm 4'' \\ 0.188 & \pm 0.48 \\ -5.8'' & \pm 4''' \end{array}$	<10" at position angle 77° 69" are double at position angle 161°
3C 435	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	30" arc double at position angle 83°

then it is possible to calculate the number of sources in this sample, which would lie at a given separation from stars having $m_{\rm pg} \le 20$. The results are plotted in Fig. 2, together with the actual separations between the radio and optical positions for the five compact sources. It can be seen that, even with the fairly small positional discrepancies given in Table 1, the number of associations is no more than can be attributed to chance.

It must therefore be concluded that while it will never be possible to exclude the hypothesis that certain radio sources are identified with galactic stars, there is now no evidence to support it. The more accurate radio positions for these seven sources do not, however, enable any alternative identifications to be made with other objects visible on the Sky Survey Prints.

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Absorption Lines in Quasi-stellar Objects

GAMOW1 has attempted to explain the lack of correlation between apparent brightness and red-shift of quasi-stellar objects2,3 as being caused by the fact that the red-shifts are measured from absorption spectra which he has argued are caused by galaxies intercepting the light from more distant quasti-stellar objects. In this case, no correlations could reasonably be expected because the apparent magnitude would refer to one object and the red-shift to another.

Gamow's conclusion, however, is based on a misinterpretation of the observational data. All the red-shifts which have been used in the analysis by Longair and Scheuer³ are emission-line red-shifts^{4,5} which cannot arise in anything other than the quasi-stellar object itself.

It is true that some quasi-stellar objects do have absorption lines in their spectra in addition to the emission lines, and we have summarized all the data now available on the red-shifts of the absorption lines. Only about twenty quasi-stellar objects out of the 104 with known red-shifts do in fact show absorption features. absorption-line red-shifts, z_{abs} , are found either to be approximately equal to the emission-line red-shifts, $z_{\rm em}$, or to have a "standard" value of about 1.95. In cases where $z_{abs} \approx z_{em}$ there is no question but that the emission lines and the absorption lines all arise in the quasi-stellar object. In cases where $z_{abs} \approx 1.95$, often $z_{em} > z_{abs}$, or $z_{\rm em} \approx z_{\rm abs}$, but there is at least one case where $z_{\rm abs} > z_{\rm em}$ by a small amount. Spectroscopic arguments^{7,8} strongly suggest that in these cases, also, the regions producing the absorption lines are very close to the source of radiation in the quasi-stellar object. This, together with the fact that a "standard" red-shift seems to have been found, led us to the conclusion that these red-shifts are largely intrinsic and not cosmological. Shklovsky, however, has argued that the "standard" absorption spectrum, zabs ≈ 1.95 , is caused by galaxies all at this (cosmological) red-shift, an idea which forces one to the conclusion that a cosmological model of the Lemaitre type is required. But in no case do the data support the idea that the absorption is taking place in galaxies with a wide range of red-shifts.

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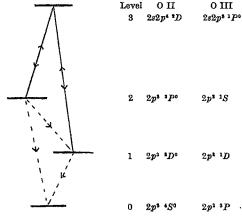
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Emission of [O II] and [O III] Forbidden Transitions by Quasi-stellar Objects

THE observation of forbidden transitions in the emission spectra of quasi-stellar objects1,2 has led previous authors to deduce limiting values for the electron density in relevant regions of these sources (for example, ref. 1). In this report I shall show that significant limits must be set on the ultraviolet radiation intensity in the emitting region, and thus on the size of the region, if the observations of certain [O II] transitions are to be explained even at low electron densities.

The level scheme relevant to both O II and O III is shown in Fig. 1. I consider the alteration in the forbidden line intensities produced by photoexcitation to levels connected by allowed transitions to both the metastable states, 1 and 2. For simplicity, only the nearest such excited level, 3, is considered. The collisional depopulation of levels 1 and 2 is neglected in comparison with radiative processes, because my interest is in conditions where forbidden transitions are emitted strongly. Spontaneous radiative transitions are assumed to be the only significant processes depopulating level 3. excitation to levels connected to a single metastable leve by allowed transitions can be ignored, because the eventual return of the electron to the original level implies that the forbidden line intensity is unchanged. Levels 1 and 2 are also populated at rates P_1 and P_2 by processes other than photoexcitation from either level and subsequent decay—the rates P_1 and P_2 need not be specified in detail in what follows, and may include either collisional excitation or radiative cascade. The ratio of the inten-



Allowed transitions; - - - . forbidden Fig. 1. Level scheme. transitions.

sities of any two of the forbidden transitions is then easily obtained from the rate equations for the populations of levels 1 and 2. Thus the relative rate of emission of photons is

$$\frac{n_1 A_{10}}{n_2 A_{20}} = \frac{C + \frac{P_1 + P_2}{P_2} \frac{\Gamma_1 I_{32} B_{23}}{A_{20}}}{1 + \frac{P_1 + P_2}{P_2} \frac{\Gamma_2 I_{31} B_{13}}{A_{10}}}$$
(1)

where C, the same ratio but in the absence of the radiation field. is given by

$$C = \frac{P_1(A_{21} + A_{20})}{P_2 A_{20}} \tag{2}$$

Here $A_{\rm nm}$ is the spontaneous transition probability, $B_{\rm mn}$ is the Milne coefficient (the absorption transition probability in terms of the intensity) of the n-m transition and $I_{\rm nm}$ is the radiation intensity at the relevant frequency. Γ_1 and Γ_2 are the branching ratios from level 3

$$\Gamma_1 = \frac{A_{31}}{A_{31} + A_{32}}, \quad \Gamma_2 = \frac{A_{32}}{A_{31} + A_{32}}, \quad \Gamma_1 + \Gamma_2 = 1$$
 (3)

Suitable values for the transition probabilities are tabulated by Wiese³, the important values A_{20} , A_{21} and A_{10} for O II being those of Seaton and Osterbrock⁴, namely $4\cdot7\times10^{-2}$ sec⁻¹, $1\cdot7\times10^{-1}$ sec⁻¹, and 9×10^{-5} sec⁻¹ (averaged values). The $\lambda3727$ Å [O II] ${}^4S^{-2}D$ (0–1 in my notation) line usually would be expected to be considerably stronger than $\lambda2470$ Å [O II] ${}^4S^{-2}P$ (0–2) in the absence of a radiation field, both because the ratio of P_1 to P_2 is large if the excitation is collisional⁵, and also (regardless of the excitation mechanism) because of the high (relative) value of A_{31} . At first sight therefore the failure to observe $\lambda2470$ Å in those quasi-stellar objects with both sufficient red-shift and strong $\lambda3727$ Å emission is not surprising.

If, however, $A_{10} \ll A_{20}$, and (as occurs in practice) the ratios of B_{13} to B_{23} , I_{31} to I_{32} and Γ_1 to Γ_2 do not differ greatly from unity, the ratio of intensities of $\lambda 3727$ Å and $\lambda 2470$ Å will be greatly reduced if the radiation intensity exceeds a critical value, and will ultimately approach a limit of the order of the ratio of the transition probabilities, that is approximately 10^{-2} . Values for B_{13} , B_{23} , Γ_1 and Γ_2 can be obtained from ref. 3. Γ_2 will depend on the exact number of excited levels considered, and for O II may vary from 0.125 to 0.5, but a suitable value when including only the $2s2p^4$ 2D level is 0.125. The cross-sections given by Aller⁵ suggest that this is about compensated by the $(P_1 + P_2)/P_2$ factor in the denominator if the excitation is collisional, and in the case of radiative cascade this compensation would be exact. It can therefore be concluded that in either case the critical intensity is given by

$$I_{31}(\lambda = 718 \text{ Å}) = \frac{A_{10}}{B_{13}} = \frac{2hv_{31}^8}{c^2} \frac{g_1}{g_3} \frac{A_{10}}{A_{31}} = 3 \times 10^{-14}$$
ergs cm⁻² sec⁻¹ (c/s)⁻¹ steradian⁻¹

This intensity is orders of magnitude smaller than that expected from several predictions (for example, refs. 1, 2 and 6) of the total ultraviolet flux and of the dimensions of the emitting region and so we can draw some interesting conclusions about those sources for which [O II] $\lambda 3727$ Å is observed (for example, 3C 249, 3C 277, 3C 48 but not 3C 273). Wampler and Oke⁶ adopt an ultraviolet continuum flux of 8×10^{29} ergs sec⁻¹ (c/s)⁻¹ and a radius of 10^{18} cm. With their value for the total flux, however, the minimum radius of the [O II] region if the critical intensity given here is not to be exceeded is

$$R > 4 \times 10^{20} \text{ cm}$$

Even assuming an intensity in the emitting region greater than the critical value by a factor of ten, the minimum radius still exceeds previous estimates by two orders of magnitude. Burbidge $et\ al.^2$ have proposed on other grounds that the forbidden transitions will be emitted in the outer layer of the source. With the parameters of their so-called region II ($T_c=3$ eV, $N_s=10^8$ cm⁻³, $R=10^{18}$ cm) the continuum flux at 718 Å due to bremstrahlung and Lyman recombination radiation is 4×10^{31} ergs sec⁻¹ (c/s)⁻¹. The minimum radius for the [O II] emitting region is then

$$R > 2.9 \times 10^{21} \text{ cm} = 10^3 \text{ parsec}$$

This radius is theoretically resolvable at the distance estimated for those quasi-stellar objects with relatively small red-shifts. Other problems are raised by so large an [O II] region. For example, Burbidge et al.² ascribe the widths of forbidden lines to electron scattering, which is consistent with their predictions on other grounds of a radius of 10¹⁸ cm and an electron density exceeding 10⁸ cm⁻³. The scattering hypothesis, however, would still require an electron density of 10⁴ cm⁻³ at the radius of 10²⁰ cm estimated here as the minimum for the [O II] region, that is an increase of four orders of magnitude in the total number of electrons in the source.

The [O III] lines are less restrictive because A_{10} is larger, but are still interesting as both $\lambda5007$ Å $^3P^{-1}D$ (0-1) and $\lambda4363$ Å $^1D^{-1}S$ (1-2) transitions are seen in some sources, the former being the stronger and the more frequently observed. Again this is, as expected in the absence of a high ultraviolet intensity, $\lambda5007$ Å, being some two orders of magnitude the stronger in planetary nebulae. Because, however, the transition probabilities differ by a factor of 80, a high ultraviolet intensity should alter the intensity ratio until $\lambda4363$ Å is much the stronger. Because A_{10} is about 2×10^{-2} sec⁻¹ the critical intensity is two hundred times larger than for [O II], that is the minimum radii are about fourteen times smaller than given here, but still an order of magnitude larger than previous estimates.

The absence of [O III] $\lambda 3727$ Å and the relative weakness of [O III] $\lambda 5007$ Å were the grounds on which Greenstein and Schmidt¹ proposed a high electron density for 3C 273. Because in this case the red-shift is too small for $\lambda 2470$ Å to be observed, a possible alternative explanation is a high radiation intensity in the emitting region and the postulate of high density and its consequences should be re-examined.

This preliminary study can necessarily yield only somewhat general conclusions. Some features at least of existing models of quasi-stellar objects need re-examining, because the predicted ultraviolet fluxes would require the regions emitting forbidden lines to be much further from the central object than previously realized. The inclusion of the many other possible levels to which photoexcitation can occur (for example, the $2p^2$ 3s and 3d configurations in O II) would increase the distances still further, and would be particularly significant for those models postulating high central temperatures, and thus a slow decrease of the ultraviolet flux with frequency. Obviously many exceptions can be taken to the estimates of the ultraviolet flux, but equally other properties of the models then need reconsideration. Estimates of the maximum size of the [O II] regions from fluctuation data would be particularly interesting, although Oke^7 has already shown that in 3C 279 and 3C 446 emission lines do not seem to vary with the continuum. Because data on the maximum size of such regions combined with observations of the relative intensities of forbidden lines can set significant upper limits on the absolute ultraviolet flux from quasi-stellar objects, the most interesting conclusion is perhaps that there exists in principle at

least a means of estimating upper bounds to the distance of such sources independently of their red-shifts.

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PLANETARY SCIENCE

Water on the Moon

THE possibility that water has existed on the Moon for varying lengths of time, both in liquid and in solid form, and both beneath the surface and on the surface, has been widely discussed during the past 10 years¹⁻⁷. The subject has been discussed repeatedly at scientific meetings and has been received mostly with great scepticism. Evidence supporting this view has recently become quite overwhelming and, in fact, no communication seems necessary to point out the evidence from the Orbiter 4 and 5 pictures. Because many people are not aware of this evidence and suggest that the effects are caused by other liquids, that is, lava, dust-gas or possibly even vodka, a brief discussion of the evidence may be in order.

Gold's pointed out that it was unreasonable to believe that all the smooth areas within the craters and between the craters were caused by lava flowing from deep in the Moon, and he suggested that dust produced by particle erosion was the origin of the material filling these craters and the maria. Because of the lack of dust river valleys, I disagreed with this and suggested that dust from the great collisions and temporary rains washed the dust into the low areas, and the water escaped into space or sank below the surface. Gilvarry⁵ postulated much more extensive oceans lasting for billions of years. Although many of Gilvarry's observations are reasonable, the long length of time assumed, with no evidence for mature river valleys, unfortunately led to definite non-acceptance of his ideas. Gold' and Kopal' have suggested that water has escaped from the lunar interior and formed permafrost similar to that found in the arctic regions of the Earth. Safronov and Rouskol¹⁰ argue that water could not have escaped slowly from the lunar interior and formed oceans because the escape rate of water from the Moon would be so great that only a very small atmosphere of water could have accumulated. This conclusion is correct, and sources of surface water, which have been suggested, have been of a catastrophic kind, that is, a degassing of the lunar surface and the colliding objects in the great collisions on the Moon¹, a great splash from the Earth during the period of accumulation of the Earth2, comet head collisions, etc. I'1 suggested that the lake-like areas near and on the walls of Alphonsus were indeed caused by water (see under Moon, Encyclopaedia Britannica, plate III, for pictures of these). Gold suggested that the great central mass in Alpetragius is a pingo*. I3 have

argued that the carbonaceous chondrites may come from the Moon, for those of the type I variety, particularly. show that they were once immersed in water and that the Moon has certainly had liquid water on its surface.

Schroeter's valley, to the west of Aristarchus, has been known since the late eighteenth century, but terrestrial observations could not establish its origin. Orbiter 4 and 5 pictures show this valley in great detail as well as many neighbouring valleys. Schroeter's valley begins in a mountainous region in an enlarged area called the "Cobra Head", and extends in a generally westerly direction into a smooth maria area. Its greatest width is some 8 km and its depth some 600 or 700 m. Other smaller valleys with "cobra heads" are found north of Aristarchus and Prinz. Branching occurs in a few cases but mostly there are no tributaries and no deltas. Possibly there are small deposits at the maria ends, but they are much too small to account for the materials eroded from the valleys. South of Aristarchus and northerly from Marius is a valley extending to more than 160 km in a very smooth maria area. It decreases gradually and uniformly in width from the south-easterly end to the north-westerly end where it runs off the picture available to me (Orbiter 4, frame 150, high resolution). Near the larger end it is about 900 m wide and apparently goes to near zero at the other end. It is a very crooked rille and cannot possibly be a physical fracture. It has no tributaries. Similar rilles are found in many places, for example, within and near the Alpine valley and many other areas. They seem to be a general lunar feature.

But why are there no sediment deposits at either end of these valleys and no tributaries? (There are a very few examples of tributaries.) The walls seem to be formed by slumping, and north of Krieger there is a row of craters which seem to form an initial stage of such slumping. Water must have run below the surface and formed a tunnel which then caved in. But where did the excavated material go? If the maria are underlaid with ice, it is only necessary to melt the ice and let the water drain into the desert sands. (The explanation offered here was suggested by Gold.) But could it have been lava? Would lava flow in a narrow stream for hundreds of kilometres and disappear without a trace? I believe that the answer to both these questions is "No". But the details of these valleys are very varied.

While flying over northern Greenland on September 9, 1967, I saw crooked rille-like structures in the Greenland ice cap. Some of these looked like fractures but others, at least over limited regions, looked much like the crooked rilles on the Moon.

Krieger, north of Aristarchus, is an irregular crater with a recent smaller collision crater on the southern wall, a curious depression in the plane south of the crater, and an irregular mass covering the north wall and an area north of the crater. There is a break in the western wall, and from this break an irregular gully or stream bed extends a short distance to the west. This seems to have been a surface stream, and a suggestion of a sediment deposit can be seen at its western terminus. This appears to be a surface stream bed quite different from the other rilles discussed. North of Prinz one of the small rilles of the Schroeter valley type crosses an elongated sunken area, transverse to this valley. It has a flat floor, and a small stream bed, similar to that coming from Krieger, crosses it. These two stream beds indicate that streams flowed on the surface and thus that an atmosphere had a pressure equal to and probably much greater than the vapour pressure of water at its melting point, that is, 4.6 mm of mercury. Could Krieger be a pingo that has melted and collapsed? If so, it is a very large example as compared with terrestrial pingos. Krieger is 25 km in diameter. I have found a picture of a terrestrial pingo 300 m in diameter¹². But in a billion years, say, it may be that pingos get very much larger. Are there unmelted and uncollapsed pingo areas on the Moon? Probably

^{*}In regions of permafrost, that is, northern Canada and Siberla, great cones of ice covered with soil are formed by water and ice being extruded from the lower regions. They often look like small volcances with sloping sides and a depression like an irregular volcanic cone at the top. In some cases the ice has melted leaving a crater much like lunar craters but of much smaller diameter^{18,13}.

some of these volcanoes which are referred to so confidently are pingos.

South of Aristarchus is a crater similar in size to Krieger (name unknown; it appears on Orbiter 4, picture frame No. 150, 1 of 3 high resolution). There is a break in the northern wall and there is a rather smooth pile of sediment outside the crater at this point. Some liquid drained out of the crater and left this material. Possibly this was lava, or mud, or a flow of dust and gas. In view of the overwhelming evidence for water as the agent producing the other valleys, I favour the view that effects seen in this crater as well as Krieger are caused by water.

In fact, as mentioned here, several serious students of the Moon concluded, or at least surmised and suggested, that water was present on the Moon at some time in the past. This evidence for lake-like structures has been presented repeatedly, each author discovering for himself the same evidence previously recorded by others. The suggestions were reasonable and the evidence valid. The fact that there seem to be no mature river valleys anywhere argues that these seas were present for only a short length of time. It is not possible to give the time at which they were present. Was it 4.5×10^9 yr ago when the Moon was captured by the Earth, and may the Moon have captured some water from the Earth at that time, or was it some 2×10° yr ago when the Moon's orbit changed from retrograde to direct and when the Moon was near Or has the surface water appeared repeatedly? Thus water may have been acquired by the Moon early in its history; it escaped into space leaving water below the surface; the atmosphere was lost and low temperatures below the surface were established. Then a comet collided with the Moon, gave it an atmosphere, the temperature became warmer as on Earth, water melted and flowed out of the surface in springs. The atmosphere escaped and the water froze again until the next comet collision. The Safronov and Rouskol arguments seem to be valid and only a catastrophic origin for the water on the Moon seems possible.

Undoubtedly, someone will eventually measure and record these stream beds in great detail. This communication is to point out that the maria of the Moon are dried-up or frozen seas3-5 and that water has aided in forming the final features of the Moon. These conclusions in no way determine what the composition of the solid materials in the maria may be or whether this material had a

volcanic or other type of origin.

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Core Convection, the Earth's Figure and Continental Drift

CERTAIN consequences of convection within the Earth seem to have been overlooked by those trying to establish satisfactory Earth model.

Variations of the geomagnetic field, and in particular the westward drift of its pattern of disturbances1, which have received considerable attention in relation to the Elsasser-Bullard hydromagnetic dynamo theory of the origin of the geomegnetic field, are widely regarded as signifying the existence of convection currents within the Earth's core. It has been argued that the heat required to drive these currents might arise from a quite small amount of radioactive material concentrated in the inner core. Another view³ is that the currents are produced by the energy released by gravitational differentiation into the inner core. Either mechanism would cause convection throughout the depth of the outer core.

The occurrence of free convection throughout the outer core would imply a uniform angular momentum per unit mass of outer core material and, because the ratio of the outer to inner core radius is 2.53, an inner core (assumed solid) rotating some $(2.53)^2 = 6.4$ times faster than the outer layer of the outer core. The moment of inertia of the Earth may be defined as that quantity which, when multiplied by the angular velocity, ω_c , of the crust, gives the Earth's total angular momentum. The slowness of the westward drift of magnetic field disturbances1 and the likelihood of tight electromagnetic coupling between the mantle and the outer layer of the outer core4,5 would then justify the view that the angular velocity of the inner core is about 6.4 wc, and that the angular velocity within the outer core decreases with increasing radius from this The angular momentum of the core as a value to ω_c . whole would thus be considerably greater than if it all rotated at ω_0 . Taking the values given by Birch⁶ as representative of recent models of the Earth's internal density distribution, an approximate calculation shows that the effective moment of inertia of the core would be doubled and the effective moment of inertia, C, of the whole Earth about its rotation axis increased by about 9 per cent. Note that the Earth's moment of inertia, A, about an equatorial axis would be unaffected (unless the core rotated about a different axis) so, in principle, observations of C-A could throw light on the amount of convection occurring in the core.

Jeffreys' has stressed that the hydrostatic theory of the Earth's figure, even when taken to the second order, yields a value for the precessional constant, H = (C-A)/C, which is significantly (about 1.1 per cent) less than that derived directly from lunar observations. The theoretical value of the quantity $J_2 = (C-A)/Ma^2$ is also about 1 per cent less than that derived directly from satellite observations^{7,8}, so the theoretical and observed values of C/Ma^2 are in substantial agreement. It follows that C-A is actually some I per cent greater than the hydrostatic theory provides, and the discrepancy is of the right sign for attributing to core convection. Because, however, H is of order 1/300, a 1 per cent change in C-A could be brought about by only a 1 in 3×10^4 change in either C or A. This is to be compared with the 9 per cent increase in C to be expected with free convection, and suggests that convective transport of angular momentum within the core is very small. It would be useful to know whether the magnetic field in the core could be responsible for this discrepancy and, if so, whether the field distribution implied by the Elsasser-Bullard theory of the geomagnetic field is of the right kind. Conversely, can that theory continue to account for the geomagnetic field if differential rotation within the core is as limited as these observations imply?

An interesting possibility in this connexion is that, if the persistent decrease of the chief geomagnetic field during the past 130 yr, amounting to more than 6.3 per

cent in all', has been associated with a general decrease in field strength within the core, there should have been a corresponding increase in the inward transport of angular momentum by the convection system. This would be expected to increase not only the westward slip of the outer layer of the core with respect to the mantle, but also the axial moment of inertia (possibly observable as an increase in J_2), and to reduce the angular velocity of the mantle and crust. The scatter of the westward slip data for magnetic disturbances is likely to be too great for a convincing correlation with the main field strength to emerge, if present, and the analysis 10 of observed variations of ωc during the past century is already beset by uncertainties as to the relative weight of the several possible causes. On the other hand, J_2 can now probably be determined from satellites with a precision of about I part in 104 (ref. 8). If we therefore regard 1 per cent of J_2 as being subject to control by the magnetic field in the core, a 1 per cent reduction in field strength might have a detectable effect on J_2 . It is unfortunate that the present rate of decrease of field strength is only about 3.5 per cent every century, because it could be another 30 yr before we know whether J_2 is affected in this manner. Any proof of lack of correlation between these parameters would imply that the chief external geomagnetic field strength is not correlated with the intensity in the inner part of the core and would place important restrictions on the nature of the geomagnetic mechanism.

This discussion shows that core convection is an extremely powerful cause of oblateness and can easily account for the observed excess over that given by the hydrostatic theory. Because it seems that core convection is essential to the generation of a geomagnetic field, and because the constraint imposed by the field is never likely to be very precisely calculable, the excess oblateness argument that the mantle has considerable long-term strength is now untenable. This does not mean, however, that the mantle does not have long-term strength, because the oblateness no longer provides evidence of continued retardation of the Earth's rotation, and Brouwer¹¹ has shown that the eclipse observations over the past few thousand years do not altogether confirm it either.

Convection within the mantle has long been favoured by proponents of continental drift, so, in view of the possibility that the mantle has very little long-term strength, the following argument may be of interest. If the outer layer of the core is the source of the observed geomagnetic disturbances drifting westward and is tightly coupled to the base of the lower mantle, it follows that the base of the mantle rotates at substantially the same rate as the crust. Thus free convection in the lower mantle, which would cause its base to rotate at something like 2.5 ωc, can be ruled out. Here again one wonders about the possible part played by the magnetic field, but the mean field will be much less than in the core, and the convective forces, with which the magnetic forces would have to compete, would need to be greater than in the fluid core, so adequate magnetic control can probably be ruled out, too. The possible argument that there is mantle convection but no core-mantle coupling can also be ruled out on the grounds that the observed nearsynchronous rotation of the crust and the outer part of the core (the angular velocities differ by only about 2 parts in 10°) would then have to be a mere coincidence. Similar arguments, much reinforced by those of Bernal^{12,13} and MacDonald14,15 in regard to upper mantle structure, and by recent evidence16 for phase transitions within the upper mantle, can be applied to convection in the upper mantle. It therefore seems that some other mechanism for continental drift will have to be found.

The proposed rotational condition within the core might have arisen as follows. Before the onset of core convection the Earth rotated rather faster. Onset of convection caused the rotation of the inner part of the core to speed up, but the corresponding slowing-down of the outer part of the core had to be shared with the mantle, because of the development of electromagnetic coupling between them.

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Radiative Thermal Conductivity in Planetary Interiors

Ir is customary to invoke radiative thermal conductivity as a mechanism for efficiently removing heat from the Earth's interior1. Clark2 derived the formula

$$C_r = 4/3 \int_0^\infty \left(\frac{n^2}{\alpha + s}\right) \frac{\partial B(\lambda, T)}{\partial T} d\lambda \tag{1}$$

for radiative thermal conductivity in planetary conditions, where n is the refractive index, B is the blackbody function, λ is the radiation wavelength, α is the absorption coefficient and s is the backscattering coefficient. He used this relationship to estimate the radiative conductivity for a number of minerals by measuring α at room temperature and neglecting s (ref. 3).

We have shown4 that, in addition to their importance for terrestrial thermal calculations, the values taken for the thermal conductivity of rocks and minerals are critical in estimates of the likelihood of volcanic activity on the Moon. We have therefore investigated radiative transfer in minerals^{5,6}, including both an experimental study of the effect of high temperatures on the optical constants of minerals, and the detailed theory of backscattering.

The scattering calculations support Clark's neglect of s for particle sizes exceeding I mm. An interesting result of the theory, however, is that the diffuse backscattering is caused almost entirely by refraction rather than reflexion when the relative refractive index across particle boundaries is less than 4.

The experimental study involved measuring reflectance and transmittance spectra of minerals at temperatures up to 1,500° K and then calculating the optical constants. The minerals studied were diopside, peridot and oligoclase. Table 1 shows values of the radiative conductivity at high temperature, calculated from equation (1), using both high temperature and room temperature values of α and n. It is seen that the use of the high temperature data can reduce the calculated conductivity by a factor of as much as 20. This results from the band broadenings which cause increased absorption in the wings of the long wavelength silicate vibrational bands near 4µ and in the wing of the 1·1μ Fe2+ electronic band in the vicinity of 2μ. For any crystal containing Fe2+ these band broadenings close down the near infrared window and thereby result in Table 1. RADIATIVE CONDUCTIVITY (W/cm °K)

Mineral	
Peridot (at 1,513° K)	
Using room temperature spectral data	0.446
Using 1.513° K spectral data	0.0212
Diopside (at 1,513° K)	
Using room temperature spectral data	0.516
Using 1,513° K spectral data	0.102
Oligoclase (at 1,303° K)	
Using room temperature spectral data	0.425
Using 1.303° K spectral data	0.255

Values were calculated from near infrared optical properties as measured

a decrease in photon mean free path and thus radiative conductivity. The oligoclase, containing no Fe2+, shows a smaller decrease in radiative conductivity (a factor of about 1.7 at $1,300^{\circ}$ K) as the band broadening only occurs on the long wavelength side of the relevant wavelength region.

MacDonald¹ was the first to indicate that estimates of the 1,500° K radiative conductivity less than 0.03 W/cm °K appeared to be necessary in calculations of the thermal history of the Earth, although the values suggested by room temperature spectra of olivines were more than an order of magnitude larger. We believe that the results reported here go a long way towards resolving this

apparent difficulty.
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Ordovician Graptolites from the Kashmir Himalayas

THE discovery of graptolites near the village of Harpatnar in the Anantnag District of Kashmir¹ was of considerable interest to geologists studying the history of the Himalayas, because the apparent absence of graptolites from the Himalayas had long been the subject of considerable debate and speculation. The graptolites (described in ref. 2) indicate a Ludlow (Late Silurian) age for the Harpatnar Beds from which they were collected2.

A continuous and apparently conformably stratigraphic succession can be recognized stratigraphically above the highest graptolite-bearing layers in the Harpatnar Beds2, which are overlain by the Naubug Beds from which fish3 and conodont faunas4, indicative of an early Middle Devonian age, have been obtained. The stratigraphic succession beneath the lowest graptolite-bearing layer in the Harpatnar Beds is, in contrast with the layers above, faulted. Despite the structural complexities, a unit of red mudstones and shales can be recognized in a slice beneath the Harpatnar Beds which is bounded by faults. A collection of graptolites has recently been made by one of us (V. J. G.) from this slice.

The newly found graptolites are poorly preserved fragments. The following forms can, however, be recognized among them: Climacograptus sp.; Didymograptus sp. (juvenile stage of an extensiform didymograptid similar to D. compressus Harris and Thomas); Glossograptus ? sp. (a glossograptid, might be a paraglossograptid).

The association of this extensiform didymograptid with a climacograptid and glossograptid indicates an age span of late Early (approximately Late Arenig) into Middle (Mid-Caradoe) Ordovician. The association of extensiform didymograptids with climacograptids and glossograptids is known in the British and Continental European Ordovician graptolite successions from the British Didymograptus hirundo Zone and strata correlative with it into the Climacograptus bicornis Zone. This association of graptolites is known in the American-Australian-Asian Ordovician graptolite successions to range from beds. considered correlative with the basal part of the British Llanvirn, into those correlative with the British Climacograptus bicornis Zone.

The presence of the extensiform didymograptid, similar to D. compressus, suggests that the fauna may have had connexions with the American-Australian Asian Ordovician graptolite faunas rather than the British-Continental faunas. This didymograptid species is restricted to the American-Australian-Asian Ordovician graptolite fauna where it occurs in faunas considered to be correlated with those of the British Llanvirn.

These are the first conclusively Ordovician graptolites to have been found in this part of the Himalayas. Their presence in this area indicates that deposition of finegrained terrigenous materials took place, at least locally in the Himalayas, during at least a part of the Ordovician. These graptolites are the oldest fossils from the area southeast from Srinagar. Their presence thus indicates that marine deposition did take place in this part of the Himalayas and that, during at least a part of both the Ordovician and Silurian, graptolites were able to live in the waters in this area. The possibilities of the faunal connexion with the American-Australian -Asian graptolite faunas are significant to the palaeozoological distribution of Ordovician faunas. The Himalayan area may have been allied faunally with Asia during at least a part of the Ordovician.

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PHYSICS

Bursting Air Bubbles studied by the Time Exposure Technique

THERE are several mechanisms by which particulate matter is transferred across the sea-air interface. Filaments of liquid are torn from the tops of whitecaps by the wind and are generated by the break-up of combers. The mechanism of greatest meteorological importance is, however, the bursting, at the interface, of bubbles of air which have been trapped under the water surface or have been released from solution.

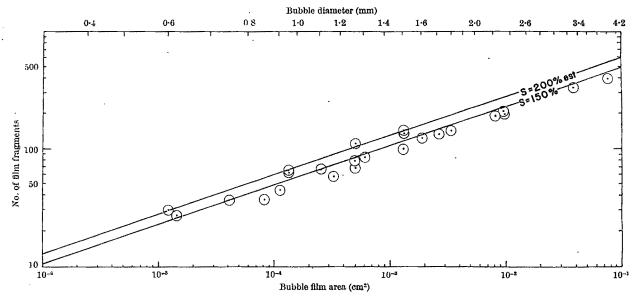


Fig. 1. Number of film fragments, counted from photographs taken in the cloud chamber, plotted as a function of bubble film area (or bubble diameter). Lines represent loci of counts made at constant maximum supersaturations, S. Supersaturation vertical profiles in the cloud chamber are extremely non-linear. At some maximum supersaturation, near 250 per cent, all bubble film fragments formed are caused to grow large enough so their scattered light exposes the photographic film.

Earlier studies¹ of bursting bubbles clearly identify two explosive actions which are intimately related, but are quite distinct. (1) The liquid surface of the ruptured bubble snaps back to re-establish a configuration of minimum surface energy. In the process a liquid jet shoots rapidly upward from the bottom of the bubble cavity, develops instabilities² and necks off into several drops and droplets (provided that bubble diameters do not exceed 5–6 mm). (2) Small fragments of liquid, torn

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Fig. 2. Time exposure of a 2-2 nun diameter bubble bursting in artificial sea water. Jet drop tracks are indicated by a broad white line. Pulsing of the mercury are lamp leaves a useful 1/120 sec time marker. Four jet drops are visible in this picture. Note the irregular bouncing of jet drops as they hit the water surface. Bubble film droplets grow to visible size by the time they reach the top of their parabolic trajectories. Then they sink slowly back to the water surface. The duration of the entire burst event is less than 2 sec.

loose from the retreating bubble film, are projected upward by the explosive release of the excess gas pressure in the bubble. These bubble film droplets are then further entrained in the wake of the ascending jet.

The principal mechanism which initiates the rupture of the bubble film is not well understood. After reaching a critical thinness by drainage and evaporation, the film may well require a triggering mechanism such as the nucleation of the gases absorbed in the liquid before it actually ruptures³.

The mean number of bubble film droplets has been found to be dependent on the size of the bubble. In an earlier investigation⁴, bubbles with a diameter of 4 mm equiv., breaking in saline solutions, were found to produce from 300 to 400 bubble film droplets while 0·1 mm bubbles produced no bubble film droplets. Fig. 1 shows that, with an improved model of the thermal diffusion cloud chamber, capable of running at higher supersaturations, somewhat larger numbers of droplets can, as expected, be counted.

I have taken time exposures of individual bubble bursts in their entirety as another means of understanding the motion of the jet drops and the bubble film drops. The type of information which a time exposure of a single bubble burst yields is illustrated in Fig. 2. One positive clarification emerges from an analysis of many of these pictures; it is that, contrary to earlier conclusions of Blanchard, myself and others, the bubble film droplets do not participate in a vortex ring circulation. The bubble film droplets seem to rise, and then fall back to the water surface along regular parabolic trajectories.

The bubble film droplets evaporate very rapidly in the free air because of their micron and submicron size range. Because of this, a film droplet of salt water is more likely to become an aerosol particle than is a jet drop, which, by virtue of its larger size, does not evaporate so rapidly. This probability factor, however, must be counterbalanced by the fact that the sea contains bubbles ranging in size from microscopic to diameters measured in millimetres, and the smaller bubbles far outnumber the larger ones. Bubbles smaller than a few tenths of a millimetre in diameter seem to produce no film droplets. Yet as the bubble diameters get smaller, so do their jet drops. This increases the probability of jet drops from small bubbles contributing substantially, along with the bubble film droplets, to the salt aerosol population of the atmosphere.

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Hysteresis Loop Experiments and the Determination of Thixotropic Properties

HARRIS¹ has pointed out the added complication in interpreting the "hysteresis" loop experiment for determining thixotropic properties which arises from the combined effect of the moment of inertia of the inner cylinder of the coaxial cylinders viscometer coupled with the stiffness of the torsion spring used in torque measurements. Huxley² has shown how this effect can be eliminated for Newtonian fluids. Steady-state oscillatory¹ and transient² experiments have been suggested for determining thixotropic properties. With this renewed interest on thixotropic behaviour, I shall summarize the results of my investigations into this phenomenon, outline work in progress and indicate the future lines of research.

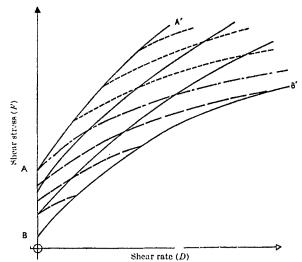
The basis of my work is a phenomenological theory of inelastic thixotropic behaviour based on the concept of thixotropic structure3. There is another theory based on the concept of relaxation spectrum due to Harris's. My theory is a generalization of three 5-7 specific model thixotropic or antithixotropic fluids in which the thixotropic behaviour is given in terms of the constitutive equations consisting of (a) an equation of state $F = \eta(D, \lambda)D$ which relates the shear stress, F, to shear rate, D, for any given state, λ , of the thixotropic structure, and (b) a rate equation which can be given in terms of the shear rate $d\lambda/dt = g(D,\lambda)$ and relates the rate of breakdown or build-up of the thixotropic structure. With certain specific restrictions on the forms of the functions n and g, these constitutive equations describe all the rheological behaviour associated with thixotropic and antithixotropic fluids.

The constitutive equations are shown in Fig. 1. can be mapped by a grid of constant structure, λ (solid lines), and constant rate, g (broken lines). The map is bounded by two constant-structure lines representing the state of maximum possible structural build-up, AA', and the state of maximum possible structural breakdown, BB'. Constant structure lines for intermediate states are between these limits. The constant-rate lines cut the constant-structure lines at an angle. The situation for a thixotropic fluid is shown in Fig. 1. The chain dotted line, running from A to B', represents zero rate and is referred to as the equilibrium curve. The dotted lines are lines of negative rate representing structural breakdown, and the dashed lines are of positive rate representing structure build-up. Thus the "maximum" representing structure build-up. Thus the structure is attained only at rest, and the "minimum" structure at very high shear rate. For an antithixotropic fluid the acceptance is fluid the equilibrium line runs from B to A' and the other constant-rate lines are similarly placed; the "minimum" structure is now attained at rest, the "maximum" structure at very high shear rate. It should be noted, however, that no real fluid has yet been clearly shown to be antithixotropic according to this description.

Experiments should ideally lead to, or be capable of being converted into, the constitutive equations. work of Billington and Huxley goes partly towards this. Billington^{8,9} first used the coaxial cylinders viscometer to observe the transient response of the inner cylinder in conditions of constant rotational speed of the outer cylinders and constant applied torques, and deduced the shear stress and shear rate variations, respectively, as the structure altered with time for "wax-containing" oils. In later papers, Billington and Huxley^{10,11} showed that. for oils containing either aluminium laurate or colloidal graphite, thixotropic breakdown did not begin until the shear stress had increased beyond a certain value. This allowed them to derive the shear stress-shear rate relation for the fluids at the state of maximum structure. They also obtained the equilibrium curves. Earlier^{8,9}, however. two factors seemed to have been neglected: the variation of shear rate in the annular gap between the cylinder and the non-uniform rate of thixotropic breakdown arising from the non-uniform shear rate and the resultant nonuniform structural states in the sample fluid. Thus, although Billington⁹ found it possible to compound the shear rate-time curves for different shear stress into one. it is doubtful whether the corresponding points on the individual curves do represent the same structural state. Later^{10,11}, the variation of shear rate was effectively allowed for, but no time variation curves were derived pertaining to thixotropic breakdown. Investigators^{2,10,11} contented themselves with stating that this is in principle possible.

Knowing that non-uniform conditions could exist within the fluid sample, my experimental work was directed towards determining constant-structure lines and deriving constant-rate lines. The use of the coaxial cylinders was first examined3. An experimental procedure was devised which involved shearing the sample at some reference speed to a steady state, suddenly changing the speed to a new value, and observing the resultant change of torque with time. Determining the constantstructure lines from the experimental data is relatively straightforward, and an example of such results, using a 'Rheomat 15' viscometer (Contraves AG, Zurich), is shown in ref. 3. It is, in principle, also possible to determine the rate equation from the experimental data, but in practice it means that some function has to be first assumed and it is the values of the parameters involved that are determined. In any case, the treatment necessary is not

The cone and plate viscometer has the reputation of giving uniform shear rate. The validity of this characteristic was therefore examined, and it was shown¹² that



during steady rotational motion, provided that the cone angle is less than about 4°, the approximation is very good. The error involved was no greater than errors inherent in any apparatus and was certainly much less than the variation possible between samples of the usual fluids that exhibit thixotropic properties. The same experimental procedure, as described here, can again be used and it is shown that the treatment of experimental data to derive the constitutive equations is very straightforward¹². It therefore seems that the cone and plate viscometer would be ideal for determining thixotropic properties. None of the commercial cone and plate instruments we possessed at the time was capable of the step-change in speed required for the experimental work. A special gearbox was therefore designed and built for use with a rheogoniometer (Sangamo Controls, Ltd., Bognor Regis), and work is now in progress to determine the constitutive equations for certain thixotropic aqueous clay suspensions.

It is appreciated that this method for determining thixotropic properties is time consuming and would not be practicable for industrial purposes, and some other rapid method for assessing thixotropic properties is therefore necessary. To this end the "hysteresis" loop test devised by Green and Weltmann (see references in ref. 1) or the oscillating experiment suggested by Harris¹ could be used. When coaxial cylinders are used for these tests, however, in addition to the objections pointed out by Harris1, there is the existence of shear rate and structural variations in the sample to take into account and this would make the detailed interpretation of experimental data even more complicated. The tests could still be used, particularly in industry, as a rapid means of ranking different thixotropic fluids or for the purpose of quality control for which detailed reduction of data is not necessary.

A commercial instrument, the Ferranti-Shirley cone and plate viscometer, is available which performs the loop test and is widely used in the clay, paints, printing ink and other industries. Its performance is also influenced by the combined effect of cone inertia and the torsional stiffness of the torque spring. This effect has been discussed earlier¹³. In the course of experiments, to test the validity of certain formulae which we have derived for the correction of these effects, it became apparent that other factors also influence the performance of the instrument. Because the loop test can also be used as a semi-automatic means of determining the flow curves of time-independent purely viscous non-Newtonian fluids, and has the added advantage that viscous heating can be minimized if it is carried out rapidly, the effect of these influences is being studied in detail.

The experiments using various Newtonian fluids covering a three-decade range of viscosity have been completed. Both positive and negative loops were obtained, depending on whether the viscosity is less or greater than certain critical values depending on the inertia of the cone involved. The retarding torque on the cone at any instance of the test, because of the fluid under test, can be deduced from these loops, after allowing for the effect of cone inertia and torsion spring stiffness. It was found, however, that the value of the torque differs from that expected from the theory for steady rotation12 using the speed of the cone at the same instant of time. It therefore seems that some other factor or factors are also involved. Two factors are being considered, both having their origin in the inertia of the fluid, but it is convenient to separate them into "secondary flow" and "inertia"

It is known that because of the geometry of the cone and plate, the primary flow usually assumed for such viscometers¹² cannot be absolutely valid when fluid inertia is not negligible, and more complex, or secondary, flow must always occur¹⁴. The effect of secondary flow, with steady rotation of the cone and plate, has been studied both theoretically and experimentally for Newtonian fluids and it was shown that the effects cannot be ignored. During the loop test, the rotation is far from steady, and it is thought that the effect of secondary flow could be even more pronounced.

During the loop test, the fluid is subjected alternately to acceleration and deceleration, and the corresponding inertia terms in the equation of motion cannot be ignored. (It can be readily shown that, in the coaxial cylinders geometry, inertia terms can be safely neglected.) If the effect of secondary flow is neglected, the equation of motion remains linear and, after using Fourier analysis on the rotational speed of the cone, the problem reduces to that of steady state oscillating motion, solutions of which are, of course, available 16,17. We are now in the process of applying these results and it is hoped to be able to judge the relative importance of the "secondary

flow" and "inertia" effects in the loop test.

From a practical viewpoint the results for Newtonian fluids using different cones can be correlated by a suitable reduction of the experimental data and the correlation can be used to correct the loop results for the various effects noted. Work is now in progress to test whether the same correlation can be applied to non-Newtonian fluids. In the future, it is planned to apply the same method of data reduction to loop tests with thixotropic fluids. The corrected loops will have to be compared with those calculable from the constitutive equations determined using the special gearbox and the rheogonio-

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Orifice Plate Calibration in a Dilute Polymer Solution

THERE have been several experiments in which the turbulent skin friction in pipe flow is reduced by adding certain soluble macromolecular polymers to a flowing solvent. The polymers do not seem to affect the turbulence in the core of the flow¹, but rather cause a thickening of the viscous sublayer². Drag reduction, it seems, requires the presence of a wall.

Gadd^{3,4} and Jackley⁵ have reported tests on free jets. In the case of initially turbulent free jets4.5, no difference was noted between the behaviour of jets of water and jets of polymer solutions. In the case of initially laminar free jets^{3,4}, however, dye tests revealed a considerable difference in the behaviour of water and polymer solutions. In polymer solutions there seemed to be a marked suppression of small-scale turbulence. Because polymer solutions apparently do not affect the turbulence away from the wall in pipe flow, the results of tests on initially turbulent free jets are to be expected. The results for laminar free jets, however, at first seem surprising. From

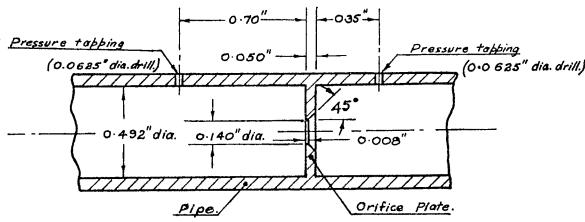


Fig. 1. Details and dimensions of the orifice plate.

the appearance of a photograph included in ref. 3, I am tempted to think that the polymer inhibits the transfer of turbulence energy from the low wave-number at which the large initial eddies were formed—because of the instability of the toroidal vortex sheet created by the jet-to higher wave numbers. This effect may be unrelated to the phenomenon of reduction of turbulent drag in pipe flow, especially because not all polymers of comparable drag reducing properties affect free laminar jets (for example, guar gum does not)4.

After carrying out some pipe flow experiments, I have noted a considerable reduction in pressure drop across an orifice plate. Workers would be well advised not to assume the calibration of an orifice plate for a dilute polymer solution to be identical with that for water. An orifice plate, of the dimensions shown in Fig. 1, was calibrated in turn for water and for a 30 w.p.p.m. (weight parts per million) aqueous solution of 'Polyox WSR-301' (polyethylene oxide) donated by Union Carbide (Australia), Ltd. The results are shown in Table I and Fig. 2. For flow rates above the order of 1.5 lb./min the pressure drop across the orifice was approximately 11 per cent less for the 'Polyox' solution than for the water. At lower rates no significant difference was noted. The results were first plotted on a log-log scale. The data for both the water and for the 'Polyox' above flow rates of 1.5 lb./min seemed to lie on curves of slope +2. Because of this, a least squares parabola was fitted to each of these sets of

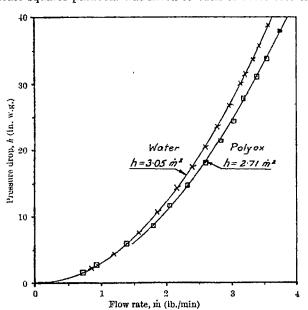


Fig. 2. Curve of pressure drop against flow rate for water (×) and 'Polyox' (□). Temperature is 61° F. Age of solution, 30 h.

Wa	ter	Table 1	'Poly	nx'
Pressure drop (in. w.g.)	Flow rate (lb./min)		Pressure drop (in, w.g.)	Flow rate (lb./min)
38·7 35·7	3·56 3·42		38·1 33·8	3·74 3·55
33·7 38·6 31·5	3·33 3·32 3·21		31·1 27·9 24·5	3·40 3·18 3·04
30·1 26·7	3·14 2·96		21·5 18·2	2·84 2·60
23·6 20·5	2·78 2·60 2·40		14·8 11·8 8·7	2·33 2·05 1·80
17·6 14·4 10·7	2·16 1·87		5·9 2·9	1·38 0·924
7·6 4·4	1·58 1·19		1.8	0.725
2.4	0.865			

points. The resulting curves are shown in Fig. 2. These results are difficult to reconcile with other experiments mentioned earlier. If it is considered that there is a region of high shear rate in the orifice similar to a viscous sublayer in pipe flow, and that this region is thickened by the addition of polymer molecules, it is questionable whether the decrease in pressure drop would be similar to that shown in Fig. 2. It should be noted that, in the case of the pipe flow, the amount of drag reduction increases with Reynolds number, while in the case of the orifice the amount of reduction in pressure drop remains constant within the order of experimental accuracy. In view of this it seems that the cause of the effect is different from that in pipe flow. Considering an orifice to be somewhat akin to a free jet, then, according to results of experiments on jets4,5, the decrease in pressure drop would be expected to become less pronounced as the flow became turbulent, that is, with increasing flow rate. Clearly, this is not the case. The mechanism seems to be different from both that present in free jets and that present in pipe flows.

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Measurement of a Mass Transfer **Boundary Layer**

DATA on momentum transfer from velocity measurements are usually applied to mass transfer processes using the accepted analogies between heat, mass and momentum Doubts about these analogies, in particular transfer1.

their application to turbulent flow, have led me to develop a method of measuring the wet bulb depression of water vapour in a mass transfer boundary layer formed by water evaporating into moving air above a flat porous plate. The efficacy of the method, in a laminar boundary layer, has been established by comparison with the Blasius boundary layer equation for mass transfer¹.

Because of the difficulty of measuring mass concentration in a small space, little attention has been given to the mass transfer boundary layer. Powell and Griffiths² used a wet-bulb thermocouple to investigate evaporation from variously shaped bodies, but they were more concerned with overall evaporation rates than detail of the boundary layer. ElWakil et al.³ used an interferometric technique.

I have developed a method, with the fine spatial discrimination required, based on the same principle as that first proposed by Hill4 for measuring vapour pressures above aqueous solutions, and developed by Spanner⁶ for measuring vapour pressures associated with leaves. The Spanner method has since been used widely for botanical studies6,7 and soil studies8 although in still air only. The Spanner method uses the thermo-electric effect to cool the junction of a thermocouple below the dewpoint of the surrounding air so that condensation occurs and the thermocouple becomes wet. Spanner used a double throw, double pole switch to connect the thermocouple alternately to a battery circuit for cooling the junction and then to a galvanometer circuit which measured the junction temperature. Because of the slow response time of the galvanometer, the ballistic throw was recorded. A suitable d.c. amplifier and an oscilloscope enable the quite rapid temperature changes of thermocouple to be observed directly and continuously.

The feasibility of a wet thermocouple for measuring humidity in moving air was suggested by the work of Wylie⁹ and Powell¹⁰, who showed that as the diameter of an ordinary wet bulb thermometer is decreased, the threshold, above which the effect of air velocity is negligible, is reduced and the wet bulb depression approaches the temperature of adiabatic saturation, or the ideal wet bulb temperature. Similarly, as the thickness of the muslin covering the wet bulb is reduced, the wet bulb depression approaches the ideal. Thus it is reasonable to assume that data from a standard hygrometer can be applied to the thermocouple psychrometer.

There are certain difficulties in using such a psychrometer in the moving air of a boundary layer. The degree of cooling obtainable with metals is limited to about 8° F, thus the probe must be returned to areas of high humidity to rewet the junction. Rather than be continually rewetting the junction and then rapidly winding the probe through the boundary layer to the desired position, the

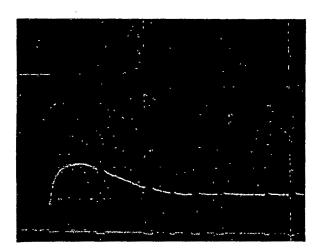


Fig. 1. The variation of wet built temperature through the boundary layer. The vertical displacement represents temperature and the horizontal scale is the height of the probe above the plate surface.

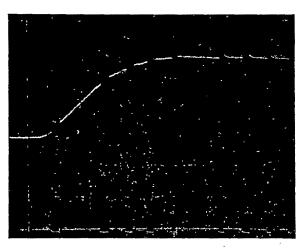


Fig. 2. The variation of temperature through the boundary layer with a thermocouple that has not previously been wet. The conditions are the same as for Fig. 1.

probe was motor driven through the boundary layer, taking measurements before all the water evaporated from the junction. The time available for the traverse was about 10 sec. It was found that the response time of the thermocouple and amplifying system was fast enough to give a true picture of the temperature change across the boundary layer.

The thermocouple used was of bismuth and an alloy of bismuth with 5 per cent tin. The wire diameters were 0-0015 in. and 0-0048 in., respectively. The thermocouple or Seebeck coefficient of the junction was 108 $\mu V/^{\circ}C$. A d.c. amplifier was used to amplify the signal from the thermocouple, which was fed to an oscilloscope and photographed.

A laminar mass transfer boundary layer was produced by evaporating water through absorbent paper placed on 50 per cent porosity sintered bronze plates measuring 24 in. \times 6 in. The plates were mounted in a horizontal position on the floor of a wind tunnel. The wind velocity was such that the air flow was laminar at the measuring station.

Fig. 1 shows the variation of wet bulb temperature through the boundary layer. The vertical displacement represents temperature (in this case 1.66° F/cm scale division). The horizontal scale is the height of the probe above the plate surface (0.064 in./em scale division indicated by the lower trace from a displacement transducer). The free stream air velocity was 3.4 ft./sec, free stream humidity 66 per cent relative humidity and ambient temperature 60° F. The initial increase in temperature is caused by the temperature of the thermocouple junction increasing from the dewpoint to the wet bulb temperature as the thermocouple is switched from the cooling to the measuring circuit. Fig. 2 is of a traverse with the same thermocouple under the same conditions but without having previously been wet. This corresponds to a dry bulb temperature traverse, but the temperature indicated is relative to the thermocouple wire supports. Thus because of a small temperature gradient between the plate and the ambient air, there is a variation in the drybulb temperature through the layer.

The difference between the traces in Figs. 1 and 2 gives the wet bulb depression and thus the mass concentration across the layer. This has been plotted in the non-dimensional form in Fig. 3. The abscissa is familiar from boundary layer theory and is given by

$$\eta = y \sqrt{\frac{U_{\infty}}{vx}}$$

where y is the height above the plate (ft.), U_{∞} is the free stream velocity (ft./h), v is the kinematic viscosity of the air (ft.²/h), and x is the distance along the plate (ft.).

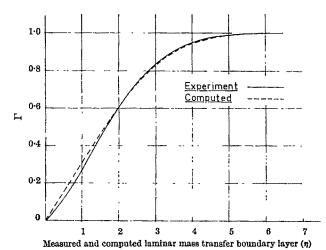


Fig. 3. The mass concentration across the layer in non-dimensional form. The continuous line is the experimental result, and the broken line is computed from Blasius boundary layer equations for mass transfer.

The ordinate Γ is a non-dimensional measure of the mass concentration and is given by

$$\Gamma = \frac{C_W - C}{C_W - C_\infty}$$

where C_W , C, C_∞ are the mass concentrations at the surface of the plate, at the position and in the free stream air, respectively (lb./ft.3).

To test the accuracy of the experimental data, a similar profile was computed from the Blasius boundary layer equations for mass transfer1. As can be seen from Fig. 3, the experimental data were in close agreement with the computed profile.

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THE SOLID STATE

Growth of Single Crystals of Silver Halides in Silica Gels at Near Ambient Temperatures

THE growth of single crystals by controlled diffusion in gels at near ambient temperatures offers intriguing advantages for substances which are sparingly soluble in the gel medium or which dissociate at the high temperatures so frequently employed for crystal growth1, or both. The gel diffusion growth method has been described as most applicable to materials with solubilities in aqueous solutions in the order of 10-1 to 10-2 weight per cent at ambient temperatures2. We report the successful application of the gel growth method to substances, the solubilities of which range from 10-5 to 10-7 weight per cent, thus enhancing its use for preparing single crystals of quite low solubility substances.

O'Connor et al. have prepared single crystals of cuprous chloride in silica gels by the controlled decomposition of a soluble cuprous chloride-hydrochloric acid complexo. The concept of precipitating single crystals can be effectively used in preparing silver halide single crystals. Such crystals have been grown from the melt by the Kyropoulos method, the Bridgeman method, etc.4. as well as from solutions, the latter methods being limited to the growth of microcrystals which are of interest in the photographic industry. High dislocation densities introduced during cooling from the melt, and contamination with silver oxide, etc., are typical problems associated with crystal growth from the melt. Growth in gels at ambient temperatures may conveniently produce larger more perfect single crystals, employing very simple equipment and preparatory techniques.

Single silver bromide and iodide crystals were grown in

silica gels at near ambient temperatures by controlled decomposition of their soluble corresponding acid complexes, for example, $H^+[Ag(Br)_2]^-$ and $H^+[Ag(I)_2]$. Silver chloride single crystals were grown by controlled decomposition of the soluble ammonia complex $[Ag(NH_3)_2]$ CI- in preference to the corresponding acid complex H+[Ag(Cl)₂]-. When silver chloride crystals were grown from the corresponding acid complex, embrittlement invariably occurred during growth. This may be associated with the adsorption of complex ions of high negative charge [Ag(Cl)₄]⁻² in the vicinity of strained surface bonds⁶. The largest silver iodide crystals grown to date were hexagonally shaped platelets approximately 10 to 12 mm in diameter. The silver bromide crystals were cubic, approximately 1 mm per edge. The silver chloride crystals were octahedral or truncated tetrahedral 6 to 8 mm per edge.

Although standard grade, not specially purified, reagents were employed in these crystal growth studies, spectrographic analyses indicated that their total cation impurity content was in the order of 10-20 p.p.m., with silicon very low. X-ray diffraction studies of silver halide crystals grown in gels indicated a high degree of crystal perfection, as will be discussed here.

Typically, crystals of silver iodide were grown by the following procedure. Silica gels were prepared by mixing equal volumes of sodium silicate (Fisher certified reagent) solution of specific gravity 1-06 and acetic acid (5 normal). Such solutions (pH 4·1) were poured into 25 mm diameter test tubes and permitted to gel in the dark at around 25° C-a process which took 10-15 h. The resulting gel was covered with additional higher acidity gel (pH < 1.0). prepared by mixing equal volumes of the sodium-silicate solution and 3.91 normal hydriodic acid (CP grade). The volume of the top gel layer was approximately one-fourth of the bottom one. About 10 ml. of solution prepared by dissolving 10 g silver-iodide (CP grade) in 50 ml. hydriodicacid (3.91 normal) was placed over the above gel and Silver-iodide crystals became allowed to decompose. visible after about an hour. Spectrographic analyses of typical crystals gave the following results: copper. 5 p.p.m.; iron, 5 p.p.m.; aluminium, 5 p.p.m.; silicon. <5 p.p.m.; magnesium, <5 p.p.m.; calcium, <1 p.p.m. Elements checked for but not found included sodium. potassium, barium, zinc and lead.

The silver iodide crystals were also examined in a two crystal spectrometer. The 2d spacing of the large hexagonal shaped faces was measured and calculated to be 7.50 Å which, within the corresponding experimental error of the measurement, corresponds to the (0001) plane of hexagonal silver-iodide with a 2d spacing of 7.494 Å. A rocking curve half width of the diffraction profile, obtained with an ethylenediamine ditartrate reference crystal, indicated crystal perfection comparable with that of the usual crystals used as X-ray analysers.

Silver bromide crystals were grown similarly except that the top gel layer was prepared by mixing equal volumes of 4 normal hydrobromic acid (CP grade) and sodiumsilicate. The soluble silver bromide complex solution was made by dissolving 10 g silver bromide (CP grade) in 50 ml. hydrobromic acid (6 normal). Crystals of silver bromide belonging to the cubic system became visible after about an hour. Their maximum size was in the order of 0.8 to 1.0 mm and was attained within 8 to 10

As noted, for the growth of silver chloride crystals, best results were obtained by decomposing silver chloride ammonia complexes. Gels were prepared by mixing equal volumes of sodium-silicate solution (specific gravity 1.06) and 2.9 normal hydrochloric acid. After gelation they were covered with a top solution prepared by dissolving 10 g silver chloride in 50 ml. ammonium hydroxide (8.33 normal). After 10 to 12 days crystals of up to 5-8 mm in edge were obtained. Most of the crystals were octahedral or truncated tetrahedral forms.

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Structural Changes of Barium Methacrylate Dihydrate under X-radiation

Schneller and Flanagan¹ have discussed the solid state decomposition of silver nitrite after irradiation with gamma rays. They noted that similar results were obtained by Boldyrev and Eroshkin³, who irradiated the compound with X-rays. We report similar work on the solid state polymerization of barium methacrylate dihydrate.

We have examined the structural decomposition of a sample of barium methacrylate dihydrate continuously irradiated with X-radiation. The experiment was performed on a finely powdered sample of barium methacrylate dihydrate left open to the atmosphere and mounted in a standard aluminium flat sample holder in a Philips PW 1051 wide angle powder diffractometer. A standard size collimator with anti-scatter slits of 1° diversion and an 0.1 mm receiving slit was used. Throughout the experiment the temperature remained constant at 25° C.

The kinetics were followed by measuring the decrease in the intensity of three powder diffraction peaks (100, 200, 002). The peaks were scanned at a speed of 0.125°/min at regular intervals of time and between scans the machine was left oscillating through the 20 range covering all three Intensities were estimated by weighing cut-out tracings of the diffracted peaks. The investigation was carried out at three different dose rates achieved by using: (a) nickel filtered copper radiation ($\lambda = 1.5418 \text{ Å}$) at 40 kV and 6 m.amp; (b) nickel filtered copper radiation at 40 kV and 20 m.amp; and (c) unfiltered copper radiation at 40 kV and 20 m.amp with a nickel filter placed between the sample and counter. The estimated dose rate (private

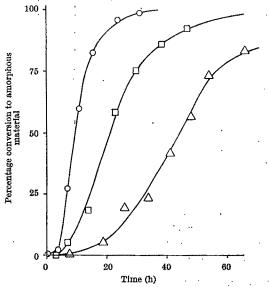


Fig. 1. Dose rate dependence of conversion of crystalline barium methacrylate dihydrate to amorphous material during continuous X-irradiation at 25° C. Mean lines for 200 and 002 reflexions. Dose rates: Ο, 556,000 rad/h; □, 205,000 rad/h; △, 69,500 rad/h.

communication from the Australian AEC Dosimetry Service) for each of these conditions is 69,500 rad/h, 205,000 rad/h and 556,000 rad/h, respectively. results are shown in Fig. 1.

O'Donnell, McGarvey and Morawetz³ have postulated, from electron spin resonance measurements, a free radical mechanism for the polymerization of barium methacrylate dihydrate induced by gamma radiation. Subsequent gravimetric determinations (results by J. H. O'Donnell and M. J. Bowden, to be published) of the kinetics of this polymerization show an expected increase in rate with an increase in the intensity of the irradiating beam. Our results show a marked similarity to those obtained by the gravimetric separation of polymer formed during the irradiation barium methacrylate dihydrate with gamma rays. As the large absorption coefficient of the compound results in a half-thickness of 20µ for the X-ray beam, the observed effect is a surface one and the comparison is only qualitative.

In view of the results of Schneller and Flanagan¹ and Boldyrev and Eroshkin², we feel that the comparison of our results with those found in the gamma ray polymerization of barium methacrylate dihydrate is justified, and that the observed structural decomposition is caused by polymer being formed. Further developments in the method we have outlined could provide a means of studying the kinetics of solid state polymerization in situ, thus overcoming a number of errors inherent in the normal gravimetric analysis techniques.

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MOLECULAR STRUCTURE

Mössbauer Spectroscopy of Some Iron Porphyrins

The Mössbauer parameters of various iron porphyrin complexes have been investigated as model systems for haem proteins1,2. Topics which require further consideration are the effect of porphyrin structure, basicity and esterification on the Mössbauer spectra. It has been shown that the basicity 'towards protons of meso and diacetyl deuterohaemins differs by a factor of one hundred whereas their isomer shifts (δ_0) and quadrupole splittings (ΔE) were insensitive to this change². We have studied the water soluble N-methyl substituted meso tetrapyridyl porphine (unpublished results of Fleischer and Hambright), which seems to be the most acidic porphyrin known. The (pK_3+pK_4) of this compound is the same as the unmethylated derivative³, whereas $pK_2 = 12 \cdot 9$. No other porphyrin has a measurable pK_2 in aqueous solution. Table 1 shows that the isomer shift of this structurally different haemin compound and protohaemin chloride are almost identical This indicates in both a 5 per cent 4s electron contribution to the d^5 configuration of Fe(III), and that regardless of structure or basicity, the metalligand bonding in these high spin square pyramid chelates is primarily ionic. It is also noted that low spin octahedral hemichromes have approximately the same sigma electron density at the iron nucleus ($\delta_0 \cong 0.041$ cm/sec) as the high spin haemin chlorides, whereas ΔE of the low spin derivatives is, as expected, larger ($\Delta E \cong 0.21$ cm/sec) than that of the high spin compounds. This unexplained similarity is novel in that typical inorganic low spin complexes have more sigma density than high spin varieties, presumably because of their increased pi bonding capacities. Thus the similarity in δ_0 values might indicate a limited role for pi bonding in low spin ferric porphyrin systems.

has described the factors giving rise to the peak asymmetry indicated in Table 1.

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BIOPHYSICS

Birefringence in Unfixed Lemon Fruit Nucleoli

CHEMICAL fixation of cells and tissues for microscopical analysis carries with it the stigma of artefact production1-5. Sjöstrand and Bakers have pointed out that the best way of checking for fixation artefacts is to employ a method of fixation or preservation of cellular components which acts on a completely different principle from chemical fixation. Cytological preparations of frozen and thawed lemon fruit tissue cultured in vitro are described here which show that the optically anisotropic nucleolar material first observed

Table 1

Compound ‡	Quadrupole splitting ΔE (cm/sec)	Diff. chemical shift* \$\delta_0 \text{ (cm/sec)} \dagger\$	Line width Higher energy (cm/s	Lower energy	Relative line areas (higher to lower energy)	Relative peak heights (higher to lower energy)
Protohaemin chloride	0.0458	+0.0446	0.044	0.042	0.84	0.81
Dicarboxylic acid of acetato haematohaemin	0.0941	+0.0580	0.092	0.066	0.81	0.58
Dimethyl ester of acetato haematohaemin	0.0631	+0.0451	0.034	0.032	1.04	1.01
Tetra N-methyl chloride chloro meso tetrapyridyl porphine	0.0625	+0.0434	0.038	0.034	1-18	1.08

- * Chemical shift reported relative to sodium nitroprusside.
- † Standard error on the basis of computed instrumental error +0.0004 cm/sec.
- ‡ All compounds gave satisfactory analytical data.

The data for protohaemin chloride were from a four times recrystallized analysed sample. It was found that commercial protohaemin from several sources contained ferromagnetic impurities (unpublished results of A. Thorpe). The susceptibility of this sample followed the Curie law to 70° K, giving a moment of 5.93 B.M. Room temperature data for the other compounds indicated high spin ferric derivatives.

Esterification of the propionic acid groups of the porphyrin has been shown to hinder the enzyme incorporation of Fe(II). It has been observed previously that with chloride or bis-imidazole groups, there is no Mössbauer difference between the acids or methyl esters. When acetate is the ligand (Table 1), δ_0 and ΔE are smaller for the dimethyl ester than the corresponding acid. These differences could be interpreted as inter- or intra-molecular hydrogen bonding between the coordinated acetate and the carboxylic acid functions changing the symmetry around the iron atom. Blume⁶

in chemically fixed tissue cultured in vitro7-11 is not an artefact of chemical fixation.

Basal nutrient solution12, modified by replacing sulphuric acid with 0.50 mmoles/l. of ammonium sulphate. was placed in Petri dishes lined with Whatman No. 40 or 42 ashless filter paper and sterilized by autoclaving in special conditions¹³ or by placing them in a deep freeze Vesicle stalks from mature lemon fruits overnight. (Citrus limon (L.) Burmann, variety 'Eureka') were inoculated aseptically onto the nutrient medium and placed in the dark at 25°C. After 24-48 h the stalks were immersed in distilled water in a Petri dish and placed in a deep freeze for 24 h or longer.

The frozen tissue was thawed to room temperature and unstained squash preparations were made as follows. Individual stalks were blotted dry, squashed between two microscope slides without any adhesives and air-dried for 10 min after separating the slides. Isopropyl alcohol was slowly added to the slide near the tissue until the tissue was flooded. After 2 min, the alcohol was drained off and the process was repeated three more times. After the last application of alcohol the tissue was mounted in 'Euparal'. If squashed tissue clung to both slides, both were dehydrated and mounted. A number of stalks were squashed and then mounted directly in corn syrup without any isopropyl alcohol dehydration.

Microscopic examination of the unfixed frozen tissue revealed the characteristic enlarged spherical or pearshaped nucleoli with the prominent inclusions or granules (Fig. 1a) as observed in interphase nuclei of chemically fixed lemon fruit tissue of previous investigations8,10,14. Each of the granules appeared to consist of a refractile body completely enveloped by a ring of contrasting material (Fig. 1a). Between crossed polars these nucleolar inclusions manifested pronounced birefringence with a maltese cross similar to that shown by starch granules in similar conditions, with the direction of maximum refractive index lying tangentially (Fig. 1b). These birefringent granules were orange yellow in colour under white light. Variation of the direction of maximum refractive index as reported for chromosomes15 was not

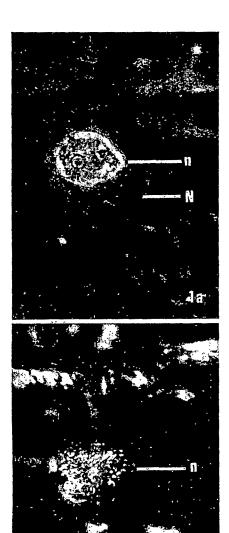


Fig. 1. a, Nucleus (N) and nucleolus (n) of unfixed vesicle stalk after 48 lin vitro showing the prominent nucleolar inclusions. Anoptral phase contrast microscopy. (×1,900.) b, Nucleolus (n) of a viewed between crossed polars showing the birefringent nature of the inclusions. The radial symmetry of many of the granules is obscured as a result of the partial juxtaposition of one granule with another. (×1,600.)

evident in the nucleolar granules, for it remained the same in both unfixed and chemically fixed specimens.

The optically anisotropic nucleolar inclusions described here are clearly not an artefact of chemical fixation. These granules withstand both the strong osmotic changes and disruptive effects of ice crystal formation associated with slow freezing and thawing of cells16 and the physical effects of squashing, and so it is very unlikely that their radial symmetry was brought about by a fixative action of the isopropyl alcohol used for dehydration. The presence of the same symmetrical bodies in nucleoli exposed to such widely divergent treatments as slow freezing and thawing and chemical fixation (CRAF, chrome-acetic, formaldehyde, and alcohol-acetic-formaldehyde fixatives) also makes it extremely unlikely that such a molecular ordering was brought about by a fixative action of isopropyl alcohol. The phenomenon of nucleolar birefringence occurring in unfixed squash preparations mounted in corn syrup without alcohol dehydration provides further evidence that dehydration and not fixation is the primary action of the isopropyl alcohol. The use of unstained squash preparations also precludes the introduction of artefacts resulting from staining reactions or contamination by embedding media such as paraffin inclusions, which have been reported as being responsible for birefringence of nuclei in animal cells17.

The granules in any given nucleolus do not lie in a single plane and seem to be oriented at distinct intervals along a strand or strand-like structure (see especially Fig. 5 of ref. 14). This seems to agree with recent investigations of La Cour¹s concerning the filamentous component (nucleolonemata) of plant nucleoli. He has shown that in a number of plant species studied, the nucleolonemata contained vacuoles along their entire length. It is therefore possible that the birefringent nucleolar granules described here may correspond to the vacuolar regions in the nucleolonemata of the plant species studied by La Cour¹⁸. The chemical and physical nature of the nucleolar material associated with birefringence is as yet unknown. Now, however, that the granules have been found not to be fixation artefacts, the way is open for their cytochemical and biophysical analysis.

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BIOCHEMISTRY

Relevance of Proton Uptake induced by Light to the Mechanism of Energy Coupling in Photophosphorylation

THE relevance of proton movements which are dependent on energy to energy conservation mechanisms in oxidative phosphorylation and photophosphorylation has recently been extensively debated, particularly with respect to the "chemiosmotic",2 and chemical,4 hypotheses. There are a few critical tests of the chemiosmotic theory. include: (a) the ratio of protons taken up to electrons transported; the value of this H⁺: e⁻ ratio should equal the number of sites of energy conservation in the electron transport path; (b) the effect of phosphorylating conditions; during phosphorylation one should observe a fall in the steady state extent of the proton gradient, as a result of the stoichiometric utilization of one or two protons² per ATP synthesized. Reversible pH changes in spinach chloroplasts induced by light have previously been described in detail^{5,8}. This communication reports experiments designed to test the chemiosmotic theory by these criteria. The results cannot be reconciled with the theory as it is at present conceived.

Figs. 1a and b show the initial rates of uptake of protons and oxygen in a suspension of chloroplasts, using diquat (N,N')-ethylene-2,2'-dipyridilium dibromide) as an electron acceptor. The most relevant experimental value of H^+ : H^+ :

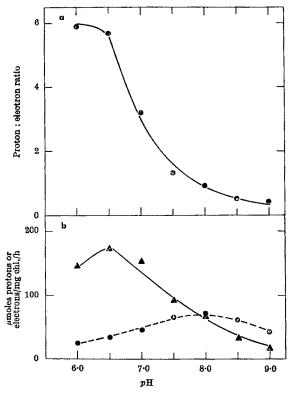


Fig. 1. Effect of pH on initial rates of proton and oxygen uptake, and on the H^+ : e^- ratio. The reaction mixture contained chloroplasts, prepared as previously described, containing chlorophyll 70 $\mu g/ml$.; sodium chloride, 15 mmolar; alquat, 3 μ molar; azide, 0.7 mmolar; pH changes were measured on a Radiometer pH electrode connected to a recorder and were calibrated by addition of a standard acid to the reaction mixture. Oxygen uptake was measured on a Gilson oxygraph. Illumination was with a 500 W slide projector fitted with a 580 $m\mu$ interference filter, of 12 $m\mu$ half-band width. The intensity of illumination was about 1.0×10^5 ergs cm⁻¹ sec⁻¹. a, H^+ : e ratio; b, h—h, H^+ uptake; h—h, electron transport.

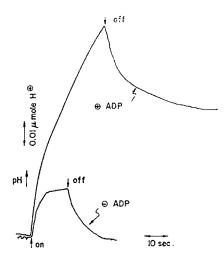


Fig. 2. Effect of phosphorylating conditions on the light-induced proton uptake. The reaction mixture included chloroplasts containing chlorophyll 50 µg/ml.; pyocyanine, 10 µmolar; sodium chloride, 30 mmolar; phosphate, 0.5 mmolar; magnesium chloride, 0.5 mmolar. ADP, 0.2 mmolar, was added where indicated. The pH was 7.8. A 700 mµ interference filter of half-band width 30 mµ was used for illumination. Other conditions as described under Fig. 1.

varies strongly with $p{\rm H}$ and has a maximum value of 6 at $p{\rm H}$ 6.0. The consequent prediction of six coupling sites in the electron transport path to diquat is difficult to accept in the light of current knowledge. It is also difficult to reconcile the observed variation of the ${\rm H}^+$: eratio with a mechanism whereby proton translocation and electron transport are obligatorily coupled. We have observed a similar variation with $p{\rm H}$ and a maximum ${\rm H}^+$: eratio value of four using ferricyanide as an electron acceptor. Values of 5 ${\rm H}^+$: erand 5 ${\rm H}^+$: hy have previously been reported using chloranil and pyocyanine. respectively.

Fig. 2 shows the effect of the complete phosphorylating system on the pH changes. In the absence of ADP, the usual reversible light-induced proton uptake can be observed, but in its presence two phases are seen in the light. The first represents the usual proton uptake and the second represents the irreversible proton consumption accompanying ATP synthesis at pH 8.

$$ADP^{3-} + HPO^{3-} + H^+ \rightarrow ATP^{4-} + H_2O$$

When the light is turned off, the yield of reversible proton uptake which has accumulated during phosphorylation is recorded. Fig. 2 shows that, surprisingly, this yield is greater than in the control curve (-ADP). In Table 1 we show the results of an experiment in which the control curve was attained with ADP, phosphate and 0·1 mmolar EDTA. EDTA at this concentration prevents the low rate of photophosphorylation due to the presence of endogenous magnesium. Increasing rates of phosphorylation can be initiated by addition of increasing concentrations of magnesium chloride. The value of these rates is calculated from the second slope of the full phos-

Table 1. EFFECT OF PHOSPHORYLATING CONDITIONS ON THE LIGHT-INDUCED PROTON UPTAKE

Mg++ concentration	ATP synthesis	Expe Assuming 2H+/ATP	Yield eted Assuming 1H+/ATP	Observed
mmolar	μ moles/h/mg chl.	•	noles H ⁺ /mg chl	•
0	0	0	0	0.13
0∙06	15	0.01	0.08	0.18
0.09	60	-0.01	0.05	0.27
0.16	120	-0.10	0-0	0.38
0.32	200	-0-34	-0.08	0.58
0-64	235	-0.39	-0.11	0.75

The system included chloroplasts containing 60 μ g chlorophyll/ml.; pyocyanine, 12 μ molar; EDTA, 0·1 mmolar; sodium chloride, 16 mmolar; ADP, 0·24 mmolar; phosphate, 0·48 mmolar; and magnesium chloride at the concentrations indicated. The pH was 8·0. Other conditions as in Fig. 1, and illumination as in Fig. 2.

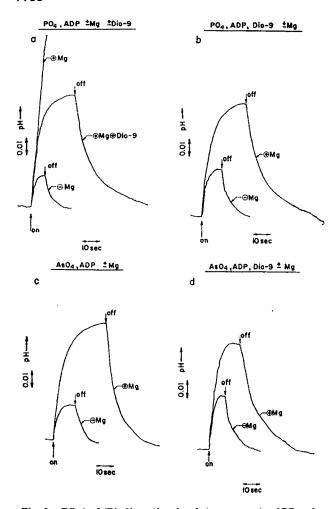


Fig. 3. Effect of 'Dio-9' on the phosphate or arsenate, ADP and magnesium stimulation of light-induced proton uptake. Conditions as in Table 1, except that arsenate or phosphate, 0-5 mmolar, magnesium chloride, 1-0 mmolar, and 'Dio-9', at 4 mg/ml., were added as indicated in the figures. Illumination as in Fig. 2.

phorylation curves, assuming 0.93 protons are consumed for each molecule of ATP formed at pH 7.8 (ref. 9). The higher the rate of ATP synthesis, the larger the steady state extent of proton uptake into the chloroplasts. Also shown are calculations of the yield which is expected on the basis of the chemicsmotic theory, assuming that one or two protons are utilized from the gradient per ATP synthesized. The calculations are based on a simple kinetic model of the light-induced proton uptake and we are now preparing the manuscript. The rate of ATP synthesis was increased to a point at which negative values of the yield of proton uptake were predicted. In other words, in such phosphorylating conditions the observed rate of proton uptake is insufficient, according to the chemicsmotic theory, to support the observed rate of ATP formation.

The combination of arsenate, ADP and magnesium was previously shown to "uncouple" photophosphorylation 10 , but unlike other uncouplers, this combination was found to increase the extent of proton uptake rather than inhibit it (Table 2). Stimulations of four-fold over the control value have been observed. The pH optimum of the stimulation was pH 8.2.

Fig. 3 shows the effect of the energy-transfer inhibitor 'Dio-9' on the pH changes induced by light in the presence of phosphate or arsenate, ADP and magnesium. Fig. 3a illustrates that the addition of 'Dio-9' to a complete phosphorylation system (+Mg), at a concentration which totally inhibits ATP synthesis (that is, the irreversible proton uptake), does not eliminate the increase in the steady state proton uptake induced by the phosphorylation system. This striking observation is re-emphasized

Table 2. EFFECT OF ADP, ARSENATE AND MAGNESIUM ON LIGHT-INDUCED PROTON UPTAKE

Reaction mixture	Yield μ moles H+/mg chl.
Control Plus Mg Plus Mg, arsenate Plus ADP, arsenate Plus Mg, ADP Plus Mg, ADP, arsenate	0·12 0·12 0·13 0·11 0·14 0·32

The system included chloroplasts containing 50 μg of chlorophyll/nl.; pyocyanine, 12 μ molar; EDTA, 0·1 mmolar; sodium chloride, 16 mmolar; ADP, 0·24 mmolar; arenate, 0·25 mmolar; and magnesium chloride, 1·0 mmolar, where indicated. The pH was 7·8. Other conditions as in Fig. 1, and illumination as in Fig. 2.

in Fig. 3b, where 'Dio-9' is included in the control (-Mg). Subsequent addition of magnesium chloride still produces a large increase in the steady state yield of proton uptake. It may be noted that in these conditions (pH 8) 'Dio-9' increases somewhat the usual (-Mg) proton uptake. In Figs. 3c and d, it can be seen that the same basic phenomena occur when arsenate replaces phosphate. Thus 'Dio-9' at a concentration which completely inhibits ATP synthesis does not markedly affect the stimulatory effect of arsenate, ADP and magnesium on the steady state proton uptake.

The finding that, in the presence of a complete phosphorylating system, the yield of light-induced proton uptake is higher than in its absence contradicts both the chemiosmotic and chemical theories. Both theories, as put foward at present^{2,4}, assume an equilibrium between ion transport and a non-phosphorylated high energy intermediate. In the former, the proton gradient is coupled to electron transport and gives rise to " $\sim X$ ", while in the latter the proton gradient is the result of "~X". The observed increase in proton uptake in the presence of arsenate or phosphate, ADP and magnesium could be interpreted as representing the sum of two processesproton efflux stoichiometric with ATP formation, as required by the chemiosmotic theory, together with a second proton accumulating process. The calculations in Table 1, however, make it unlikely that there is a sufficient rate of proton uptake in the control sample to support the observed rate of ATP synthesis as demanded by the chemiosmotic theory.

We were prompted by these observations to consider a model in which the prime function of the proton pump is to enable co-transport into the chloroplast of a negatively charged complex of HPO?—or HAsO?—Mg³+ and ADP³-(see Fig. 4). The increase in proton uptake observed in our case with arsenate or phosphate, ADP+Mg present, is a result of the fact that these are the "natural substrates" of the ion transport system and thus permit a higher activity than other "substrates" with a lower affinity, such as chloride. This co-transport may be of an effec-

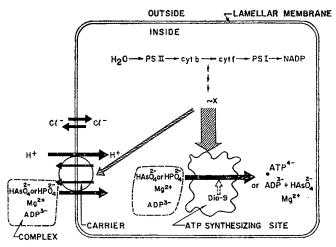


Fig. 4. A hypothetical model describing the proposed function of light-induced proton uptake in chloroplasts; see text for details.

tively uncharged complex of protons and HPO, Mg2+ and ADP3-. It has previously been shown that the uncharged species of several weak acids is that which best penetrates passively into the chloroplasts¹¹.

The model envisaged in Fig. 4 allows protons and a phosphate or arsenate, magnesium and ADP complex to be simultaneously transported on the same or different "carrier" proteins. ATP or ADP+AsO₄ will be formed subsequently on an ATP synthesizing enzyme complex. 'Dio-9' has been shown to inhibit the induced ATPase of the EDTA extracted coupling factor¹², and is therefore assumed to act at the ATP synthesizing site. The experiments shown in Fig. 3 are consistent with the hypothesis presented, which allows the arsenate or phosphate, ADP, and magnesium effects to lie before the site of action of 'Dio-9' (that is, on a carrier in our hypothesis, Fig. 4).

Finally, it must be remarked that the hypothesis requires that the actual energy expenditure in transporting the phosphate or arsenate, ADP, magnesium complex is a small fraction of that involved in the subsequent ATP synthesis reaction. 'Dio-9' has been shown to inhibit the uncoupling of photophosphorylation caused by arsenate ADP and Mg (ref. 12). Hence no significant uncoupling of photophosphorylation (that is, stimulation of electron transport) can be envisaged as occurring as a result of the energy expenditure involved in the arsenate or phosphate, ADP+magnesium, uptake process.

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Disappearance of Ribosomes and Polyribosomes during in vivo **Erythroid Maturation**

Burka and DeBellis¹ have compared the disappearance of ribosomes and polyribosomes during in vivo erythroid maturation in the rabbit. In order to study in vivo maturation of circulating erythroid cells labelled with isotope, unobscured by the continued release of new cells from the marrow, they injected labelled cells from a donor animal into a lethally irradiated recipient which continued to receive colchicine. In contrast to previous work² which they cite on this subject, they failed to find a disproportionate loss of polyribosomes and an increasing proportion of single ribosomes during maturation.

They provide sucrose density gradient patterns (Fig. 1, top) as evidence that the proportion of polyribosomes is

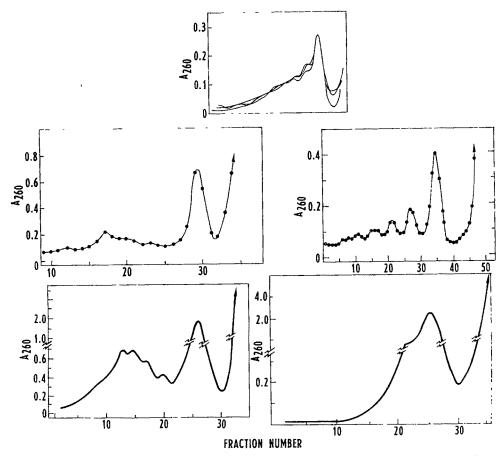


Fig. 1. Sucrose gradient analyses of ribosomal composition of rabbit reticulocyte lysates. (The ordinate scales have been adjusted to facilitate comparison.) Top: From Burkai. The individual lines represent profiles obtained 15 min, 1 h and 5 h, respectively, after reticulocyte infusion. Middle: From Warner et al.². Left, native; right, degraded. Bottom: From Elkenberry et al.³. Left, control; right, ribonuclease treated.

constant as a function of time. These, however, resemble the patterns obtained for partially degraded polyribosomes by Warner, Knopf, and Rich3 (Fig. 1, right centre) or those obtained by the addition of ribonuclease (Eikenberry and Rich4, Fig. 1, bottom right), rather than those density gradient patterns for polyribosomes in the native state (centre and bottom, left). In the gradient profiles of Burka there are more aggregates of two ribosomes than of three, more aggregates of three than of four, and more aggregates of four than of five, whereas the native lysate contains more aggregates of five ribosomes than aggregates of four, three or two ribosomes. In view of the possibility of degradation in the patterns of Burka, one might conclude that the similarity in his patterns as a function of age simply represents degradation to a uniform state. PETER T. ROWLEY

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Metabolic Effects of Methionine in Schizophrenic Patients pretreated with a Monoamine Oxidase Inhibitor

ORAL administration of a large amount of methionine to schizophrenic patients who had been treated with a monoamine oxidase inhibitor (MAOI) was reported to produce psychic symptoms which have been interpreted either as an intensification of schizophrenic symptoms or as superimposed toxic symptoms 1-4. The effects of methionine and MAOI have been explained as an increase in the amounts of methylated amines, such as bufotenin, N,N-dimethyltryptamine, 3,4-dimethoxyphenylethylamine or unknown amines which have been claimed to be psychotogenic substances. One of the purposes of the present experiments is to determine whether methionine increases the rates of N or O-methylation of catechol and indolic amines.

Asao and Yamano have re-investigated the clinical effects of MAOI and methionine. Eight hospitalized Eight hospitalized schizophrenic females aged between 31 and 57 who had the disease for more than 13 yr were selected. Treatment was stopped during the experimental period. Isocarboxazid (1 mg/kg/day) was administered in three divided doses from the eighth day of the experiment for 2 weeks, and 0.3 g/kg/day of L-methionine was given in three divided doses from the fifteenth day for I week. The symptoms observed were different from those characteristic of schizophrenia, but were the superimposed symptoms of intoxication such as delirium, visual hallucination, ataxia, speech disturbance, increased salivation and hyperhidrosis. Urine specimens (24 h) were collected on days 6, 7, 13, 14, 20, 21 and 30 in a bottle containing 200 ml. of isopropyl alcohol and 0.5 g of ethylenediamine tetraacetate (EDTA), and frozen until analysed.

A sample of urine containing 600 mg of creatinine was partially purified by the method of Kakimoto and Armstrong⁵, then dried eluate from a column of 'Dowex 50' was dissolved in 5 ml. of water, applied to a 2×4 cm column of 'Amberlite CG-50', diethylamine form, the resin was washed with 40 ml. of water and the amines were eluted with 40 ml. of 3 normal ammonia. The dried eluate was dissolved in 3 ml. of 70 per cent ethanol, passed through a column of 2.5 g of alumina suspended in ethanol and the column was washed with 15 ml. of 70 per cent ethanol. The total effluent was combined and the dried residue was dissolved in 90 per cent ethanol, and three equal portions were applied to three sheets of Toyoroshi No. 51 which is of a similar quality to Whatman No. 1. The recovery rates of normetanephrine and serotonin added to urine were greater than 70 per cent. One chromatogram was developed with solvent systems consisting of a mixture of isoamylalcohol, acetic acid and water (60:27:15) for the first dimension and a mixture of n-butanol, pyridine and 3 normal ammonia (50: 25: 20) as the second. The other two chromatograms were developed with the same solvent systems as used by Kakimoto and Armstrong⁵. The paper chromatogram developed with the first two solvent systems was visualized with ninhydrin, and the other two chromatograms with the diazotized p-nitroaniline and with Ehrlich reagent for phenolic and indolic amines, respectively.

Seventeen ninhydrin positive compounds were constantly observed, but the chromatograms were unchanged after treatment with isocarboxazid and methionine. 3,4-Dimethoxyphenylethylamine^{6,7} was not found in any urine specimens. Paper chromatograms of phenolic and indolic amines showed that the intensities of the amines did not change significantly after the loading of methionine under the influence of the MAOI, and the compounds which suggest accelerated formation of N-methyl amines were not found. From the rate of recovery of bufotenin and N-methyltryptamine when added to urine, daily amounts of these amines should be less than 10 µg, if they occurred. Failure to detect other N-methylated amines such as N-methylserotonin, bufotenin, N-methyltryptamine and N,N-dimethyltyramine after loading with methionine does not support the hypothesis that methionine exerts its action through increased methylation.

If methylation is stimulated by methionine, catecholamines should be methylated at an increased rate in the body and the amounts of normetanephrine and metanephrine should increase more intensely than noradrenaline and adrenaline. Adrenaline and noradrenaline were determined by a modification of the method of Von Euler Eluate from an alumina column was and Floding⁸. passed through a 0.3×1.0 cm column of a freshly prepared acetate form of 'Dowex 1', and the effluent was assayed fluorimetrically⁸. The introduction of the step with 'Dower 1' could remove the interfering substances described by Berlet et al.4, who reported difficulty in assaying catecholamine in the urine of subjects with methionine loading. Metanephrine and normetanephrine were measured by the method of Taniguchi et al.³. When methionine was administered to the patients under the influence of MAOI, the amounts of these four amines increased, but the ratio of the amount of metanephrine to that of adrenaline and the ratio of normetanephrine to noradrenaline did not rise, suggesting that the O-methylation of catecholamines was not accelerated by loading with methionine. These results are shown in Table 1.

For the determination of amino-acids an amount of concentrated urine corresponding to 1 mg of creatinine was applied to a Toyoroshi No. 50 which is of a similar quality to Whatman No. 3 MM. Chromatograms were run two-dimensionally with solvent systems devised by M. D. Armstrong of the Fels Research Institute, Yellow Springs, Ohio. The first solvent system is a mixture of pyridine, acetone and 3 normal ammonia (50:30:25) and the second one is a mixture of isopropylalcohol. formic acid and water (8:1:1). The paper was dipped in an 0.2 per cent ninhydrin solution in acetone, acetic acid and pyridine (18:1:1) and heated at 90° C for 3 min. Coloured areas of amino-acids were cut, eluted with 5 ml. of 50 per cent ethanol and the solution was assayed colorimetrically at 570 mu. The chromatographic pattern of urinary amino-acids was not changed by the administration of isocarboxazid, but was changed markedly after the loading of methionine in all eight individuals. The change in the intensities of the spots was not confined to methionine and its metabolites such as cystathionine and methionine sulphoxide, and greater changes were observed in a few other amino-acids. The amino-acids, which increased markedly (two- to sixfold) were serine, threonine, glutamine and histidine. The result was further confirmed by determination with an amino-acid analyser, which showed increases in the amounts of these amino-acids and These findings may reflect a disturbance of the metabolism or membrane transport of the amino-acids.

The identities of cystathionine and methionine sulphoxide were confirmed by the isolation of the compounds followed by the comparison of their infrared spectra with the authentic amino-acids. The spectrum of the isolated cystathionine coincided with that of L-cystathionine but not of L-allocystathionine. The spectrum of the isolated methionine sulphox. Je coincided completely with that of L-methionine-d-sulphoxide, but different from the l-sulphoxide. The authentic compounds were prepared by the method of Lavine¹⁰. This indicates that the sulphoxide found in urine is formed by an enzyme in a stereospecific

Table 1. Amounts of adrenatine, noradrenatine, metanephrine and normetanephrine in trine

	,	None	Isocarboxazid	Isocarboxazid +methionine
Adrenaline Noradrenaline Metanephrine Normetanephrine Metanephrine/adrenaline* Normetanephrine/noradrenaline*		4·8 (3·2) 20·0 (9·3) 7·6 (4·0) 11·7 (3·9) 2·1 (1·2) 1·3 (1·6)	3·1 (1·3) 12·4 (7·3) 10·3 (5·6) 27·1 (7·5) 3·3 (1·1) 3·4 (2·1)	8·8 (6·3) 25·6 (22·1) 18·2 (9·6) 36·9 (17·4) 2·6 (1·3) 2·1 (1·3)

Numbers not in parentheses represent the mean of the amounts of amines in two 24 h urine specimens from eight patients (μ g/g of creatinine), and numbers in parentheses are standard deviations. None, isocarboxazid, and isocarboxazid+methionine represent experimental periods of 0 and 7, 13 and 14 and 20 and 21 experimental days respectively.

* Mean of the ratios of the amount of a 3-0-methylated amines to that of its precursor amines in each urine specimen.

manner, and is not an artefact formed from methionine by spontaneous oxidation. The occurrence of methionine sulphoxide in large quantities in urine (2·7 μmoles/day on average) is interesting from the point of view that the sulphoxide has been thought to play a pathophysiological part in hepatic coma¹¹. Amounts of free sulphate were 7.1 ± 1.9 and 79 ± 24 mmole/day before and after methionine loading respectively. These results suggest amino-acid imbalance and ionic disturbances under the influence of methionine, and these may have to be taken into consideration before the psychic effects of methionine can be understood.

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Possible in vivo De-acetylation of N-Acetylhistamine in Man

AUTHENTIC N-acetylhistamine is degraded by human faeces in vitro and a histamine-like activity concurrently evolves, so the underlying mechanism for the decay seems to be de-acetylation.

The so-called conjugated histamine in urine consists solely or partly of N-acetylhistamine². The only observations on in vivo stability of conjugated histamine have been carried out on dogs by Anrep et al.3. After subcutaneous administration, conjugated histamine could be recovered almost quantitatively from the urine, but in these experiments unphysiologically high dosages of conjugated histamine seem to have been used. I have now examined the fate of small quantities of parenterally administered N-acetylhistamine in man.

N-Acetylhistamine (Calbiochem) was dissolved in isotonic saline, and the solution was sterilized by heating or by sterile filtration. A portion of the sample was subjected to two-dimensional paper chromatography1, and biological assay for free histamine and N-acetylhistamine, the latter after acid hydrolysis. There was no evidence of decay of the N-acetylhistamine before administration.

N-Acetylhistamine (205-37,000 µg, calculated as the base) was injected subcutaneously (twelve experiments on four healthy individuals). No untoward sympon four healthy individuals). No untoward symptoms followed the injections. Urine was collected for 24 h in bottles containing 100 ml. of 1·2 normal hydrochloric acid and the amount of histamine in the urine was estimated as described by Dunér and Pernow4. Mean recoveries of small amounts of histamine diphosphate (Nutritional Biochemicals Corporation) and Nacetylhistamine added to urine were 72 ± 8.3 (S.D.) and 74 ± 8.6 per cent, respectively. The mean urinary excretion of free histamine (unweighted values) in a control series was 12.6 ± 0.9 (standard error of mean), and the range 2-31 µg base/24 h. The corresponding values for conjugated histamine were 30.0 ± 5.9 and 1-99 (ref. 5).

The results are presented in Table 1. No corrections for losses during the procedure have been made for the urinary excretion of free and conjugated histamine. The recoveries of N-acetylhistamine in urine, however. have been calculated from corrected values.

It is evident that only a fraction of the administered dose of N-acetylhistamine is excreted unchanged in the The mean percentage not recovered with urine in 24 h.

Table 1. URINARY EXCRETION OF FREE AND CONJUGATED HISTAVINE AFTER

SUBCUTANEOUS INJECTION OF A ACETYLHISTAMINE										
	Urinary histamine (µg base)									
Test	AH*			\mathbf{Fr}			oujugat		recov-	
subj.	(μg		0-6	6-24	0-24	06	6-24	0-24	ered	
No.	hase)		h	lı	h	h	h	lı	(%)	
1	205	A			14			56	53	
•		\hat{B}	4	4	8	100	28	128		
2	205	Ā	-	_	15			4()	92	
-		$_{B}^{A}$	4	11	15	45	7	52 15		
3	205	A			17			15	57	
		\boldsymbol{B}	3	อี	8	74	7	81	_	
1	224	$\stackrel{A}{B}$			14			56	61	
		\boldsymbol{B}			26			120		
3	224	A			17			15	46	
		B			15 15			105	۸-	
2	410	A		_				40	67	
		A B A B A B A B	3	3	6	115	26	141	00	
3	410	Ą		_	17	125		15	32	
		В	3	7	10	175	45	220	5.1	
4	410	Ą			11	105	39	14 164	50	
_	w	B	2	2	4	125	39	40	44	
2	7,200	Ą	20	00	15 (6-22)†	2,700	320	3,020	**	
	* 000	35	26	22	48 17 (9–30)†	2,700	320	15	55	
3	7,200	$\stackrel{A}{B}$	12	15	27	2,100	300	2,400	547	
2	9,000	D _A	12	70	15 (6-22) †	2,100	000	40	34	
2	¥,000	n	19	11	30	3,700	750	4,450	٠.	
3	37,000	A	10	11	17 (9-30)+	0, 100	, ,,,,	15	52	
.,	31,000	A B A B	24	34	58	10,200	2,000	13,100		
		1.0	44	O-E	00	~~,	-,500	,		

Control series: mean free histamine, $12\cdot 6\pm 6\cdot 3$ (S.D.); mean conjugated histamine, $30\cdot 0\pm 23\cdot 6$.

A. Control studies, mean of excretion.
B. Study with N-acetylhistamine.

AH, N-Acetylhistamine.

Range in parentheses.

doses of less than 224 μg of N-acetylhistamine was 62 ; with doses of more than 7,000 μg the corresponding figure was 46. The ratio of excretion at 6 h to that at 24 h makes it unlikely that appreciable excess excretion of conjugated histamine takes place after 24 h.

Only 2-3 per cent of histamine administered parenterally to healthy individuals is excreted unchanged in the This may explain why there was no definite increase in the free fraction in the urine in experiments with small doses of N-acetylhistamine. With larger doses, an increase in urinary output of free histamine was found (P < 0.05, both with hypothesis null andStudent's t test). There are at least two feasible explanations for these findings: (I) the mechanism could be that of a de-acetylation of N-acetylhistamine; (2) the administered N-acetylhistamine could hinder the catabolism of endogenous histamine along the N-acetylhistamine pathway by product inhibition. The second possibility seems unlikely for the following reason. If a quantitatively rather unimportant pathway of histamine catabolism, such as acetylation, is blocked, the more important histamine-degrading enzymes that is, histaminase and the methylating enzymeshould be able to handle the small extra quantity of histamine. If this were the mechanism, the free fraction in urine should not increase. De-acetylation therefore seems to be the most likely explanation for the present observations.

It may seem peculiar that N-acetylhistamine is excreted unchanged to a higher extent when ingested, even in small doses7, than when administered parenterally. It seems reasonable, however, to assume that this depends on one of the following mechanisms: either orally administered N-acetylhistamine is conveyed through fluids and tissues with a relative lack of the de-acetylating system, or N-acetylhistamine is protected against deacetylation by some sort of binding.

These observations may necessitate a reappraisal of the supposed unimportant role allotted to acetylation in the catabolism of histamine6,8.

It would also be interesting to investigate whether N-acetylhistamine and histamine are formed from Nacetyl-L-histidine in vivo as they are in faecal specimens with a high capacity to form histamine1.

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Molecular Weight of Human Follicle Stimulating Hormone

FOLLICLE stimulating hormone (FSH) was obtained from powdered human pituitary dried with acetone and purified in two ways: (a) by the method of Amir, Barker, Butt and Crooke¹, and (b) by the cellosolve extraction procedure of Butt, Crooke and Wolf² followed by fractionation on DEAE-'Sephadex A-50'. Haemagglutination inhibition tests showed that luteinizing hormone (LH) and albumin were present to an extent of less than 1 per cent by weight of protein. FSH obtained by both procedures seems to undergo reversible association and dissociation, as the following experiments show.

The material gave two peaks when chromatographed on a column of 'Bio-Gel P-30' (22 \times 1-6 cm) in 0-01 molar phosphate buffer, pH 7.1 (Fig. 1A), the first peak being totally excluded (molecular weight greater than about The position of the second peak suggests a 30,000). molecular weight of 16,000-17,000 when compared with the elution diagrams for a-chymotrypsin (molecular weight 23,000), ribonuclease dimer (25,400) and ribonuclease monomer (12,700), assuming that the relationship log_{10} (molecular weight) = k (elution volume) holds for this column within the appropriate range. Chromatography on 'Bio-Gel P-100' (17×1 -6 cm) gave three peaks (Fig. 1B). The first peak was totally excluded, representing a molecular weight of greater than about 55,000. The third peak was totally included and thus represents material of molecular weight less than 20,000. The position of the second peak implies a molecular weight of approximately 34,000 by comparison with carboxypeptidase A (34,500) which was eluted in a similar position. On 'Bio-Gel P-150' (20 \times 1.6 cm) two peaks were obtained, the second being totally included as expected (molecular weight less than 50,000). The position of the first peak was the same as that of albumin, which indicates a molecular weight of about 68,000 (Fig. 1C).

The material thus seems to be a mixture of three species with molecular weights of approximately 68,000, 34,000 and 17,000. When a solution of FSH in the buffer was made I molar in sodium chloride, stored for several hours

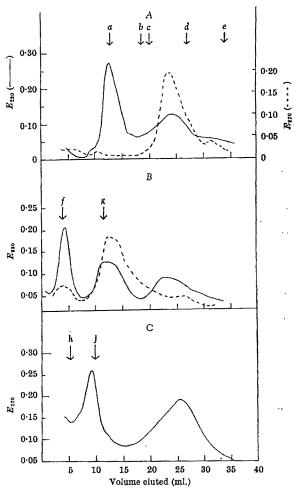


Fig. 1. Gel filtration of FSH. A, Elution diagram on 'Bio-Gel P-30'.

—, FSH; ---, FSH after treatment with 1 molar sodium chloride.

a, Position at which blue dextran was eluted; b, ribonuclease dimer (molecular weight, 25,400); c, chymotrypsin (molecular weight, 23,000); d, ribonuclease monomer (molecular weight, 12,700); e, salts. B, 'Bio-Gel P-100'.

—, FSH; ---, FSH after treatment with, and subsequent removal of, sodium chloride. f, Blue dextran; g, carboxypeptidase (molecular weight, 34,500). C, FSH on 'Bio-Gel P-150'. h, Blue dextran; j, albumin (molecular weight, 68,000).

at 0° C and then chromatographed on the 'Bio-Gel P-30' column, a single peak was obtained which corresponded to a molecular weight of 16,000-17,000 (Fig. 1A).

The peak from the 'Bio-Gel P-100' column which corresponded to a molecular weight of 34,000 was concentrated to 1 ml. by ultrafiltration, made 1 molar in sodium chloride and, after several hours, was chromatographed on the 'Bio-Gel P-30' column, giving a single peak again corresponding to a molecular weight of 17,000. When this peak was again concentrated to approximately 1 ml. (the sodium chloride having been removed during the chromatography) and rechromatographed on the 'Bio-Gel P-100' column, it gave a major peak at molecular weight 34,000 and a minor one totally excluded (Fig. 1B). The peak and a minor one totally excluded (Fig. 1B). The peak corresponding to a molecular weight of 68,000 from the 'Bio-Gel P-150' column underwent the same interconversions (that is, to molecular weight 17,000 in 1 molar salt and then to 34,000 on removal of the salt), as also did the first and third peaks from the column of 'Bio-Gel P-100'. When the preparations were stored in the absence of sodium chloride, those which showed single peaks of molecular weight 34,000 were converted into mixtures containing the two higher forms. The original preparations of FSH which contained all three forms had been obtained by chromatography using elution with salt gradients, and, on prolonged storage after dialysis, these were converted principally into the high molecular weight form.

Thus FSH apparently exhibits association and dissociation according to the salt concentration of the medium; in 1 molar salt the molecular weight is about 17,000 and in 0.01 molar phosphate buffer association occurs giving dimeric and tetrameric forms. No evidence of the presence of a trimer was observed. The association dissociation process seems to have no effect on the biological activity of the hormone; peaks corresponding to all three molecular weight forms were active in the ovarian augmentation assay.

This observation agrees well with data reported previously. Butt, Crooke and Cunningham³ have estimated the molecular weight as approximately 68,000, based on ultracentrifuge-sedimentation measurements, and Odell, Swain and Nydick⁴ have used a radiation-inactivation technique to obtain an average value of 31,400 (30,000–34,000). Li and Starman⁵ have demonstrated the phenomenon of dissociation in another gonadotrophin, luteinizing hormone, which apparently has a monomer form of molecular weight 16,300.

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Nucleotide Rhythms in the Mature Rat Heart

CARDIAC cells in adult humans or animals, like neurones, do not divide in the mature state¹, in contradistinction either to cells which continue to proliferate throughout the life of the organism (haematopoietic, oesophago-intestinal cells), or which show a gradual waning of this capacity with maturity (liver)². Mitotic figures have been seen in certain human infant hearts but these cases are rare and serve as exceptions to the rule.

Pelc^{1,3}, in a study of DNA synthesis in the rat heart, demonstrated the incorporation of tritiated thymidine into cardiac cells in an amount deduced to be eight to twelve times greater than that needed for cell division. The administration of 'Colcemid', followed by killing and autoradiographic examination of heart muscle, revealed no labelled divisions after 49 h, 73 h or 7 days. Pelc suggested that such incorporation of thymidine, as indicative of DNA synthesis, implies either DNA metabolism and turnover or a progressive movement towards a hyperdiploid cell. Such cardiac hyperdiploid cells have been well demonstrated in human cardiac hypertrophy³.

Our approach to a study of biochemical synthesis in the heart has been to observe rhythmicity in certain chemical components of the cardiac cell. We used 6 month old Sprague—Dawley male rats which had been conditioned on a 12 h lighting regimen and fed a standard laboratory diet from time of weaning. The hearts were removed from groups of six at 0800, 1500, 2000 and 2400 h. The atria were removed, the blood blotted off and the remaining entire ventricular myocardia frozen until individually analysed for protein and the nucleic and free amino-acids by methods described by Wannemacher et al.⁵ and Squibb⁶.

Table 1. BODY AND HEART WEIGHTS AND TOTAL CONTENT OF DNA

Hours	0800	1500	2000	2400
Heart weight (g)	0.836*	0.751	0.725	0.719
Body weight (g)	337*	312	312	309
Heart weight/body weight	0.0025	0.0024	0.0023	0.0024
Total DNA (mg)	1.04	0.94	0.99	0.90*

^{*} Significance at 5 per cent level.

The average heart weight of the rats was greater at 0800 h (P < 0.05), and the ratios of heart-body for the periods sampled averaged 0.0025, 0.0024, 0.0023 and 0.0024, respectively (Table 1). The biochemical results, shown in Fig. 1, demonstrate a definite rhythm for DNA (P < 0.05), RNA (not significant), total protein (P < 0.05)

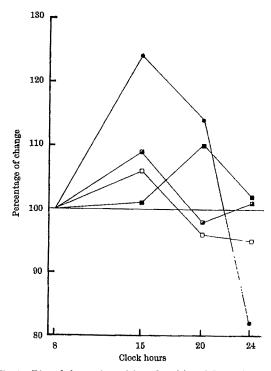


Fig. 1. Diurnal changes in protein and nucleic and free amino-acids in the mature rat heart.

DNA; , RNA; , protein; , free amino-acids.

and free amino-acids (P < 0.05). Furthermore, they delineate a period of an increase in DNA synthesis which, although it covers a span of 9 h, in all likelihood could be narrowed somewhat by more numerous within-period samplings. When the total quantities of DNA are calculated (heart weight \times mg of DNA/g wet tissue) an actual diurnal change in cellular DNA can be seen, with quantities significantly (P < 0.05) lower at 2400 h (Table 1). Increases and decreases in DNA synthesis in each gram of tissue have been noted in seminal vesicles1 and in chick livers, and intestines (Squibb, unpublished results). Total DNA (in mg) in relation to the heart-body weight ratio demonstrates an absolute change in quantities of this nucleic acid. This would suggest a diurnal rhythm which is the result of an increased synthesis of DNA apparently unrelated to the production of new cardiac cells. No determinations of ventricular water content were made in this experiment, but previous analyses by other investigators have revealed a constant diurnal water content for liver and heart⁸⁻¹⁰. We have analysed chicken liver every 4 h over a period of 48 h in controlled diurnal experiments and found a 1 per cent variation in liver cells with a water content of 70 per cent (unpublished results).

The curves in Fig. 1 indicate there was an accumulation of RNA, total protein and free amino-acid which preceded and partially continued into the zone of early DNA increase. It has been demonstrated11,12 that DNA, RNA and protein synthesis is integrated, with in fact a doubling of DNA, total RNA and cell mass (total protein) before cell division. Our analyses on virtually the entire cardiac ventricular mass, and the lack of any data on what percentage of the cardiac cells is involved in these processes, do not allow such an oversimplification. The data show the free amino-acid pool to be highly dynamic in cardiac tissue, having a distinct diurnal rhythm. We would speculate that changes in these free amino-acid levels relate to protein production as manifested in the protein pattern.

A number of investigators, unaware of this DNA rhythm, have used the nucleotide RNA/DNA relationship between experimental and controlled animals as key data for certain hypotheses. Such workers must now consider the point in diurnal time for the normal and abnormal rhythm.

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IMMUNOLOGY

Synthesis of the Antigens of Influenza Virus in Chick Embryo Kidney Cultures as studied by Fluorescent Antibody Techniques

The production of S and V antigens of influenza virus has been widely studied $^{1-6}$. We have investigated the accumulation of influenza virus in susceptible tissue cultures by both the direct and indirect fluorescent antibody techniques and the S and V antigens were simultaneously detected by the indirect method.

Tissue cultures were prepared from the kidneys of chick embryos 18 days old by the method described by Lippelt and Mannweiler and monolayers were grown on tube walls and on cover slips. Cultures were inoculated with 104 or 106 EID₅₀/ml. of influenza A2 virus (strain 2226). After 90 min the cultures were washed twice with Hanks solution, maintenance medium was added and they were incubated at 37° C.

The virus infectivity of the culture medium was studied at intervals by inoculating ten-fold dilutions into the allantoic cavity of 10 day old chick embryos. At the same time the haemagglutination titre was measured by a standard method. Cover slip preparations were dried, fixed in acetone for 10 min and then stained in one of the following ways: (1) direct Coons technique using the globulin fraction of a type-specific anti-influenza horse serum conjugated with fluorescein isothiocyanate; (2) indirect method using the unconjugated globulin of the same serum and a rabbit anti-horse conjugate; (3) indirect method using specific antisera to S and V antigens (given by Drs Vonka and Zavadova of the Serum and Vaccine Institute, Prague) and rabbit anti-guinea-pig conjugate.

Standard methods were used to prepare and purify the conjugates8,9 and to stain the preparations and controls.

Table 1. Characteristics of the infectivity of influenza virus produced in cultures of chick embryo kidney at different multiplicities

Multiplicity		tious act EID_{50}/r		Haen activit	ID/HA		
(EID_{60}/cell)	24 h	48 h	72 h	24 h	48 h	72 h	
5.0-10.0	5.25	4.0		1.1	1.2	1.1	4.15
0.05-0.1		6.33	6-0		1.1	1.0	5-23

Table 1 shows the results of the titrations of the infectivity and haemagglutinating activity. It can be seen that the use of high multiplicities (5-10 EID₅₀/cell) leads to the production of a virus population with a relatively lower infectivity than does the use of 0.05-0.1 EID 50/cell.

Immunofluorescent studies with anti-S serum and high multiplicities showed that specific fluorescence first appeared in the nucleus after 4 h. The brilliance of fluorescence increased after 5 h and more fluorescent nuclei were detectable (Fig. 1). After 6 h, S antigen appeared in the perinuclear cytoplasm. By 8-10 h there was fine granular fluorescence throughout the cytoplasm while nuclear fluorescence became gradually weaker (Fig. 2).

Cultures infected with the same dose of virus were stained for V antigen and after 5 h showed diffuse perinuclear fluorescence which spread gradually towards the periphery of the cell and by 8 h included the whole cytoplasm. There was no V antigen in the nucleus.

The appearance and localization of the S and V antigens in preparations inoculated with 10^4 EID_{50} were similar to those in preparations inoculated with 10^6 EID_{50} , but the first fluorescence was observed later (Table 2). The brilliance of nuclear fluorescence was the same after both inocula.

The character and dynamics of appearance of the nuclear and cytoplasmic fluorescence were very similar when antiserum against whole virus and the indirect method were used. Using this antiserum and the direct

Table 2. DYNAMIOS OF THE REPRODUCTION OF INFLUENZA A VIRUS IN CULTURES OF CHICK EMBRYO KIDNEY BY MEANS OF DIRECT AND INDIRECT FLUORESCENT ANTIBODY STAINING

Dose inoculated (EID_{zo})	Multiplicity of infection $(EID_{\delta 0}/\text{cell})$	S ant Nucleus	igen Cytoplasm	Indire V Nucleus	ect method antigen Cytoplasm	Who Nucleus	de virus Cytoplasm	Direct Nucleus	method Cytoplasm
10°	5.0	4 h+				4 h+		*****	-
		5 h++			5 h+	5 h++	5 h+		5 h+
			6 h+ 8 h++		01		6 h+++		6 h + +
101	0.05	6 h+			8h++			4.1.	8 h+++
10-	0.09					6 h+	6 h+	6 h+	
		8 h + + +	8 h + +		8 h +	gh + +	8 h + + +	8 h + + +	8 h + +

method the same sequence of fluorescence was observed after low multiplicities of infection, but after high multiplicities (5.0 EID₅₀/cell) intranuclear fluorescence was not found during the earliest periods, and the specific fluorescence appeared in the perinuclear cytoplasm (Fig. 3).

The data show that using different multiplicities of infection we obtained virus with different levels of infectivity and these results agree with those of others who have used influenza virus and tissue cultures2,8,10. There were no differences in the dynamics of accumulation of S and V antigens with the two multiplicities; this supports the data of Sugiura⁸ and Fraser¹, who found identical appearances in complete and incomplete and in complete and

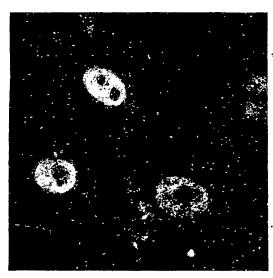


Fig. 1. Chick embryo kidney cells infected with massive doses of influenza A_2 virus. Treated with fluorescent antibody by the indirect method with specific anti-S serum. 5 h after infection. Specific fluorescence on nucleus; note dark "vacuoles".

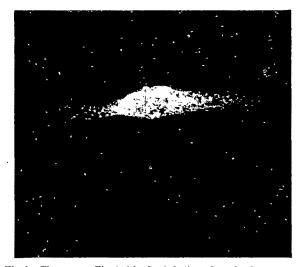


Fig. 2. The same as Fig. 1, 8 h after infection. Granular fluorescence in all cytoplasm.

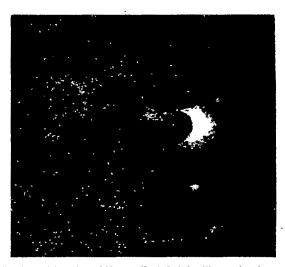


Fig. 3. Chick embryo kidney cells, infected with massive doses of influenza A_2 virus. Treated with specific conjugate by the method of Coons, 5 h after infection. Perinuclear fluorescence.

abortive cycles of reproduction, respectively. This implies that qualitative differences in the nucleoprotein component of the virus do not show themselves in the immunofluorescent picture.

On the other hand, our results differ from those of some authors who have detected in incomplete virus production either diminished production of S antigen^{2,5} or delay in its appearance in the nucleus⁴. This difference may be associated with the slight degree of incompleteness of the influenza virus we used.

The localization and appearance of influenza virus antigen in cells depend on many factors¹¹. We consider that the absence of nuclear fluorescence in incomplete virus production examined by the direct fluorescent antibody method can be explained either by the lesser sensitivity of the direct as compared with the indirect method. by inhibition of intranuclear fluorescence or by a reduction in the titre of the antibody against S antigen during the preparation and purification of the conjugate. In any case it seems that apparent differences between the results of earlier authors could be a reflexion of minor differences in the immunofluorescent technique used.

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Maternofoetal Transfer of γ G Immunoglobulins

Brambell has suggested that the mechanisms which underlie the transmission of antibodies and other plasma proteins from mother to foetus are similar to those which operate in the catabolism of these proteins. This concept was supported by the observation that maternofoetal transfer of homologous and heterologous γG globulins in the rabbit decreased with the half lives of these proteins; the species order of decreasing maternofoetal transmission and decreasing half life in the rabbit was rabbit > man > ginea pig > horse > bovine. In this study, the maternofoetal transfer of γG globulin was examined in the mouse, rat and guinea-pig.

Human serum albumin, human γG globulin and bovine γG globulin were obtained by low temperature ethanol-water fractionation^{4,5}, and labelled with iodine-125 by a modification of the nitrous acid method⁶; mouse, rat and guinea-pig serum γG globulins were isolated by means of DEAE-cellulose column chromatography⁷, and labelled⁶ with iodine-131. Of the total radioactivity in each of the labelled protein preparations, more than 98 per cent was precipitable in 10 per cent trichloroacetic acid, and 88–99 per cent was precipitable with specific rabbit antisera

against the unlabelled proteins.

Albino rats, smooth haired guinea-pigs and albino mice were used; all were pregnant and near term. Their drinking water contained 250 mg per cent of sodium iodide beginning at least 24 h before injection of labelled protein to inhibit thyroidal uptake of radioiodine. Each animal was given one of the following labelled protein mixtures, intravenously in the mice and rats and intracardially in the guinea-pigs: mouse $\gamma G^{-125}I$ and human albumin⁻¹³¹I, guinea-pig $\gamma G^{-131}I$ and human $\gamma G^{-125}I$, guinea-pig $\gamma G^{-131}I$ and bovine $\gamma G^{-125}I$, or rat $\gamma G^{-131}I$ and human $\gamma G^{-125}I$. The total labelled protein injected into each animal was less than 0·1 mg for a total of 1-2 μc. The animals were anaesthetized 24 h later, bled, and samples of serum were precipitated in 10 per cent trichloroacetic acid. The guinea-pig and rat foetuses were bled in situ by section of the axillary blood vessels; serum from the foetuses of a given litter were pooled and samples were precipitated in 10 per cent trichloroacetic acid. The mouse foetuses of a given litter less the placentas were weighed, then homogenized in 10 per cent trichloroacetic acid and the final volume was adjusted to 200 ml.; the concentration of labelled protein in foetal serum was determined as the amount of radioactivity for that amount of protein in each g of mouse foetus divided by the average volume of distribution⁸ for human $\gamma G^{-125}I$ which was determined All trichloroacetic acid precipitates were washed twice with a 10 per cent solution of the acid. Assay for iodine-125 and iodine-131 was carried out with a 400 channel spectrometer in conjunction with a 3 in. well type crystal of sodium iodide, each channel being 10 keV in width.

The concentration, F, of labelled protein in foetal serum relative to the concentration, M, of that protein in the maternal serum, or F/M, was taken as a measure of net maternofoetal transfer. The results are shown in Table 1. It must be emphasized that the F/M ratios in Table 1 represent the relative transfer efficiencies for the different proteins, or the relative protein clearances of maternal serum resulting from maternofoetal transfer, in these experimental conditions; that is, tracer amounts of labelled protein in the presence of normal maternal serum concentrations of the homologous γG globulin.

It will be noted that guinea-pig γG traversed the maternofoetal barrier in each of the three species more readily than did any of the other γG globulins, and that the efficiency of maternofoetal transfer in each species was least for human serum albumin. In decreasing order, the efficiency of maternofoetal transfer of γG globulin in

the mouse was guinea-pig \geq human > rat > bovine = mouse; in the rat, the order was guinea-pig > human = mouse > rat \geq bovine. In the guinea-pig, the order for those γG globulins studied was similar to that in the mouse and rat, that is, guinea-pig > human > mouse, but the maternofoetal transfer of these immunoglobulins in the guinea-pig was less than that for any γG globulin in the mouse and rat; the maternofoetal transfer of γG globulin in the guinea-pig was comparable with that of human albumin in the mouse and rat, the least efficiently transferred protein in the latter species. Thus, although selectivity in protein transfer was noted in each of these species, such transfer was far less efficient in the guinea-pig than in the mouse and rat.

The average half lives of guinea-pig γG , human γG , mouse γG , bovine γG and human albumin in the mouse are, respectively: 4.93, 4.53, 4.03, 1.610 and 0.7511 days. Although the species orders for half life and maternofoetal transfer for these proteins are similar, the data do not give much support to a possible relation between the two processes in the mouse. The differences in maternofoetal transfer of guinea-pig γG , human γG and mouse γG in the mouse are much greater than the relatively small differences in the average half lives of these proteins. In addition, labelled mouse YG and bovine YG have similar maternofoetal transfer rates in the mouse but quite different half lives. Nor can the differences in maternofoetal transfer that appeared between species be explained in terms of differences in half lives: the F/M ratio for guinea-pig γG and human γG in the mouse averaged fifty times the ratios for the same proteins in the guinea-pig, whereas the half lives of these proteins in the mouse, 4.9 and 4.5 days, respectively, were virtually the same as in the guinea-pig, 4.2 and 3.8 days, respectively. Of course, a possible similarity in underlying mechanisms of the two processes is not precluded by these data, but it is apparent that there are specific kinetic differences between plasma protein catabolism and maternofoetal transfer.

The half lives of these proteins and the maternofoetal transfer efficiencies were determined in similar conditions, that is, with tracer amounts of labelled protein in the presence of normal concentrations of homologous protein, and so comparison between these half lives and the F/M ratios is valid. Nevertheless, it should be noted that the F/M ratio for human γG , at least, is dependent on concentration, and thus the F/M ratios may not remain the same at higher maternal concentrations of the heterologous γG globulin or at lower concentrations of the homologous γG globulin (our unpublished work). At a maternal serum concentration of human γG in the mouse comparable with that for mouse γG , approximately 1,000 mg per cent, the F/M ratio for human γG is less than a fifth that for mouse γG ; thus, at this concentration, the rate of transfer of human γG is less than that of mouse γG .

The high F/M ratios for human and guinea-pig γG in the mouse compared with those for mouse γG indicate that mouse γG at normal maternal serum concentrations competes little, if at all, in the transport system for human or

guinea-pig YG. It is therefore interesting that the mouse

Table 1. Serum concentration of labelled protein in foetus relative to that in mother 24 h after injection into mother

Labelled	Mo . F/M	×100	Recipient s R_i F/M	ùt × 100	Guine F/M	× 100
protein	Litter	Mean	Litter	Mean	Litter	Mean
Guinea-pig γG	149 300	224	37 177	107	3·7 5·3	4.5
Human yG	78 138 142	150	28 44	36	$\frac{2\cdot7}{3\cdot2}$,	2-9
	247		\mathcal{E}_{i}	* 1,		
Rat yG	31	. 31	11 17	14		
Mouse γG	9 11	10'	30 32	31	1·8 2·0	1.9
Bovine yG	- 9 19	14	9 11	10	20	
Human albumin	3·6 4·0	3.8	2·4 3·2	2.8	1·0 1·1	1.1

has a maternofoetal transport system which is relatively specific and more efficient for human and guinea-pig YG globulins than for its own immunoglobulin, although the mouse presumably never came into contact with these molecules before.

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Transference of Maternal Passive Immunity to Vaccinia Virus in Mice

ADULT mice are highly resistant to experimental vaccinial infection, but suckling mice succumb to relatively low doses of virus introduced into the inter-scapular fat pad1. Transfer of protective antibody from immunized dams to their offspring has been described in this species for the viruses of variola2, herpes3, foot and mouth disease4 and Friend leukaemia⁵. In this communication, I report the transfer of antivaccinial immunity from actively immunized pregnant mice to their young. The Porton strain of albino mice and the WR strain of vaccinia virus were used throughout. The biological characteristics and method of assay of WR vaccinia virus are recorded by Turner⁶. A single batch of virus (titre 2×10^7 plaque forming units (P.F.U.)/ml.) was used, collected from chick embryo fibroblasts and stored in small volumes at -70° C.

The first experiment was designed to determine how late in pregnancy mice could be treated with virus so as to give birth to pups resistant to challenge with live virus. Breeding pairs of mice were kept in separate cages. At different times after mating, pregnant mice were inoculated intraperitoneally with a single dose of 0.1 ml. of virus

Table 1. TRANSFER OF PROTECTION TO LITTERS FROM MICE IMMUNIZED DURING PREGNANCY

Interval between vaccination of pregnant mouse and	No. of litters	Total progeny dead
parturition (days)	challenged	Total challenged
1 2 3 4 5 6 7 8 10 15 18 20	2 3 1 1 2 3 4 1 1 1 2 2 1	19/19 20/21 4/4 6/8 6/18 17/17 13/16 2/9 0/4 0/5 0/15
21 22*	1 2	2/5 8/11
 Vaginal plug seen. 		

suspension containing 105-106 P.F.U. (Table 1). The male mice were removed on about the tenth day of pregnancy. The baby mice were challenged within 24 h of birth by an injection into the fat pad of 0.05 ml. of virus suspension containing 10^4 P.F.U. (equivalent to $100 LD_{50}$). Deaths were recorded daily for 3 weeks. Control litters from unvaccinated mothers were similarly challenged; the mortality was 100 per cent. Female mice vaccinated between 8 and 18 days before parturition synthesized and transferred protective amounts of maternal antibody to almost all their foetuses. The decrease in protection with intervals of 20-22 days is presumably a result of the decline in the concentration of circulating antibody. This single injection conferred protection on three to four subsequent litters; but the mothers may have been re-immunized by contact with the live virus used for challenge of the litters.

These vaccinated mice were used to assess the role of the prenatal and mammary routes of antibody transfer in a cross-fostering experiment. At the same time, a second group of virgin females was mated, one breeding pair to a cage. The pregnant mice were inspected frequently and within 8 h after parturition litters were exchanged between the two groups. Litters which remained with their mothers for longer than this time were treated as controls. All progeny were challenged at the time of exchange. Deaths were recorded daily for 21 days. The difference in the degree of protection between the two cross-fostered groups was very significant ($\chi^2 = 15.7$ with l d.f.; P < 0.001; Table 2). The distribution of daily mortality in the two groups was also different (Fig. 1) The difference between the mean times to death, 5.5 days for group A and 7.8 days for group B, was highly significant (t=19 with 95 d.f.; P < 0.001).

Table 2. Transfer of protective antibody by the uterine ani-mammary routes

	Route of passive immunity	Status of mothers	Status of foster- mothers		surviving/No. of
В	Mammary	Unvaccinated	Vaccinated	10	8/66
	Uterine	Vaccinated	Unvaccinated	9	28/66
	Both	Vaccinated	None	2	14/15
	Neither	Unvaccinated	None	3	0/16

Other workers have challenged the mice at 3 days2. 5 days4 or 14 days3 after birth, by which time the suckling mouse has absorbed a considerable amount of antibody through the intestine. By challenging the litters within 8 h of birth the protective effect of the antibody secreted in the milk was negligible ($\chi^2 = 2.2$ with 1 d.f.; P > 0.05. Table 2). Thus, although it is not possible to compare the relative maximum efficiencies of the pre- and postnatal routes of transfer of antibody, the results of these experiments indicate that there is a substantial prenatal absorption of antibody by the foetal mouse, greater than has hitherto been suspected?. Kempes found that the titres of haemagglutinin-inhibiting antibody of cord blood of newborn human infants were significantly higher than those of their mothers who had been immunized with smallpox vaccine. It would be interesting to determine

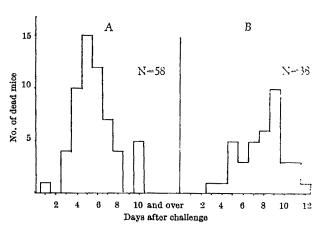


Fig. 1. Histograms of the daily mortalities in two groups of cross-fostered litters. A, Progeny of unvaccinated mothers fostered by immunized mothers. B, Progeny of immunized mothers fostered by unvaccinated mothers.

whether a similar mechanism for antibody concentration exists in the pregnant mouse.

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PHYSIOLOGY

Coelomic Water Volume Control in the Antarctic Sea-star Odontaster validus

ASTEROIDS are usually considered to have little or no control over the volume of water in the perivisceral coelom, the body wall being freely permeable to water and most ions¹. The Antarctic asteroid *Odontaster validus* Koehler, however, apparently regulates coelomic water volume to the extent of maintaining seasonal fluctuations2. This species is a "cushion" type asteroid with small, loosely scattered ossicles in a thin body wall permitting considerable flexibility in body shape. Individuals often markedly swollen with coelomic water were collected, while others were "normal" or even shrivelled. Many animals lost weight during successive weighings, and in some cases the decrease was more than 40 per cent of the original wet weight. Other animals maintained their original weight, or gained up to 30 per cent of their original wet weight, during successive weighings. Animals in the field had significantly less coelomic water in March and April, and significantly more in June, than at other times during the year. This seasonal fluctuation did not correlate with fluctuations in gonadal, pyloric caecal or intestinal caecal size. Several experiments, carried out at McMurdo Station, Antarctica, and showing other possible aspects of coelomic water volume regulation in O. validus,

are described here.

Two series of weighings, using three animals each, were followed with the animals placed first in sea water diluted 50 per cent with distilled water, and then returned to full strength sea water (Fig. 1). Wet weight rose slowly in one animal after it was placed in the 50 per cent sea water solution, while in the others wet weight fluctuated with little real change. The lack of expected marked increase in weight as a result of water uptake1 could be caused by holes broken through the body wall allowing water to escape, as occurred in similar experiments with Asterias rubens3. The animals recovered, however, and became active when returned to full strength sea water, and they all lost weight. The loss of weight probably resulted from the coelomic fluid becoming hypo-osmotic to sea water while in the 50 per cent sea water solution, and indicates either that the body wall remained intact or that the possible holes were quickly sealed.

When collected, most specimens of O. validus were turgid as if water in the perivisceral coelom was held at a slightly positive pressure. After being "relaxed" for 18-24 h in a solution of 1 part isosmotic magnesium chloride to 1 part full strength sea water, most specimens lost water (Fig. 2A), became flaccid and sluggish, and reached a constant weight, that is, they seemed to have lost their ability to hold coelomic water under pressure. When replaced in sea water after the treatment with isosmotic magnesium chloride, most animals regained activity and

returned to their original weight within 20 h, and some even gained much over their original weight (Fig. 2B). Binyon⁴ found a similar drop in weight in A. rubens in isosmotic glucose and choline chloride solutions because of salt and water loss as a result of the impermeability of the glucose molecules and choline ions to the body wall. But he also showed that magnesium ions in the perivisceral fluid remained isoionic to the outside medium. The effect of magnesium ions on O. validus is therefore probably caused by something other than their being impermeable to the body wall.

The loss of the ability to control water volume in isosmotic magnesium chloride solution is further demonstrated by the fact that the wet weight of animals treated with magnesium chloride showed an almost linear relation to dry weight, whereas the original wet weight to dry weight relationship was very irregular because of variable coelomic water content in the different animals The smallest animals, however, did not lose weight in the magnesium chloride solution, and some even gained weight. These very small animals are the ones shown to have gained weight in Fig. 2A.

A final series of weighings, shown in Fig. 4, illustrated another aspect of control of coelomic water volume in

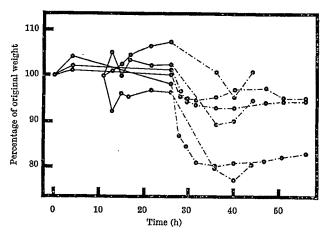


Fig. 1. Wet weight changes in six specimens of O. validus in 50 per cent sea water (——) and after their return to full strength sea water (——). Initial wet weights ranged from 4.0 to 10.8 g.

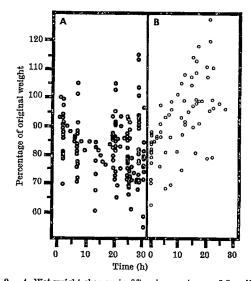
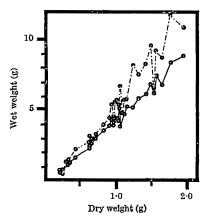


Fig. 2. A, Wet weight changes in fifty-nine specimens of O. validus in isosmotic magnesium chloride solutions. Different series of weighings, varying in exposure and weighing times, are shown. Two animals, both weighing 0.7 g, gained weight as did a third weighing 3.3 g. All the other animals, ranging in initial weight from 1.5 to 35.4 g, lost weight. B, Wet weight changes in twelve specimens of O. validus when returned to sea water after 18-24 h exposures to isosmotic magnesium chloride solutions. Initial wet weight ranged from 4.0 to 14.3 g.

O. validus. The weights of three animals, maintained in sea water at -1.5° C and fed on beef and seal meat every 3 to 5 days, were followed for 1-2 months. The smallest animal followed a striking periodicity in wet weight with a period of 10 days. The other small animal had a periodicity in wet weight of 15-20 days, while a suggestion of a periodicity of about 60 days occurred in the large animal. These periodicities did not correlate with the feeding or water changing regimes, nor with any other recognized fluctuation.

Gemmill⁵ found weight increase in the asteroid Porania pulvillus during flagellary-mucoid feeding activity. validus is also a flagellary-mucoid feeder2, and the turgidity found, and its fluctuations, may be a consequence of such feeding activity. From evidence of pyloric caecal growth, however, feeding was most intense during the late summer when the coelomic water volume was lowest2.

Another possible means of controlling volume is by the regulation of small osmotically active organic molecules. Such molecules are important for the control of coelomic water volume in some sipunculids, and the control of at least intracellular volume in asteroids, and echinoids.



Relation to dry weight to initial wet weight (----) and to wet after 18-24 h exposures to isosmotic magnesium chloride solutions (-----) in twenty-nine specimens of O. validus.

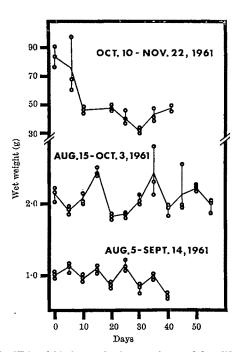


Fig. 4. Wet weight changes in three specimens of O. validus maintained in the laboratory. Each dot represents one weighing, and three weighings were made 1 h apart every 5 days.

Being common in very shallow Antarctic waters2, specimens of O. validus are exposed occasionally to summer fresh water run-off from the surrounding lands. ability to control body volume and withstand hypo-osmotic conditions may be particularly important at these times.

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Haemostatic Plugs in Experimental Scurvy

Born and Payling Wright¹ found that the blood platelets of weanling guinea-pigs fed on a scorbutogenic diet show decreased adhesiveness. These results suggested to them that the haemorrhages of scurvy might be caused either by the failure of the platelets to adhere to form effective haemostatic plugs, or because of some unexplained mechanism, to maintain normal capillary resistance. We have repeated these experiments and, in addition to measuring the adhesiveness of platelets to glass, we have studied the formation of haemostatic plugs by direct observation.

Weanling guinea-pigs, Hartley-Dunkin albino strain, weighing 167-220 g, were divided into two groups; one was fed a standard diet, the other received a scorbutogenic diet2 supplemented with autoclaved reconstituted milk and green vegetables. Control animals gained weight, while those on the scorbutogenic diet were almost at their initial body weight when clinical signs of scurvy appeared. The animals were anaesthetized with intraperitoneal urethane. A loop of intestine with its mesenteric vessels was withdrawn through a small incision in the abdominal wall, and the arteries were observed and major injuries inflicted as described by Honour and Ross Russell3. Several arteries were subjected to such injuries in each animal. Blood was withdrawn from the abdominal vena cava and mixed with one part in ten of 3.8 per cent trisodium citrate. Platelet adhesiveness was measured by a modified rotating bulb technique4; platelet counts were made using the method of Brecher and Cronkite before and after rotation of the blood for 40 min. Packed red cell volumes were measured by the haematocrit method. The animals were divided into two groups of six. One group was maintained on the scorbutogenic diet, the other on a standard diet. All animals on the scorbutogenic diet showed loss of hair, had haemorrhages and were in such poor condition by the fourth week that three were lost through anaesthesia during preparation. In the remaining three, several mesenteric arteries in each animal were subjected to major injuries which resulted in profuse bleeding, but at all sites a firm haemostatic plug formed and the bleeding ceased. White bodies

developed at all injury sites within the lumen of the vessel. Of the control group, two died as a result of anaesthesia. In each of the remainder several mesenteric arteries were injured and at each injury site firm haemostatic plugs and intraluminal white bodies formed. There were no detectable differences in the formation of haemostatic plugs and white bodies between the two groups of animals. Platelet adhesiveness was measured in too few animals for any great weight to be attached to the results, but the scorbutic animals showed lower adhesiveness than the controls (27 per cent compared with 36 per cent). There was a small difference in packed red cell volumes (scorbutogenic animals, 36; controls, 38).

These observations agree with those of Born and Payling Wright in the matter of platelet stickiness, but they also show that, despite the presence of a defect in the blood platelets, the haemostatic plugs produced by injury are firm and have their integrity unimpaired. It would thus seem that the haemorrhages of scurvy are not attributable to failure of haemostatic plugs to form, and the maintenance of a normal capillary resistance as suggested by Born and Payling Wright may still be implicated.

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Vascular Organization of the Human Placenta

The currently accepted theory of maternal blood flow through the intervillous space of the primate placenta1 is that maternal blood entering through basally situated spiral arterioles spurts up towards the chorionic plate under the influence of the vis a tergo of the maternal arterial pressure, spreads laterally and falls back to drain through basal venous openings. Short circuiting from artery to adjacent vein is believed to be avoided purely by virtue of the arterial pressure. This theory based on cineangiographic evidence takes no account of the structure of the foetal portion of the placenta. The latter consists of numerous lobules or cotyledons of which the principal fifty to sixty are arranged in the form of hollow centred bush-like structures as seen in corrosion casts of the foetal vasculature^{2,3}. Each lobule arises by a main chorionic stem, and is supplied through a major branch of an umbilical artery. Subsequent branches supply an extensive capillary network within the mass of terminal villi which crowd round the central intralobular space. This lobular pattern can be recognized in histological sections in the form of dense masses of villi orientated round cavities. Interlobular and subchorial areas show greater separation between the villi.

It has been suggested, on theoretical grounds, that the hollow centres of the lobules are the site of entry of the maternal blood into the intervillous space3,5,6 with some recent experimental confirmation derived from eineangiography7,8, but this has been denied for the human placenta by evidence based on placental reconstructions.

The work reported here indicates that the anatomical relationships of the maternal and foetal circulations can be determined in the delivered human placenta by the use of simple techniques and that the pattern displayed throws light on the nature of the circulation through the intervillous space.

If the maternal surface of a freshly delivered placenta is examined under a good light the thin-walled spiral arteri-

oles can be defined by squeezing the placenta gently so as to fill them in retrograde fashion from the intervillous space. It is then possible to inject a number of the vessels with barium gelatine solution ('Micropaque', Damancy and Co., Ltd., with 3 per cent gelatine), through a fine nylon intravenous cannula (size 2-3, French) so that any desired quantity of contrast enters the intervillous space. injection mass is adjusted to a viscosity of about twice that of blood by addition of the "neutral medium" of Damancy and Co. The injected vessels are surprisingly large with a diameter of 2-3 mm (Fig. 1). Separate injection can be made into the placental veins and into the foetal vessels of the umbilical cord. The placenta is then fixed in 10 per cent formal saline before further

study by radiology, dissection and microscopy.

Examination of injected specimens shows that the spiral arterioles can enter the intervillous space either over the centre or at the periphery of the foetal lobules. On dissection it is apparent that the injection mass invariably collects in the loose centre of the lobule irrespective of its site of entry. Injection through the decidual veins selectively fills the subchorial lake and interlobular spaces with little tendency to percolate to the centre of the lobules. X-ray studies confirm the orientation of the



Fig. 1. Portion of fixed placenta viewed from maternal surface showing several injected spiral arterioles.

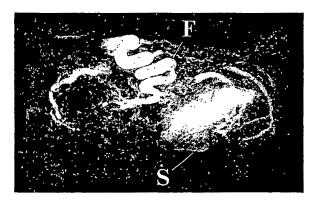


Fig. 2. Lateral X-ray of portion of fixed placenta. Contrast has entered intervillous space through spiral arteriole (8) and is surrounded in basket fashion by branches of foetal artery (F). Note an unfilled intralobular space on the left. ...

foetal placental vascular tree round the sites of spiral arteriolar inflow (Fig. 2). Histological sections show that the injection mass is surrounded by the densely packed masses of villi of the lobular shell which separate it from the loose interlobular zones (Fig. 3).

This investigation supports the hypothesis that maternal blood enters the centre of the foetal lobules although it is not yet possible to state whether each lobule is always supplied by a single spiral arteriole. The vascular pattern has important implications. Because the spiral arterioles of the decidua develop from those of the endometrium it follows that to achieve the structural relationship observed near term, the foetal lobules must have developed round the arteriolar entries. This has several predictable results. First, most of the terminal villi come into contact with the richly oxygenated maternal blood as it enters the intervillous space. Second, the dense masses of villi are likely to form sufficient resistance to flow to allow a substantial arterial pressure to be achieved in the intralobular space as envisaged by Reynolds. Third, the terminal villi act as baffles which result in uniform lateral dispersion of blood flow throughout the lobule. Finally, as growth of the placenta proceeds, loose spaces are left between the lobules and will form natural pathways for venous drainage. The circulation through the intervillous space may be envisaged as in Fig. 4.

All conditions essential for development of a logical maternal placental circulation and for achievement of

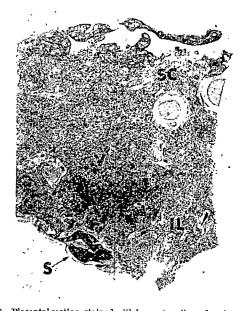


Fig. 3. Placental section, stained with haematoxylin and eosin, \times c. 3.5. Contrast has entered placents through spiral artery (S) and collected in the intralobular space where it is separated from interlobular space (IL) and subchorial space (SC) by dense masses of villi (V).

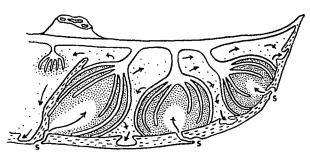


Fig. 4. Circulation through intervillous space. Diagram indicates main chorionic stems with spiral arterioles (S) discharging into the centre of the lobules. Density of stippling indicates density of villi and arrows show directions of flow through intervillous space.

optimal conditions for placental transfer may well be fulfilled purely by preferential growth of the foetal lobules round the entries of maternal spiral arterioles into the intervillous space. The central role of the spiral arteriolar entries in the organization of the intervillous circulation receives further support from a recent study of aortograms performed on pregnant women.

Current arguments about the existence of an intervillous capillary space¹⁰ may perhaps be resolved on the basis of this pattern. If the areas filled readily by high viscosity injection media in the delivered placenta accord with conditions in the uterus, then the intralobular, interlobular and subchorial spaces are all of greater than capillary dimensions, whereas the crowded masses of terminal villi making up the shell of the lobule may well form a functional intervillous capillary space.

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Static Method for determining **Blood Yield Stress**

THE yield stress or shear strength of a suspension is the critical stress which must be applied before the suspension will flow. This property of blood has been determined in the past by the extrapolation to zero shear rate from viscometry at higher shear rates, for example, (a) the extrapolation of low shear rate Couette viscometric data1-3, (b) the extrapolation of capillary tube viscometric data and (c) the torque-decay curve of the Couette viscometer2,3

These methods for determining yield stress have been open to question. Some workers have noted that blood does not seem to have a yield stress if sufficiently low shear rates are achieved. On the other hand, if torque decay is measured, a yield stress may be detected. It has been suggested that this discrepancy may be caused by the development of a marginal plasma layer at low shear Thus a method based on a different principle is needed. A settling technique for determining yield stress has been suggested before. This approach has been developed to provide a method for determining blood yield stress that is independent of blood viscometry.

A glass capillary tube of inside diameter about 0-20 cm was drawn to a fine taper of about 30µ inside diameter, and the tip was broken to allow the tube to be filled with liquid. The tapered tube was then filled with blood, sealed at the bottom, for example, with clay, and allowed to stand in a vertical position for about 5-10 min (Fig. 1). In this time, settling of cells may be observed. There will, however, be a diameter for the inside of the tube above which no settling occurs. This

is known as the yield diameter, D_y . No settling occurs above the yield diameter because the weight of the red cell network is capable of being supported along its lateral surface. It is essential to note the first break in structure and to avoid trapping bubbles in the blood which disrupt the suspension structure. If allowed to stand too long, the cell suspension structure may slip and cause secondary breaks.

From a consideration of the force balance involved, it is possible to calculate the yield stress from the yield diameter. At equilibrium the force acting along the lateral surface just equals the weight of the cells supported. This is expressed by

$$\tau_{y} = \frac{(g)(\varphi)(\rho_{c} - \rho_{p})D_{y}}{4\cos\theta} \tag{1}$$

where ρ_p is plasma density; ρ_c is cell density: D_y is yield diameter; τ_y is yield stress; g is the gravitational constant; φ is the volume fraction of red cells, and θ is the angle of taper, which is very small in the drawn capillary tubes, so that $\cos\theta \cong 1$.

The density difference between plasma and cells was determined by the copper sulphate method by noting the solution densities which just suspend plasma and centrifuged cells. This density can be measured to 0.001 g/ml. by this method. The density difference $(\rho_c - \rho_p)$ does not change substantially for a relatively large range of temperatures, and remains nearly constant for many samples measured. The density difference error is within 4 per cent.

4 per cent.

The yield diameter was measured using a low power microscope with a graded eyepiece. A drop of immersion oil on the glass tube aids the measurement. At least three replicates were measured and the error was within 5 per cent. The blood yield stress is then calculated from equation (1).

The values obtained by this method were compared with those for yield stress obtained by extrapolation of a plot of (shear rate) against (shear stress) from shear rates of between 1 and 1,500 sec⁻¹ (Fig. 2). Brookfield cone and plate viscometers covering this shear rate range were used. The viscometers were calibrated with NBS oil and checked with Brookfield standard oil. The blood samples were drawn either from healthy donors or from

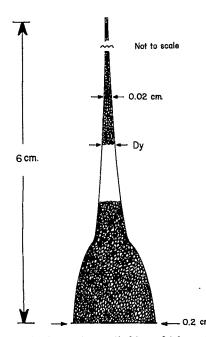


Fig. 1. The yield diameter in a vertical tapered tube containing a red cell suspension.

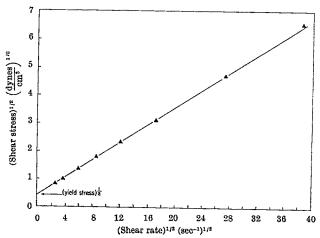


Fig. 2. A typical plot of cone and plate viscometry for determination of yield stress.

patients under anaesthesia, undergoing surgery. The blood from healthy donors was drawn in ACD buffer while that from surgical patients was placed in heparin as anticoagulant. Dilutions of cells were made by suspending centrifuged packed cells in an appropriate volume of plasma obtained from the same blood.

In Table 1, the static yield stress of various samples of blood with varying haematocrit and at different temperatures is compared with the yield stress derived from viscometry determined by a Brookfield cone and plate viscometer.

The results indicate good agreement between the yield stress determined by the static method and yield stress determined by extrapolation in sixteen of nineteen experiments (Fig. 3). The density difference ($\rho_c - \rho_p$) is nearly constant at 0.061 g/cm³.

The agreement between the static method and the extrapolation of shear rate information from above 1 sec⁻¹ suggests that Casson's equation applies below 1 sec⁻¹ as well as above 1 sec⁻¹ for blood.

The yield stress is a measure of the aggregation function of cells and may be of critical importance in determining flow in response to small stresses. This method makes possible easy determination of this property of blood in health and disease.

The values in the literature for blood yield stress determined in a GDM Couette viscometer seem to be about five times less than those determined with a cone and plate viscometer^{4,7}. Yield stress determined by the static method described here, however, agrees with the yield

Table 1. Comparison of yield stress determined by static method with yield stress determined by viscometry

Sample		(gc - gp) (g/ml.)	Tem- perature (°C)	Dy (cm)	method yield stress (dynes/ cm²)	metry yield stress (dynes/ cm²)
5/17/67	0.179	0.061	22.5	0.0460	0.122	0.292
*5/12/67	0·250	0·062	24·5	0-0306	0·116	0·116
5/ 3/67	0·290	0·064	38·0	0-0324	0·109	0·0361
*5/12/67	0·325	0·062	24·5	0.0324	0-160	0·0625
5/17/67	0·338	0·061	22·5	0.0510	0-258	0·270
*5/23/67	0·319	0·063	37·0	0.0279	0-137	0·123
5/29/67	0·379	0·062	28·5	0.0432	0-249	0·292
5/30/67	0·399	0·062	28·5	0.0486	0-295	0·336
*4/10/67 *4/24/67 *5/ 2/67 *5/ 2/67 *5/ 2/67 5/ 3/67 *5/12/67 5/19/67 \$/19/67 *6/12/67	0-460 0-440 0-450 0-450 0-470 0-436 0-482 0-482	0.058 0.061 0.062 0.062 0.064 0.062 0.060 0.060	22·0 21·0 24·5 37·0 38·0 24·5 37·0 24·5	0.0265 0.0380 0.0276 0.0234 0.0203 0.0441 0.0428 0.0504	0·174 0·250 0·276 0·160 0·149 0·360 0·305 0·348	0·164 0·260 0·381 0·160 0·152 0·335 0·250 0·360
6/ 5/67	0·130	0.066	29·0	0·0252	0·053	0·054
6/ 5/67	0·201	0.066	29·0	0·0298	0·095	0·102

* Blood from normal donors.

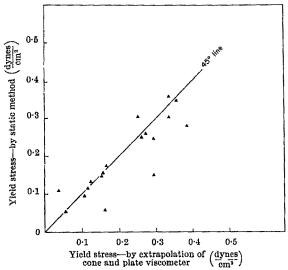


Fig. 3. Correlation of yield stress by static method with extrapolation of viscometry (Table 1).

stress determined by extrapolation of cone and plate

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Teratogenic Effect of Cadmium and its Inhibition by Zinc

DURING a study of the teratogenic effects of heavy metals on mammalian embryos, we have been able to produce severe developmental malformations in golden hamster embryos by the intravenous injection of 2 mg/kg of cadmium sulphate into pregnant mothers on the eighth day of gestation. These malformations consisted of a specific effect on the face and upper jaw, ranging from a simple mid-line cleft to almost complete obliteration of normal facial architecture. A few other malformations apparently also induced by cadmium in this dose were occasionally found. These consisted of anophthalmia, digital and other limb defects, rib fusions and exencephaly. The simultaneous administration of 2 mg/kg zinc sulphate almost completely inhibited this marked teratogenic effect of cadmium. The particular susceptibility of the germinal epithelium of the testis and the rodent placenta to the toxic effects of cadmium has been described1.2. This toxic effect of cadmium has been attributed to alterations in the endothelium of the vascular beds in these affected organs2,3.

Timed matings of female hamsters were obtained by a manner previously described4. On the eighth day of gestation, the animals were injected intravenously with

either 2 mg/kg of cadmium sulphate, 2 mg/kg of zinc sulphate or a mixture of 2 mg/kg of cadmium sulphate plus 2 mg/kg of zinc sulphate made up in distilled water. The volume of the injected solutions was standardized at 1.0 ml./100 g of body weight. On the tenth, eleventh or twelfth day of gestation the embryos were collected and examined for gross external malformations. At this time, the number of resorption sites was also ascertained.

Table 1 shows that zinc alone provoked a very mild teratogenic response. In identical conditions, the intravenous injection of cadmium caused a marked embryocidal and teratogenic effect; approximately 66 per cent of the embryos showed developmental malformations. The most striking malformation was a facial abnormality affecting the upper jaw and resulting in varying degrees of facial When zinc was combined with cadmium at the time of injection, however, the teratogenic and embryocidal effects of cadmium were markedly reduced. The maternal animals survived the injections well and no pathological changes were noted when they were killed.

L. EFFECT OF CADMIUM, ZINC AND CADMIUM-ZINC ON EMBRYONIC MORTALITY AND MALFORMATIONS IN THE GOLDEN HAMSTER

	No. of mothers treated	No. of embryos recovered	No. of embryos resorbed	No. of embryos malformed
Controls (distilled water)	10	116	7	1
Cadmium (2 mg/kg)	20	204	44	131
Zine (2·0-6·0 mg/kg)	12	142	4	2
Cadmium-zine (2 mg/kg of each)	10	120	6	5

The protective effect of zinc could be related to a critical physiological cadmium: zinc ratio which has been demonstrated in a variety of ways. Schroeder has shown that high renal ratios of cadmium: zinc have been associated with arterial hypertension in rats and that this hypertension can be reversed by a zinc chelate. Administration of zinc to animals loaded with cadmium will also prevent the testicular lesions previously described6

Recent studies of the teratogenic effect of lead ions have emphasized the marked teratogenic effect of lead on the tail bud of hamster embryos7. The specificity of the teratogenic effect of cadmium on embryonic facial architecture points to another specific organogenetic effect of a heavy metal teratogen. It is tempting to hypothesize a localized specific effect on some metalloenzyme which competes for both cadmium and zinc. These experimental results point the way to further research concerning the specificity of the teratogenic effects of heavy metals.

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Cerebral Potentials evoked by Pattern Reversal and their Suppression in Visual Rivalry

SINCE the introduction of techniques to improve the signal to noise ratio1, the number of publications on human visual evoked potentials has been considerable; usually the stimulus has been a brief flash or series of flashes (flicker) on an unstructured field, but recently the interest of patterned fields has become apparent²⁻⁴. Following a suggestion by Gross et al.⁵ we have demonstrated the effectiveness of "pattern reversal", without change in the total luminous flux, in evoking visual cortical responses and have applied the method to the investigation of binocular rivalry.

As in our previous experiments on binocular rivalry, the target area was limited by a hole 3.75° (2.5 cm at 37 cm distant) in diameter, or less, in a black surround, usually viewed by one eye. The target was a thin transparent 'Cellophane' sheet with a regular pattern of black horizontal bars printed on it, lying immediately behind the hole and moved vertically up and down by a linear transducer. It was transilluminated by a tungsten filament lamp behind a diffusing screen of white paper; the luminance of the clear parts of the target was 200 ft.-lumens or less. For rivalry experiments the second eye viewed a congruent target, similar in all respects except that its parallel black bars were vertical; it could be stationary or moved from side to side by a second transducer.

The electroencephalogram was recorded from an electrode on the midline 3 cm above the inion, with reference to an electrode at the vertex or on one ear lobe; in the former case an ear-vertex derivation was used to check the absence of response from the vertex lead. After amplification the responses were averaged by a Biomac 500 averaging computer, usually 128 sweeps of 500 msec duration. The averaged responses were stored in digital form on punch tape and written out by an X-Y plotter.

The target used most often had fifty-eight alternating black and white bars of equal width and was 2.5 cm in diameter; the transducer was adjusted to move the target by one bar width, a distance of 0.43 mm or 4' of visual angle, so that the black-white pattern was wholly reversed; the rise or fall time was 3 msec (10-90 per cent amplitude). In these conditions good evoked responses were obtained in all the twelve subjects examined for frequencies in the range from 2 to 20 c/s (Figs. 1 and 2). It should be noted that the frequency of movement was 1-10 c/s; upward and downward displacements of the transducer gave similar responses so that the response frequency was twice the transducer frequency. Exactly similar responses were obtained when the bars were placed vertically and moved horizontally (Fig. 1, channel The maximum amplitude of response was about 12 μV peak to peak, obtained at 12 c/s.

Three sizes of target (25, 12 and 6 mm in diameter) have been used and the same trends are clear in all subjects although the effect of target size has so far only been studied systematically in one. When the visual angle of the target was increased from 56' to 1° 52' the response amplitude rose by 55-75 per cent, depending on frequency; a further increase to 3° 45′, however, caused only a 16 per cent increase in amplitude, or less, except at the highest frequency (20 c/s) at which the responses with the smaller targets had been ill defined. In this and other subjects, looking at the edge of the target caused marked decrease in response amplitude; it fell almost to zero when the fixation point was a few degrees to either Thus the response, like those to flash stimulation, must be largely the result of foveal stimulation and probably requires good resolution of the pattern; this is further suggested by the sharp decrease in amplitude when the target is thrown out of focus by a suitable lens.

With the largest target, a ten-fold reduction of the luminous flux caused little reduction in the amplitude of the response. Some other target materials have been used and a chequer pattern (one black-white pair = 27') seemed particularly effective (Fig. 2). The response to two-eyed stimulation was larger and could be nearly twice the voltage of that to a unilateral stimulus.

Two types of control have been made. The theoretically more satisfactory is to set the transducer to move the target by a black—white pair width so that there is no apparent movement. In these conditions, the responses were greatly reduced although a small component at the stimulus frequency might remain, possibly caused by target overshoot, resulting in some visual stimulation. An alternative control was to cover the target with a sheet of plain white paper; when the movement of the target was through one bar width no response was recorded at the stimulus frequency.

The term binocular rivalry implies that when different images fall on congruent areas of the two retinae the subject usually perceives first one image, then the other; one eye is said to be dominant while the other is said to be suppressed. It has been reported. It has been reported. It has been reported evoked responses than when it is applied to the suppressed eye. In our experiments we used the same target material as that described except that the patterns of horizontal or vertical bars were stationary and transilluminated by gas-discharge flashes or 100 per cent square wave modulation of a fluorescent source. We found no consistent differences in amplitude between responses to stimulation of the

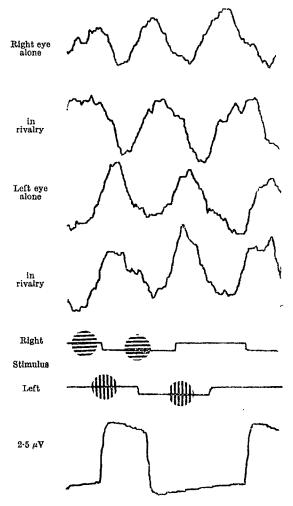


Fig. 1. Pattern reversal in visual rivalry. Each trace is the average of 128 sweeps and lasts 250 msec; response frequency, 12 c/s. The targets, 6 mm in diameter (56'), have alternating black and white bars 0.43 mm wide; for the right eye they are horizontal and move vertically by one bar width; for the left eye they are vertical and move laterally. The two traces above the calibration signal, if viewed at 37 cm, show the actual size of, and change in, the targets and the temporal sequence of their movements, which are arranged so that the responses from stimulating the eyes separately are 180° out of phase (channel 1 = right eye, channel 3 = left eye). When the eyes are stimulated together rivarity occurs and the subject sorts the responses by pressing either of two switches, according to whether the right eye (channel 2) or left eye (channel 4) is dominant. It will be seen that the responses during rivalry are as large as or larger than during single-eyed viewing, which suggests that the non-dominant eye has not contributed to the trace.

dominant or suppressed eyes, confirming the negative results of Kaufman et al.10.

Using pattern reversal as the stimulus, we have so far investigated seven subjects, two of whom found it impossible to sort dominance and suppression reliably. In one type of experiment only one of the targets moved and the subject indicated by two switches which eye was dominant, causing the responses to be stored in the corresponding channel of the Biomac⁶. Thus the amplitudes of the two stored signals represented the mean amplitudes of the responses to stimulation of the one eye when it was dominant or when it was suppressed. In twenty-four experiments of this kind on five subjects (2, 2, 5, 10 and 5) a clear reduction in amplitude, easily seen without measurement, was present in twenty-two cases; one subject gave two doubtful results out of ten; in no case was the opposite effect seen. No such consistent effects were found with conventional flicker.

In a second type of experiment, stimuli were presented 180° out of phase to the two eyes (Fig. 1); the subject sorted the responses as described. If suppression were complete and if this sorting were perfect it would be expected that the two traces would be similar to those obtained for stimulation of either eye alone, whereas without suppression or without sorting each channel would contain an equal mixture of the out-of-phase responses and both traces would approximate to straight lines. This was the result when using conventional flicker, whereas with pattern reversal the four subjects investigated (twenty experiments: 1, 8, 4 and 7) gave clearly positive results apart from three out of seven in one subject which were equivocal. Two of the subjects gave out of phase responses as large as or larger than those

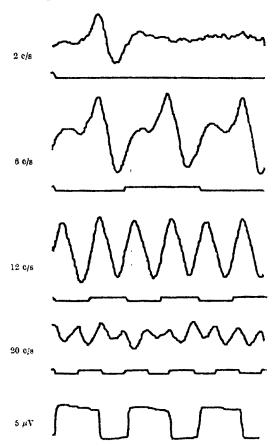


Fig. 2. Responses in one subject to stimulation of the right eye by vertical movement of a chequer pattern through one square, or 13.5'. Field diameter, 12 mm or 1° 52'. Each trace is the average of 128 responses and lasts 500 msec. Channel 1 is the response to downward movement of the pattern, indicated by the trace immediately below it; the remaining channels show the similarity of responses to up or down movements at various frequencies. Note that the transducer frequency is half the response frequency, the latter being given in the figure.

obtained when the eyes were stimulated separately (Fig. 1). Sorting cannot be perfect because of indecision and reaction time delays, so this is a surprising result which, if confirmed, raises interesting possibilities regarding the interaction of these responses at the cortical level.

If, as seems likely, the amplitude of the visual evoked response is dependent, in the first instance, on the number of approximately synchronized post-synaptic potentials in a given area, the work of Hubel and Wiesel¹¹ should have given an indication that a large uniformly flashed or flickering field was unlikely to be the optimum stimulus; possibly it will be found that the greatest number of clearly resolvable contrast borders gives the highest probability of activating the largest number of cortical cells.

Spehlman² has shown that the addition of pattern to a previously uniform flashing field can modify the form of the evoked potentials, but the present work suggests that reversal of the pattern, although without any change in the overall luminance of the field, is a particularly effective stimulus. This observation goes some way to explain the occurrence of lambda waves, as potentials evoked by scanning patterned fields, which have always seemed disproportionately large in comparison with the potentials evoked by bright flashes in the same subjects.

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Central Patterning of a Vocalization in Fowl

Brown¹ has described a region of the lateral tegmental gray in red-winged blackbirds where focal electrical stimulation regularly produces alarm calls with very low stimulus intensities. We have found that stimulation of the same structure in chickens (stereotoxic co-ordinates A 4, L 4.5, V 4; ref. 2) produces clucking, which is an alarm call in this species. We present here evidence that this clucking is patterned in the central nervous system and does not depend on phasic input for its timing. This behaviour is favourable for analysis because the response is easily obtained under deep anaesthesia, making possible surgical manipulations.

The importance of central, as opposed to peripheral. control of the organization and timing of behaviour in animals has been emphasized by Bullock3. The subject is interesting because of its broad implications for the control of behaviour, but well documented examples are

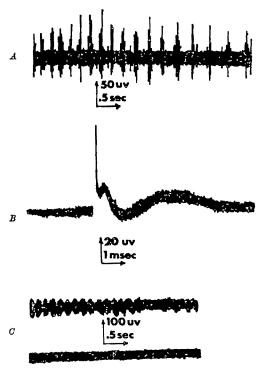


Fig. 1. Tracings of the response obtained while stimulating in the "calling" site and recording from: A, peripheral nerve T_4 ; B, ventromedial medulla, evoked response, and C, ventro-medial medulla—upper, bursting response and lower, no stimulation in "calling" site. Records A and C are from different birds with stimulating electrodes in similar but not the same sites.

still scarce, especially in vertebrates. Most of the experimental evidence for central control comes from studies of respiration or locomotion. Some examples which are often cited are rhythmic electrical discharges in the medulla of goldfish which match the frequency of respiration4, patterning of swimmeret movements in crayfish5, patterning of wing movements in flying locusts6 and fin movements in teleosts. The apparently centrally controlled locomotor rhythms in toads proved, on finer analysis, to be dependent on phasic sensory inputs.

The behaviour which we have studied, that is, clucking in chickens, resembles these examples in that it is quite regular and rhythmical, but it has additional interest as part of a highly evolved system of social communication. Our experimental procedure has been to implant electrodes in brain areas where stimulation produces clucking, then to stimulate these areas while recording electrical activity of motor nerves to the most important muscles involved in the production of the calls. Clucking is largely the result of brief contractions of the abdominal muscles, and spinal cord transections indicated that sections below T-4 had little or no effect while those at T-2 completely blocked it.

To determine the role of sensory feedback in the periodic activation of the abdominal muscles during clucking, nerve activity was recorded in nerves T_4 and T_3 while stimulating in the "calling" site (0.5 msec monophasic square wave pulses at 60 pulses/sec and 1.5-2.0 V). Recordings were made by impaling the nerve on electrodes made from double 0 insect pins, and the impulses were amplified by a Grass P_5 preamplifier and displayed on a cathode ray oscilloscope. Throughout the recording sessions the bird was anaesthetized with urethane (3 g given intravenously), and immobilized with tubocurarine chlorine (Abbott) (3 mg initially, 1.5 mg each 0.5 h as needed). The birds were artificially ventilated by passing warm moist air into the cannulated trachea and out through a hole in a thoracic air sac. In some cases, the curarine was allowed to wear off so that the rhythmic muscle movements reappeared and the movement artefact

on the cathode ray oscilloscope trace was used to confirm the phase relationship of the movement to the neural response. An example of the response in the nerve is illustrated in Fig. 1A. The rhythmic bursting in the absence of phasic proprioceptive or auditory input indicates that the stimulation in the brain elicits a rhythmic response which does not require peripheral feedback for its patterning.

The gross level of the central nervous system at which the patterning occurred was then examined. Analysis of degeneration from lesions in the calling site indicates that some fibres from the "cluck" site terminate both ipsi- and contra-laterally in the ventro-medial pons and medulla. This area was probed with semi-micro electrodes with a tip diameter of $10-20\mu$ insulated to within 0.15~mmof the tip. In two birds evoked responses were obtained (Fig. 1B) and in one of the birds bursts of activity could be detected at slow sweep speeds (Fig. 1C). No attempt was made to show directly that the response is in phase with the calling movements (again tubocurarine was used) but indirect evidence indicates that it is. The rate of bursting falls within the range of rates of calling usually obtained from the calling site (4-6/sec) and in this particular bird the site showed fairly rapid decay in calling and associated body movements in the noncurarized bird. The recorded response showed a similar decay (Fig. 1C).

These results indicate that the rhythmic body movements of the fowl which produce clucking calls in response to intracranial stimulation are centrally patterned and not dependent on phasic feedback for their timing. On the basis of our data we cannot evaluate the possible role of sensory input in modulations of the calls, but clearly the basic pattern is determined in the central nervous system.

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Filling Gaps in the Vascular **Endothelium with Blood Platelets**

In thrombogenesis the interaction between platelets and the wall of the blood vessel is very important. It has been found that after a slight injury to the layer of endothelial cells, platelets adhere to the basement lamina which has been denuded of endothelial cells¹. Other platelets adhere to this first layer and form aggregates. Evidence from in vitro investigations has aided the elucidation of the mechanism of platelet-platelet interaction. This mechanism involves ADP, calcium, fibrinogen and possibly some other agents² in a biological chain reaction³. The mechanism of platelet—vessel wall interaction is not clear. It has been demonstrated in vivo and in vitro that platelets can adhere to collagen fibres4,5. Nevertheless, in experimental thrombosis where the layer of endothelial cells is the only visible site of injury, platelets adhere exclusively to the basement lamina. Collagen fibres are not involved in this process of adhesion1. It is not clear from these experiments, however,

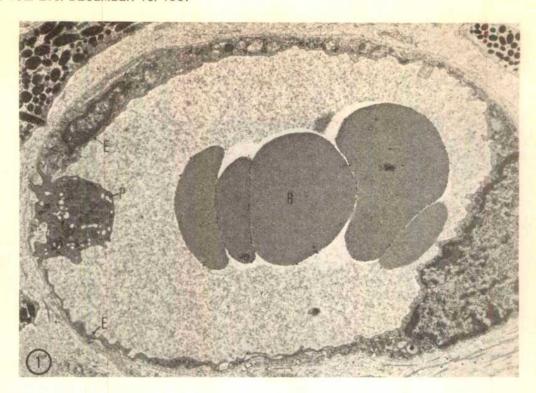


Fig. 1. Dilated capillaries from the iris of a cat. A platelet (P) fills a "gap" in the endothelial cell layer (E); R, erythrocyte (×8,000).

whether injury to the endothelial cells and the eventual release of tissue factors were necessary for the interaction of platelet with basement lamina.

Ultramorphological investigation of the cat iris showed that similar interaction between platelet and basement lamina can occur in capillaries which show no visible injury to the endothelial cell layer. We have therefore made a comparative study of capillaries from the iris of normal animals, of animals pre-treated with reserpine (2 mg/kg given intraperitoneally, 20 h before the experiment) and of other animals 1, 2 and 7 days after sympathetic denervation.

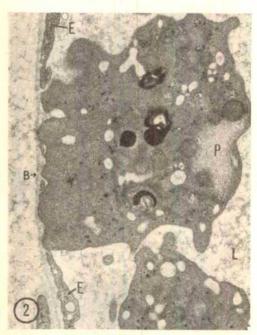


Fig. 2. Dilated capillaries from the iris of a cat. A platelet (P) in close contact with the basement lamina (B) in a "gap" between two endothelial cells (E); L, capillary lumen $(\times 18.400)$.

In the capillaries of untreated animals the endothelial cells completely covered the underlying basement lamina. Platelets were occasionally found in the lumen but there was no visible interaction between platelets and other blood cells nor between platelets and endothelial cells. Gaps appeared between endothelial cells in the very dilated capillaries of the animals which had been treated with reservine and of those which had been sympathectomized. The basement lamina thus became exposed at several points and was in direct contact with blood elements. At the exposed site a platelet, but never another blood cell, was found in close contact with the basement lamina (Figs. 1 and 2). When there were small gaps in the layer of endothelial cells only the "foot-like" process of a platelet contacted the basement lamina; the body of the platelet dipped freely into the lumen. Wider areas of denuded basement lamina were in close contact with more than one platelet, but platelet aggregates as seen during the formation of platelet thrombi were never observed. Other ultramorphological signs of thrombi formation, such as platelet degranulation and fibrin formation, were also absent.

Thus it would seem that in certain physiological conditions platelets function as "bouche-trou" when for any reason the endothelial cells separate and the underlying basement lamina becomes exposed. It is reasonable to assume that this phenomenon is reversible, that is, whenever the endothelial cells move back to their original position they can displace the platelet.

Platelets also adhere to the denuded basement lamina when there has been no injury to the endothelial cells other than vasodilatation. It seems unlikely that substances which amplify the ability of platelets to adhere are liberated from these endothelial cells. Because of the morphological similarity of the interactions between basement lamina and platelet and between basement lamina and endothelium¹ it is suggested that both are mediated by analogous forces, perhaps an electrical attraction.

With respect to observations in experimental platelet thrombosis, it seems that only the interaction between platelet and platelet and the subsequent platelet degranulation are related to endothelial damage and the release of tissue factors. The interaction between platelet and basement lamina seems to be related to endothelial injury only in as far as the basement lamina becomes exposed. We therefore suggested that this interaction and that between platelet and platelet are mediated through two fundamentally different mechanisms and should be thought of as two different phenomena.

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Absence of Testosterone in Urine of Rats

Camacho and Migeon¹ established that testosterone which had been recovered from the urine of normal subjects was largely conjugated with glucuronic acid. Recently, measurement of testosterone in urine after β-glucuronidase hydrolysis has been proposed as a satisfactory means of evaluating testicular function2 and testosterone produc-

tion³ in male subjects. Testosterone has been tentatively identified in testicular venous blood of the adult rat4. From this observation, it was suggested that testicular function in the rat could be assessed by the excretion of testosterone in urine. To test this hypothesis we used the method of de Nicola, Dorfman and Forchielli⁵ to measure testosterone in urine. We modified their method to provide increased sensitivity, precision and specificity. The most important changes were: (1) acetylation of testosterone after paper chromatography, followed by thin-layer chromatography of testosterone acetate on silica gel in ligroin : ethyl acetate (5:2); and (2) addition of cholesterol as an internal standard before gas-liquid chromatography using 3 per cent OV-1 and 3 per cent OV-17 as the liquid phases on separate 6 ft. columns. In optimal conditions, 12.5 mug

biological samples. The results of our measurements of testosterone in urine are shown in Table 1. Testosterone was not identifiable after β-glucuronidase hydrolysis of urine which had been collected from adult rats or from rats which had been given human chorionic gonadotrophin. Urine from

of testosterone acetate was detected and measured in

a normal male subject and from a male pseudohermaphrodite gave results comparable with those reported by others5,6.

An attempt was made to determine whether a testosterone conjugate which could be hydrolysed by acid was present in the spent urine from adult male rats. After incubation with β-glucuronidase and extraction, the spent urine was taken to pH 1 with hydrochloric acid and incubated for 24 h (ref. 7) and analysed as described. testosterone could be identified. We concluded that little or no testosterone was present in the β-glucuronidase-hydrolysed or pH 1 fraction of urine from adult male rats or in the fraction from the urine of rats treated with human chorionic gonadotrophin which had been hydrolysed with enzyme.

Fishman and Sie⁸ demonstrated that slices of rat liver can conjugate testosterone with glucuronic acid in vitro. Comparison

Table 1. COMPARISON OF TESTOSTERONE EXCRETION IN URINE OF THE RAT

Experiment	Time of urine collection (h)	Recovery of *H-testo- sterone (%)	Testo- sterone measured (µg)	Calculated maximum testosterone/ sample (µg)
Twelve rats (250 g) Ten rats (80 g) 200 IU human chorionic gonadotrophin daily for 5 days	48 72	31·4 27·4	0	<0.281 <0.347
Normal man, aged 21 yr Male pseudoherm-	24	43.0	61·6/24 h	
aphrodite, aged 14 yr	24	29-9	66·0/24 h	

of the excretion of testosterone by the rat and man indicates that, although testosterone may be a secretory product of the rat testes, it does not seem to be excreted as testosterone glucuronide in the urine of the rat as it is in man.

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Mode of Early Shell Growth in the Ammonite Promicroceras marstonense Spath

Some authors have suggested that the nature of the contiguity between the ammonoid proseptum and the shell wall indicates that there is no junction, but that the two are continuous resulting from contemporaneous formation1-3. This contrasts with the relationship between "normal" septa (that is, all septa excluding the proseptum) and the shell wall to which they are attached. There is abundant evidence in the case of "normal" septa that they were secreted by the animal's adapical soft parts to the inner surface of previously formed shell. The

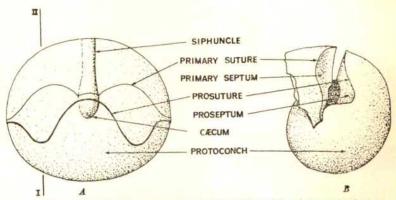


Fig. 1. Internal mould of the protoconch and early chambers of *Promicroceras marsionense* Spath: specimen No. DP 35. A, Ventral view showing the siphuncle and caecum which, although internal, can be seen through the transparent calcite infilling. The line I-II shows the approximate position of the sections illustrated in Fig. 3. B, Side view: the caecum has been omitted from this illustration. (×80.)

time relationship between each septum and the shell to which it is attached will therefore be the amount of time the animal takes to secrete shell proper equivalent in length to its body chamber, as shown for Nautilus pompilius Linné by Eichler and Ristedt⁴. Other authors⁵⁻⁸ maintain that the proseptum was inserted in an identical manner to normal septa. House⁶ (page 88) points out that the protoconch apparatus (prosiphon and caecum, Figs. 1A and 2E) is attached to the protoconch wall and must have been formed after this wall, but have preceded the proseptum.

During the present investigation, I prepared the nuclei of ten specimens of the Lower Jurassic ammonite *P. marstonense* preserved as aragonitic shells (determined by X-ray diffraction). The specimens were sectioned parallel to the median plane, to a position approximately mid-way between the median plane and the outer flank, in the region where the proseptum and primary septum are furthest apart (Fig. 1*A* and *B*). In exact median section the close proximity of these septa together with the bulbous caecum may confuse their interpretation (Figs. 1 and 2*F*).

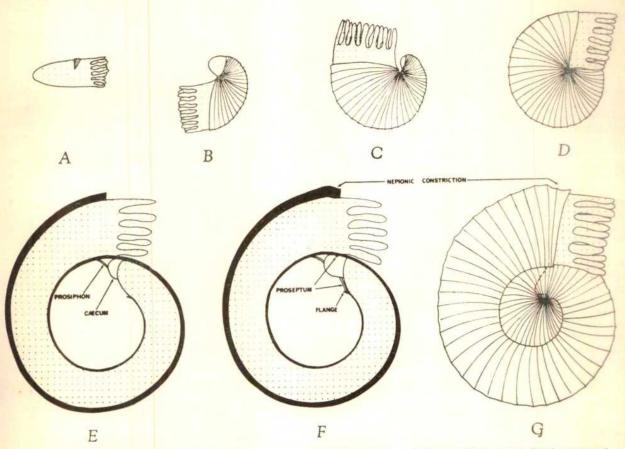


Fig. 2. Suggested mode of early growth of Promicroceras marstonense Spath, in particular, and of ammonoids in general. For the purpose of diagrammatic illustration the shell remains in the same position (A-G) and the soft parts are shown to revolve about the fixed shell. In life, the orientation of the entire animal would almost certainly have been different from that in the figure. Throughout the diagram, the lines on the shell are meant to represent growth lines; soft parts are stippled; shell (in E and F) is solid black. E and F are cross-sections in the median plane to show internal development. In G the position of the proseptum (dashed line) has been superposed on the shell. (All $\times c$. 68.)

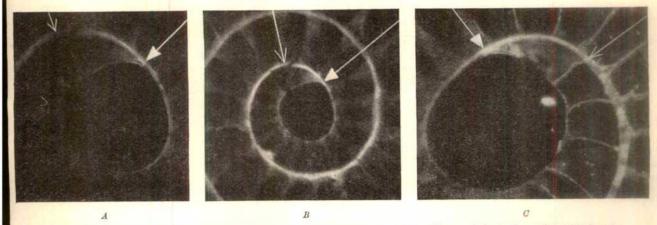


Fig. 3. Photomicrographs of polished nuclei of *Promicroceras marstonense* Spath. In A-C the solid arrow indicates the position of the junction between the proseptum and the inner shell wall, and the open arrow indicates the position of the junction between the primary septum and the inner shell wall. Sections are parallel to the median plane (see Fig. 1A). A and B, Specimen No. DP 25: $A_1 \times 100$; B_1 as A_2 , but with lower focus to show the convergence of the proseptum and primary septum towards the median plane (see Fig. 1B), \times 50. C_2 , Specimen No. DP 25, \times 100. In A_2 and A_3 the junction between the proseptum and the inner shell wall can be seen clearly.

The nuclei were highly polished and examined under reflected light. In several specimens the junction between the proseptum and shell wall could clearly be seen (Fig. 3A and C). This junction appears to be identical to that between all successive septa and the shell wall, and it is considered that the proseptum, of this species at least, was laid down by the adapical soft parts of the animal in a manner identical to that of all later septa.

In general, planispirally coiled ammonoids do not secrete a dorsal wall, unlike the nautiloids; shell secretion is therefore restricted to the venter and flanks of the animal. Invoking shell secretion from these areas only, it is shown how an ammonoid can produce the protoconch which houses the embryonic animal (Fig. 2). It is envisaged that the earliest formed shell is strap-like in shape and that this corresponds to the flange of the fully formed protoconch (Fig. 2A and F). Using only shell secreting glands situated at the adoral end of the soft parts, as in living Nautilus, the animal would be able to produce the protoconch in which it gradually encapsulated its soft parts. Growth lines on the protoconch of well preserved ammonoids are identical with those of the first whorl of growth ". . . and hence [the protoconch and phragmocone were] formed by shell secreting glands of similar type" (ref. 6, page 88). These growth lines indicate that the protoconch, like the phragmocone, was formed by the addition of successive shell increments.

It is considered that the protoconch actually housed the embryonic ammonoid and that after its formation the soft parts were permanently protected by shell. After secreting about three-quarters of a whorl of phragmocone shell, the prosiphon would be laid down, and this is followed by the caecum. After the completion of about one volution of phragmocone shell1,6,9-13, the nepionic It has been suggested that the constriction occurs. formation of the nepionic constriction by the adoral soft parts, and the secretion of the proseptum by the adapical soft parts, were contemporaneous. Eichler and Ristedt⁴ show that, for Nautilus pompilius, secretion of the proseptum may have preceded the formation of the "nepionic line" [=nepionic constriction]. As Erben' has pointed out, however, there are two important changes during early ammonoid ontogeny: the first occurs in the oral part of the protoconch, the second at the nepionic con-Both these changes are most convincingly shown in electron micrograph pictures by Birkelund¹⁴ for the species Saghalinites wrighti Birkelund. Erben relates the change in shell growth after the nepionic constriction with the change from the proseptum to "normal" septa, and suggests these changes indicate that the formation of the proseptum and nepionic constriction were contemporaneous. These changes are thought to indicate a metamorphosis¹⁵ in the ammonoids; the first as denoting the end of the embryonic stage (herein Fig. 2D), the second as ending the larval stage (herein Fig. 2G).

If this account of early ammonoid growth is correct, it would explain why ammonoids, which secrete shell in curved crescentic strips, do not have a cicatrix16, which is characteristic of the nautiloid apex formed by the secretion of successive concentric shell layers.

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Factor VII Deficiency in Beagle Dog Plasma and its Use in the Assay of Human Factor VII

During routine screening tests on a group of beagle dogs before a toxicological test, it was noted that several dogs showed a prolonging of the one-stage prothrombin time. Some of these dogs were found to be related (Fig. 1). The results of more detailed tests showed that intrinsic prothrombin activation was normal (Table 1) and that extrinsic prothrombin activation could be corrected by adding aliquots of normal human plasma or serum, normal dog plasma or serum, and Russell viper venom (Table 2). In addition, liver function tests were normal and the presence of an inhibitor was excluded (Table 3). Finally, factor VII activity assays, using congenitally deficient human plasma, confirmed that this factor specifically was reduced.

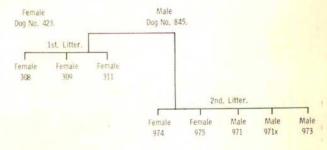


Table 1. RESULTS OF COAGULATION FUNCTION TESTS ON PARENTS AND OFFSPRING

Stock No.		ood clotting me Non-contact	Pro- thrombin time (sec)	Kaolin/ cephalin time (sec)*	Factor V assay per cent normal Average normal dog †	Factor X assay per cent normal Average normal dog ‡	Fibrinogen mg/ per cent	Factor II assay per cent normal§	Factor VII assay per cent normal	Thrombo- plastin generation test
0.45	Wante FF and	5 min 34 sec	44	20-5	105	100	245	108	< 1	Normal
845	2 min 55 sec		53	21.5	100	100	280	96	<1	**
423	2 min 10 sec	5 min 50 sec	65	19	95	80	267	105	<1	11
971	2 min 35 sec	7 min 48 sec		10	95 90	90	280	88	< 1	**
973	4 min 45 sec	7 min 12 sec	67	19	115	80	236	88	< 1	**
971x	2 min 45 sec	3 min 26 sec	59	19	90	100	191	88 88	<1	
308	4 min 55 sec	9 min 10 sec	54	22 22	100	90	198	80	<1	
309	3 min 10 sec	6 min 47 sec	50	22		120	397	105	<1	,,
974	4 min 10 sec	5 min 15 sec	55	18	100			105	<1	"
975	2 min 55 sec	5 min 29 sec	44	15	120	115	343	100	<1	**
311	3 min 15 sec	5 min 40 sec	45	22	110	100	315	100	100	**
Controle	9 min 10 sec	6 min 00 sec	15	20	100	100	270	100	100	5.5

^{*} Diagen Diagnostic Reagent Ltd. † Artificially prepared human plasma substrate. ‡ Diagen substrate (see ref. 1). § Tiger snake venom assay (see ref. 2). || See ref. 3.

Table 2. EFFECTS OF SUBSTITUTION ON ONE-STAGE PROTHROMBIN TIME

Material added	One-stage prothi	rombin time (sec) Pooled control
1/5 vol. normal dog plasma or serum	22	17
1/5 vol. normal human plasma or serum	22	16
1/5 vol. factor VII deficient plasma	44	16
1/5 vol. absorbed dog plasma	44	17
R.V.V. 1/200,000 cephalin	23	13

Table 3. One-stage prothrombin times of normal dog plasma after incubation with normal 423 and 845 plasma or serum

Incu- bation			1	/3 P	lasma	or sert	ım d	ilution	* 1	/12		
time (min)	C	4° C 423	845	C	37° (423	845	C	4° C 423	845	C	37° C 423	845
45 90 180 400	9·5 8·5 9·5 10·5	9·5 9 9·5 10	9·5 9 9·5 10	7.5 9.5 10 10	8 8·5 10·5 10·5	8·5 9 10·5 10	9 8 9 10	9·5 9 9 10	9 9 9	8 8-5 9-5 9-5	8 9 10 9.5	8 8·5 9 9·5

The use of the defective dog plasma in the assay of human factor VII requires an effective and readily obtainable thromboplastin. The obvious choice is human brain thromboplastin, which is in common use in most laboratories. The efficacy of this was compared with dog thromboplastin and shown to be adequate (Fig. 2). An incidental finding of interest here was that human brain thromboplastin was more efficient than dog brain thromboplastin in a normal dog system, and more sensitive to VII deficiency.

Dilutions, ranging from 1:10 to 1:1,000, of pooled normal human plasma were made with glyoxaline buffers and one-stage prothrombin times performed using 1:20 human brain thromboplastin in 0.025 molar calcium chloride and dog VII deficient plasma. Clotting times

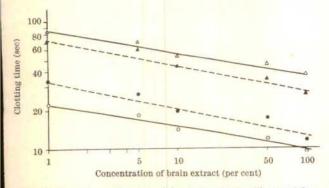


Fig. 2. Comparison of human and dog brain extracts with normal dog (○, ●) and VII deficient (△, ▲) plasmas. — — — , Dog brain; ——, human brain.

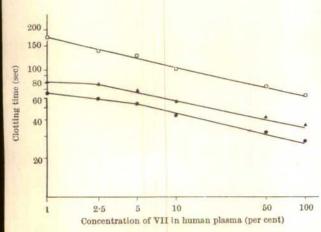


Fig. 3. The influence of factor V on human VII assays in deficient dog plasma. ☐, Factor V 1 per cent (average normal dog); ♠, factor V 6 per cent (average normal dog); ♠, factor V 100 per cent (average normal dog).

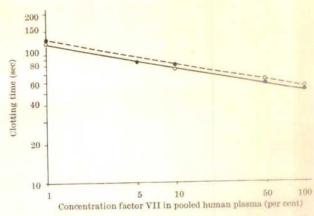


Fig. 4. Comparative VII assays in human (● - - - ●) and dog (○ — ○) VII deficient plasmas.

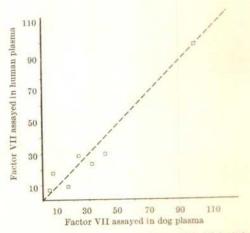


Fig. 5. Comparative VII assays in dog and human VII deficient plasmas,

were found to be shorter than predicted when the plasma concentration was less than 10 per cent. Dilution of thromboplastin did not affect this, but reduction of factor V content of the substrate plasma by incubating overnight at 40° C produced predicted values (Fig. 3). Comparing the assay of factor VII activity in diluted normal human plasma, using a known VII deficient human plasma and the deficient dog plasma with artificially depleted factor V, showed virtually identical results (Fig. 4).

So far, a comparison of seven factor VII assays, using human deficient plasma and the dog plasma, has been performed on patients under anticoagulant therapy or with liver failure. (The number of comparisons is limited by the unavailability of factor VII deficient plasma.) Good correlation has been obtained (Fig. 5).

It is suggested that this plasma provides a suitable substrate for the assay of factor VII in human plasma, a procedure until now dependent on access to a patient with the rare factor VII deficiency, or the less satisfactory methods involving the use of artificially depleted plasmas.

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CYTOLOGY

Influence of the Injection of 'Triton WR-1339' on Lysosomes of a Rat Transplantable Hepatoma

Lysosomes are subcellular particles in which several acid hydrolases of various specificities are localized. One of their main intracellular functions is to digest exogenous compounds taken up by the cell. The lysosomal system can be overloaded with substances which cannot be degraded, at least at a sufficient rate, by hydrolases1. This occurs in rat liver lysosomes2 and to some extent in kidney3 and spleen lysosomes after injection of 'Triton WR-1339'. a non-ionic detergent. This compound is taken up by liver, kidney and spleen cells, and accumulates inside the lysosomes because it is not subjected to the lytic action of lysosomal enzymes. Accumulation causes increase in granular size and decrease in granular density.

This phenomenon can be used to study lysosomes in a tissue5. 'Triton WR-1339' has antitumoral activity6, and this has prompted us to investigate whether the detergent accumulates in the lysosomes of some tumours. We have used a chemically induced transplantable hepatoma in the rat (hepatoma HW) where a well developed lysosomal

system has been found7.8.

An injection of 'Triton WR-1339' causes a decrease in the equilibrium density of rat liver lysosomes in a sucrose gradient². The effect is also found in hepatoma lysosomes (Fig. 1). The distribution patterns of two acid hydrolases. cathepsin and acid phosphatase, which are reference

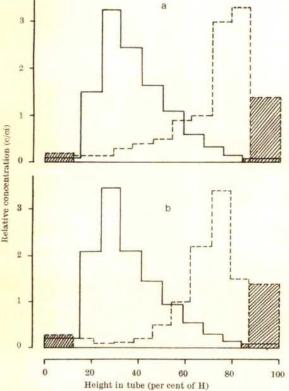
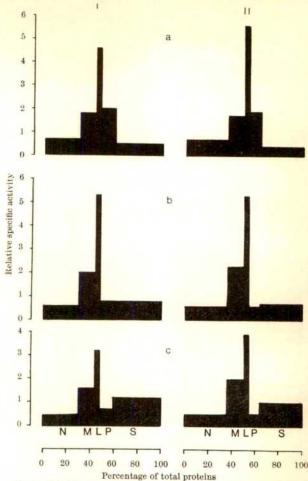


Fig. 1. Distribution of cathepsin (b) and acid phosphatase (a) after centrifugation (for 2-5 h at 39,000 r.p.m. in head SW 39 of the Spinco model L-HV preparative ultracentrifuge) of a mitochondrial fraction of hepatoma HW through a 0-776-2-419 m sucrose gradient in water. The particles were initially layered on the top of the gradient. Abscissa, percentage of the height of the liquid column in tube (H); ordinate, relative concentration, that is, the ratio of the observed activity (c) to that which had been found if the enzyme had been homogeneously distributed throughout the whole gradient (ci). Filled blocks are used for the top and bottom subfractions to indicate that they include material falling beyond the limits of the gradient, ..., Preparations from a hepatoma of a control animal; ..., preparations from an animal killed 4 days after an intravenous injection of 170 mg of 'Triton WR-1339' in 1 ml. of saline.



Fercentage of total proteins

Fig. 2. Distribution patterns of cathepsin (b), acid phosphatase (a) and radioactivity (c) in hepatoma HW, after an injection of 170 mg of 'Triton WR-1339' labelled with '131. Differential centrifugations: a nuclear fraction N, a "heavy" mitochondrial fraction M, a "light" mitochondrial fraction L, a microsomal fraction P and a soluble fraction S. Ordinate, relative specific activity (percentage of total recovered activity/percentage of total recovered proteins; abscissa, relative protein content of fractions (cumulatively from left to right). I, Animal killed 48 h; II, animal killed 4 days after the injection.

enzymes for lysosomes, after density equilibration in a sucrose gradient are shown in Fig. 1. The experiment was performed, using techniques similar to those of Beaufay et al.9 and de Duve et al.10, on a mitochondrial fraction isolated from hepatomas from a rat injected with 'Triton WR-1339' and from an untreated rat. The injection of Triton WR-1339' causes a striking shift of the distribution curves of the two hydrolases towards the low density

regions of the gradient.

The change may be explained by supposing that some of the injected 'Triton WR-1339' is stored in the hepatoma lysosomes as it is in liver lysosomes. An injection of Triton WR-1339' labelled with iodine-131 showed that some of the detergent was taken up by the tumour. After differential centrifugation using the method of de Duve et al.10, the radioactivity exhibits a distribution pattern comparable with that found for acid hydrolases (Fig. 2). A high percentage is associated with the mitochondrial fractions M and \bar{L} , and a peak of relative specific activity is found in the light fraction L. The most important difference is observed in the soluble fraction S, but this can be explained by a contamination of the homogenate with blood still containing radioactive deter-Proteins were measured by the method of Lowry et al.11 and radioactivity was measured in a liquid scintillation system using a Nuclear Chicago scintillator.

The radioactivity present in the mitochondrial fractions is not associated with mitochondria (Fig. 3). Cytochrome

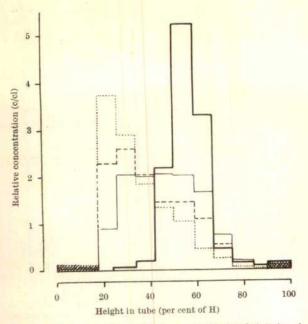


Fig. 3. Distribution of cathepsin (---), acid phosphatase (--), cytochrome oxidase (--) and radioactivity $(\cdot \cdot \cdot \cdot)$. Experimental conditions were as in Fig. 1. Mitochondrial preparation from a hepatoma of a rat injected with 170 mg of "Triton WR-1339" labelled with ^[41] and killed 4 days after the injection.

oxidase was measured by the method of de Duve et al. 10. After equilibration in a sucrose gradient, the radioactivity is associated with particles found in the low density regions of the gradient, where the lysosomes are localized as ascertained by the distribution of acid hydrolases. The detergent engulfed by hepatoma cells is therefore

probably confined in the lysosomes.

Because of the effect of 'Triton WR-1339' on lysosomes it is possible to label lysosomes with an exogenous substance, to produce an experimental overloading of the lysosomal system and to obtain a purified preparation of lysosomes. Results obtained on hepatoma HW suggest that rats injected with 'Triton WR-1339' may be used to study the lysosomes of the tumour. The detergent may have a similar effect on the granules of other tumours. The possibility of isolating purified preparations of lysosomes from the tumour would permit the analysis of their digestive capacities, of their membrane properties, of their immunological characteristics, and the comparison with lysosomes of normal tissue. The effect on a tumour cell of an overloading of its lysosomes could also be studied. The sensitivity of a cancer cell to injurious effect induced by overloading could be different from that of a normal cell.

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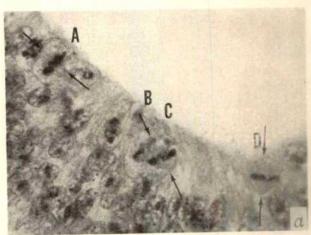
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Significance of Mitotic Spindle Fibre Orientation in the Neural Tube

CELLS in the neural tube are usually said to divide with their spindle fibres oriented parallel to the luminal sur-The plane of cleavage is always at right angles to the direction of the spindle fibres2, and so the cleavage between daughter cells would always be perpendicular to the luminal surface of the neural tube.

Examination of serial cross-sections of neural tube in early chick embryos, stages 14-20 (ref. 3), cut at 5μ and stained with haematoxylin and eosin, has revealed, however, that mitotic cells may have their spindle fibres oriented at various angles relative to the free surface of the tube. For example, the oil immersion photomicrograph in Fig. 1a shows a short segment from the neural tube of a twenty-two somite chick embryo-stage 14 (ref. 3), which contains four metaphase figures. Of these, the mitotic spindle fibres of cell A are clearly parallel to the luminal surface, but those of cells B and \hat{C} are obliquely oriented while the fibres of cell D are perpendicular to the luminal surface. Similar orientations of spindle fibres



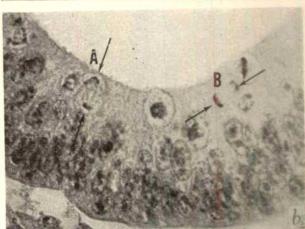


Fig. 1. Segments of cross-sections through the neural tubes of two twenty-two somite chick embryos; the arrows indicate the orientation of the spindle fibres. a, The spindle fibres of cell A are parallel to the free surface of the neural tube, those of cells B and C are oblique and the fibres of cell D are perpendicular to the free surface (\times 680). b. The spindle fibres of cell D are perpendicular to the free surface of the tube while those of cell B are parallel to the luminal or free surface (\times 640).

can be found at later stages of mitosis, for example, Fig. 1b, which shows a mitotic cell (A), in anaphase, with its spindle fibres perpendicular to the surface of the tube. The neuroepithelial cells which initially form the wall of the neural tube are attached to one another near the lumen by terminal bars and this terminal bar network remains intact during cellular division4-6. Thus when the cleavage furrow in a dividing cell is perpendicular to the luminal surface as in cell A (Fig. 1a), each of the daughter cells will receive part of the original terminal bar network and therefore probably remain attached at the lumen. When, however, the spindle fibres are oriented perpendicular to the epithelial surface as in cell A (Fig. 1b), the cleavage furrow will be parallel to the surface and it seems probable that the daughter cell furthest removed from the lumen will have no attachment to the terminal bar network. Such a cell would then be free to migrate away from the lumen and out of the epithelium.

If this is so, then the orientation of the spindle fibres is of considerable importance in maintaining, on the one hand, a population of proliferating neuroepithelial cells at the luminal surface of the neural tube and, on the other hand, providing a mechanism for the detachment of other cells during neuroblast differentiation. In this respect, it is no doubt significant that at the neural groove stage, before any differentiation of neuroepithelial cells is apparent, perpendicular spindle fibres are not found. With the appearance of neuroblasts, however, at about stage 14 of development3, spindle fibres perpendicular to the luminal surface are noted and remain apparent until approximately stage 20 (ref. 3), at which time most neuroblasts, destined to become motor neurones in the spinal cord, have been formed.

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Action of Cytochalasin B on Cultured **Human Lymphocytes**

COMPOUNDS which effect cell division are interesting because they have the potential of increasing our knowledge about this poorly understood biological process. The cytochalasin compounds fall into this category because they can influence both cellular and nuclear division. Cytochalasins are metabolites derived from moulds, and have recently been isolated by Dr W. B. Turner and their molecular structure reported by Aldridge et al.1. Carter has shown some of the effects of the cytochalasin B on Earle's L strain of mouse fibroblasts in culture². In varying conditions of concentration and time, the compound inhibited cell motility, produced extrusion of the nucleus and blocked cytoplasmic cleavage, nuclear division continuing with the production of multinucleated

Some of the effects of these compounds seemed to be dependent on the culture environment of the fibroblasts, and so we decided to test the effects of cytochalasin B on human cells grown in suspension.

Peripheral blood was obtained by venipuncture and prepared for lymphocyte cultures, using a modification of the technique of Moorhead et al.3. Cytochalasin B was dissolved in dimethyl sulphoxide (DMSO) and refrigerated at 4° C as a 0.1 per cent stock solution. The stock solution

Table 1. Relationship of multinucleated cells to concentration of cytochalasin ${\cal B}$

Concentration of cytochalasin B (µg/ml.)	Peak response of cells (per cent) Two nuclei More than two nuc				
1	2	θ			
2	13	2			
3	21	11			
4	28	31			
6	49	37			
10	46	38			

Table 2. RELATIONSHIP OF MULTINUCLEATED CELLS TO DURATION OF TREAT-MENT WITH CYTOCHALASIN B

Duration of treatment	Peak response of cells (per cent)					
with cytochalasin B (h)	Two nuclei	More than two nuclei				
24	4	0				
48	3	0				
72	22	. 8				
96	49	19				
168	35	38				

was added, at the start of the culture, so that the final concentrations in the cultures varied between 0.5 to 20 μg/ml. The lymphocyte cultures were incubated for up to 7 days. At intervals, samples of culture were fixed in glacial acetic acid and absolute ethyl alcohol (1:3) and examined microscopically. Cells were spread on clean slides using the air drying technique of Rothfels and Siminovitch⁴. The cells were stained with Leishman's stain. Control cultures without cytochalasin and with DMSO added alone were set up.

There was initial failure of cultures because of an apparent toxicity of DMSO at certain concentrations. This was eliminated by keeping the final concentration of DMSO not greater than 0.1 ml. in 10 ml. of culture When the concentrations of DMSO and cytochalasin B were standardized, the lymphocyte responses varied only slightly in different cultures, and a general pattern was discernible.

Only an occasional multinucleated cell was seen in the cultures with a final concentration of cytochalasin B of less than 2 µg/ml. A significant response, as judged by the production of multinucleated cells (Figs. 1 and 2), was obtained when the compound was used at a final concentration of 2-20 µg/ml. (Table 1). There was increasing

response up to 6 µg/ml.

A very small proportion of binucleated cells were detected in the cytochalasin cultures up to 48 h, with a major response at 72 h rising to a peak at 96 h (Table 2). There seemed to be an increase of cells with more than two nuclei (Fig. 3) between 72 and 168 h (7 days). The

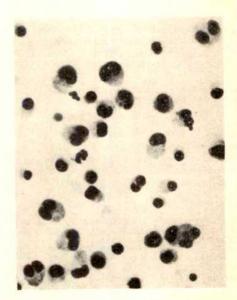


Fig. 1. Preparation showing chiefly binucleated cells after exposure to 6 μ g/ml, of cytochalasin B (\times 300).

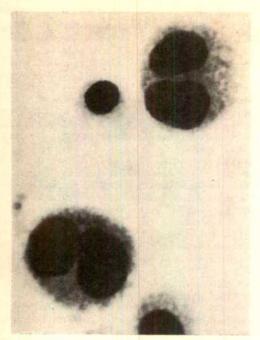


Fig. 2. Typical binucleated cells after exposure to cytochalasin $B = (\times 1, 264)$.

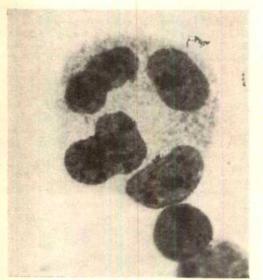


Fig. 3. Multinucleated cell showing variations of nuclear morphology (\times 1,264).

7 day cultures also showed evidence of cellular degeneration, probably secondary to nutritional depletion of the

Cells with between one and eight nuclei were clearly observed; however, it is possible that cells with more then eight nuclei were present. In some cells with more than three nuclei, it was difficult to determine the exact number because the nuclei were closely packed and there was some overlapping. The proportion of cells containing ncreasing numbers of nuclei was inversely related to the number of nuclei in each cell. In many multinucleated cells, the nuclei varied in size and shape (Fig. 3), and nicronuclei and nuclear bridging were seen. Cytoplasmic bridging also occurred but much less frequently than nuclear bridging. Only rarely was nuclear extrusion letected.

While this study of the effects of cytochalasin B n cultured human lymphocytes demonstrates that the compound produces multinucleated cells, and is in agree-

ment with some of the observations of Carter for mouse fibroblasts, there are certain important differences. Lymphocytes required a higher concentration (2-6 µg/ml.) of cytochalasin than fibroblasts (0.5–1.0 µg/ml.) in order to produce multinucleated cells. Nuclear extrusion could easily be observed and controlled in mouse fibroblast cultures, but this phenomenon was only rarely observed and was unpredictable in lymphocyte cultures. Based on Carter's observations, it seems unlikely that a difference in dosage could totally account for lymphocyte cultures only rarely showing this phenomenon. A more likely explanation might be based on different growth patterns of the two cell types. Mouse fibroblasts are cultured as monolayers while lymphocytes are grown in suspension. Carter has postulated that extrusion of nuclei is related to a surface tension effect on the cellular membrane of the growing fibroblast.

The nuclei in dividing multinucleated cells appeared to be in the same stage of mitosis, although in some cells the dividing nuclei were slightly out of phase with each other. There were no mitotic figures associated with an interphase nucleus (asynchronous division). Carter has used the term "pseudomitosis" to account for the observation that the nuclear complement of each cell increases by increments of one nucleus instead of the doubling which would be expected with a failure of cytoplasmic cleavage. Lymphocytes also appear to show a gradual build-up of the number of nuclei per cell, and cells with between one and eight nuclei have been defined. The observed variations in the size and shape of the nuclei are difficult to explain by the "pseudomitosis hypothesis" alone. Mitotic figures often showed disorganized aggregates of varying numbers of chromosomes which may be related to the morphological variations seen in interphase nuclei. Evidence from mitotic figures suggests that, at least in some cells, variation in nuclear morphology may be due to abnormal division of the chromosomes with multipolar mitotic apparatus, for binucleate cells of normal appearance were produced after the first nuclear division. While it may be possible to postulate other effects of the compound on the mitotic apparatus, there is no direct evidence, at this time, on the specific mode of action of the compound in producing the observed variation in nuclear morphology.

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BIOLOGY

Longevity of Rhinolophid Bats

Numbered aluminium bands which are used to study the movements of bats are also of value in providing specific information concerning age1. In Britain, the first large scale bat-banding experiment was commenced in Devon in 1948 (ref. 2) and during recent years it has been gratifying to note the continued survival of a number of

greater horseshoe bats (Rhinolophus ferrumequinum) banded during the early stages of that experiment. Of particular interest has been the finding, on October 28, 1967, of greater horseshoe No. 918 (male). This bat, found in a small mine adit near Holne, in south Devon, was originally banded in a cave at Buckfastleigh, about 3 miles away, on March 4, 1949. Its age, when first handled, was not known, but because for this species birth invariably occurs only in mid-summer, usually early in July, this bat could not have been born later than July 1948. At the time of its most recent finding it must therefore have been at least 19.25 yr old.

This is the greatest age so far recorded for a Rhinolophid or indeed for any other species of bat in Britain. It seems probable, however, that such longevity, at least for R. ferrumequinum, may not prove unusual for during the past 3 yr two ringed bats of this species aged at least 17.5 yr and a further six individuals aged at least 16.5 yr

have been recorded in Devon.

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"Booby-trapping" as an Alternative to Sterile Males for Insect Control

ATTEMPTS are now being made to apply to other pests the sterile male technique, so successful in the suppression of the screw-worm fly Cochliomyia hominivorax in the southern United States. There is, however, a need to devise similar methods which may be even more appropriate in particular cases. One such method which is similar to, but simpler than, the "booby-trapping" method of Morgan¹ was suggested as a result of experiments with the Australian sheep blowfly, Lucilia cuprina. Female flies, resistant to dieldrin, which survived 0.5 µl. of 2 per cent dieldrin dissolved in kerosene and diisobutyl ketone (1:1) applied topically to the thorax, were exposed to males from a susceptible strain (LD_{50} < 0.005 per cent dieldrin). Each female killed up to one hundred males through contact during attempted mating. Treated resistant males were capable of killing susceptible females, but the effect was of a lower order of magnitude.

How might this sort of system be used for insect control? There are clearly a number of possibilities of which we shall mention two. The most obvious is to take advantage of the development in one region of a strain of pest resistant to a particular insecticide, or to develop such a strain in the laboratory. These resistant insects could then be mass reared, sterilized, loaded with the insecticide to which they had become resistant, and released among susceptible populations. Another possibility is to load insects with a non-lethal topical dose of a stable chemosterilant, of such a type that it will nevertheless exert a sterilizing effect on the carrier itself and also on those with which it mates or attempts to mate.

There are several points worth noting in a system of this sort. (a) Instead of females being of no particular advantage, as in the case of the screw-worm fly, both sexes are effective in destroying or sterilizing their partners. (b) Multiple matings are an advantage. In species in which the female tends to mate once only, attempted matings by males treated with insecticide will destroy a female even though copulation is not achieved. In the sterile male technique the reproductive capacity of fertilized females is not influenced by attempted matings by sterile males. (c) Relatively minute quantities of insecticide are employed, thereby reducing the danger of accumulation of toxicants in soil and water. (d) Tl range of possible insecticides is increased, because que tions of toxicity to man and domestic animals are large irrelevant.

There are several problems inherent in the use of resis ant insects in a "booby-trap" method. Thus, as a resu of incomplete sterilization, genes for insecticide resistan might enter the gene pool of field populations. Th probability of this could be minimized by supplementing sterilization with other systems, such as condition lethals, perhaps governing nutritional requirements f the released strain.

If resistance to a pesticide has developed in one regie it is very likely to emerge also in other regions und selection pressure. Its development might be delaye however, if the strain released were selected to be "supe resistant" so that the wild, susceptible individuals wou tend to encounter a lethal dose of the chemical if the were exposed to it at all.

If a choice were available the genetic basis of the resistance itself would also be an important factor f consideration. Resistance which is polygenic at balanced, as described by Crow2, would seem to be mo difficult to incorporate accidentally into the field popul tion. Chance outcrossing of resistant to susceptib strains would probably destroy the particular combination of genes necessary for resistance.

By contrast, the use of an appropriate chemosterila

seems not to involve any of these disadvantages.

The "booby-trap" technique seems particularly suits to insects with the following characteristics: (a) amen bility to cheap laboratory propagation; (b) a low natur population density; (c) the adult stage not attracted humans (if highly toxic insecticides are to be employed (d) behavioural characteristics leading to consideral and frequent bodily contact between individuals-1 example, a readiness of males to attempt mating with females encountered.

The Australian sheep blowfly possesses all these chara teristics. In species where the adult constitutes the 'nuisance' stage the sterile male method may be und sirable because of the need to flood the natural population with ten or more times the number of sterile insect If, however, the killing power of insects loaded with chem sterilant or insecticide is sufficient, the "booby-traj method may be practicable because it would not be nece sary to release nearly so many individuals.
We thank Dr D. F. Waterhouse for helpful criticism

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Regeneration of the Axial Organ of Arbacia punctulata and its **Implications**

LITTLE is known of the regenerative powers of echinoic so that it is of interest to report the regeneration of the

most enigmatic organ.

The axial organ is a complex spongy structure lying the mesentery which carries the oesophagus. The lor standing controversy over its function has recently be revived by the studies of Boolootian and Campbell¹ Millott and Vevers³, Millott^{4,5} and Farmanfarmaian⁶ So far, only one function has been adequately demo strated, namely, activity in defence against injury as

infection⁴. The axial organ has often been regarded as a heart and, despite evidence to the contrary^{3,6,7}, the idea dies hard.

One argument against such a view stems from the fact that urchins can survive complete removal of the organ^{3,8}. Our studies of its regeneration in *Arbacia* amply confirm this. More significantly in this connexion, we have shown that animals so treated usually remain healthy and active. No case therefore remains for regarding this structure, or any part of it, as an important circulatory organ in the accepted sense of the term.

Axial organs with their attendant "head processes" were excised through the small hole in the test remaining after removal of the madreporite. If animals so treated emain in highly aerated running sea water the wound soon heals and the axial organ is usually regenerated Figs. 1 and 2) although not necessarily completely or orecisely in its original form.

The "head process" is reformed independently of the est, the regeneration of which is preceded by de-differntiation and histolysis of the remaining portions of the

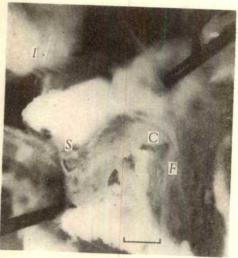


Fig. 1. An axial organ which had been completely regenerated in the course of a year. C, Contractile vessel; F, floor of regenerated axocoele which marks the approximate area of the wound through which the original organ was withdrawn; I, intestine; S, stone canal curling over the main glandular mass of the axial organ. Scale, 1 mm.

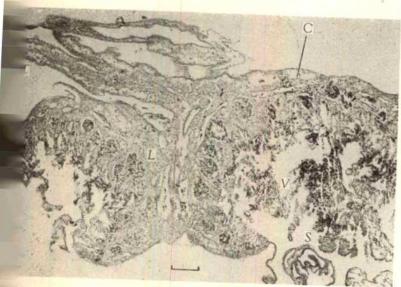


Fig. 2. Portion of a parasagittal section of the axial organ shown in Fig. 1 to illustrate the completeness of the regeneration. C, Canaliculus; L, portion of lacunar system; S, stone anal; T, orally directed process of organ; V, contractile vessel. Fixed with formalin and stained with Mallory. Scale, 122 μ m.

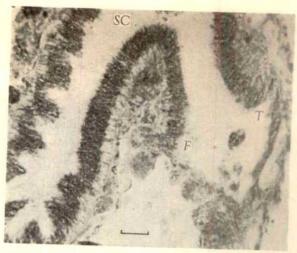


Fig. 3. An early stage in regeneration showing the de-differentiation of a portion of the stone canal (SC). At F, the cells of the lining (appearing as a dark border) are seen leaving the disintegrating epithelium to enter the subjacent tissue. At T, the epithelial cells are changing to become migratory. Fixed in Bouin and stained with Mallory. Scale, 10 μ m.

stone canal and mesentery. This is accompanied by active proliferation and migration of cells within the mesentery, so that a blastema is formed, which receives contributions in order of decreasing importance, from the surrounding peritoneum, the lining of the stone canal and the so-called haemal vessel of the mesentery. Cellular de-differentiation is extensive (Fig. 3), yielding fusiform cells which migrate in such numbers that they often denude the stone canal and mesentery, respectively, of lining and covering.

The migrating cells fuse to form a stroma of delicate fibrous connective tissue within the blastema forming a plate- or cup-like rudiment (Figs. 4 and 5). Some of these cells become greatly distended by vacuoles or pigment and their subsequent disintegration produces deep stromal fissures in which amoebocytes and impressive quantities of pigment accumulate.

Cells derived from the peritoneum and lining of the stone canal now align to form strands or tubes which invade the stromal fissures (Fig. 5). In the case of cup-like rudiments the stroma is invaded from both the outside and the hollow interior (Fig. 5). Cells invading from the

outside come to line pre-existing spaces forming canaliculi⁵, and also contribute, together with any haemal elements remaining in the mesentery, to the contents of the lacunae⁵. Cells invading the hollow of the cup assemble on its surface as a dense mass of strands or tubules, some of which, destined to enclose the ramifying channels of the contractile vessel⁵, extend to join the lacunae forming within the stroma. Others penetrate the stromal fissures which they come to line and so form the characteristic embayments of what will become the central lumen of the organ⁵.

The process of reconstituting the stone canal has yet to be discovered. The extent to which it disorganizes seems to influence both the level at which the organ is regenerated along the oral-aboral axis of the mesentery and the form of the early regenerate. There are hints of a morphogenetic influence, for in the two cases where there was no evident regeneration, little, if anything, could be found of the canal.

The work was in part supported by a grant from the US National Science Foundation to the Department of Invertebrate Zoology at the Marine Biological Laboratory,

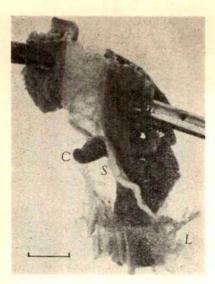


Fig. 4. A cup-like regenerate (C), portion of which is shown in section in Fig. 5, excised attached to a piece of oesophagus. L, Roof of lantern coelom; S, stone canal. Scale, 1 mm.

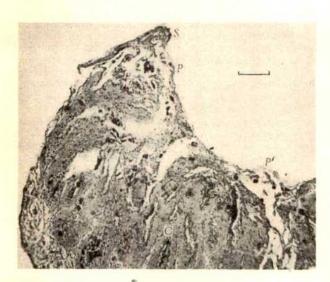


Fig. 5. Portion of a regenerate of 41 days showing invasion of the hollow of a cup-like stroma (C) by peritoneal cells (P) proliferated from the de-differentiated tip of the stone canal (S). At P' the strands of cells are penetrating a stromal fissure, Fixed with Susa in sea water and stained in Mallory. Scale, 65 μ m.

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Nature of the Magnetic Radula in Chitons from Eilat on the Red Sea

Ir has been noted^{1,2} that the radula (rasping tongue) o certain chitons, molluscs, are ferromagnetic. Examples o Polyplacophora in which the magnetism is reported to be caused by magnetite in the dentical caps are species of Cryptochiton, Chiton, Acanthopleura, and Mopalia1 and Chaetopleura apiculata².

We have studied the radula of Acanthopleura haddoni the largest intertidal species to be found at Eilat on the Red Sea coast, and have found that it is ferromagnetic The magnetic moment measured at room temperature was found to be 7 EMU/g of radula; for the dentical cap at a magnetic field of 10 Koersteds, it is more than 75 EMU/g. The Curie point is 570 ± 5° C. Hysteresis curves at room temperature and dependence of magnetization on temperature are shown in Figs. 1 and 2, respectively. All magnetic measurements were made on a motor-driven vibrating sample magnetometer.

There was an irreversible loss in magnetic moment on heating (see Fig. 2), and also when the samples were heated to 600° C in a helium atmosphere. A long and wide ferromagnetic resonance (full width at half height approximately 500 oersteds) absorption was found as expected; measurement was carried out at the X-band.

X-ray diffraction photographs were taken with a Norelco 'Straumanis' camera, with a diameter of 114.6 mm and cobalt radiation filtered through an iron foil. The pattern shows the existence of a spinel phase with a unit cell of 8.400 ± 0.003 Å. It is of interest that no magnetic phase other than that of the same spinel with the unit cell of 8.398 ± 0.003 Å appeared after the specimens had been heated to 500° C.

The magnetic and crystallographic data are on the whole consistent with the magnetic properties and the crystal

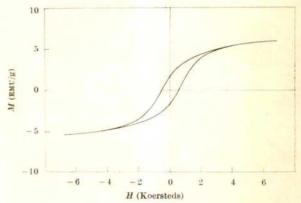


Fig. 1. Magnetic hysteresis curve of radula,

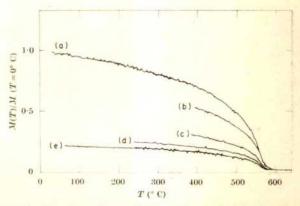


Fig. 2. Dependence of magnetization on temperature at subsequent measurements. a, First; b, second . . . e, fifth. Further cycles do not essentially change the magnetization.

structure of synthetic magnetite (Fe₃O₄) (ref. 3). It should be noted, however, that while the reduction of the magnetic moment on heating in synthetic magnetite is caused by oxidation to ferric oxide, this is probably not the case with Acanthopleura haddoni.

We also found that the remanence in virgin state of the radula was less than 0.1 per cent of the maximum remanence (that is 30 EMU/g) in this material. This negates, in our opinion, the speculation that the magnetic material might serve as a guidance device for navigation1.

We thank Professor S. Shtrikman and Dr H. A. Lowen-

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Non-thermal Effects of Microwave Radiation on Birds

MICROWAVE radiation produces both thermal and nonthermal effects in biological systems^{1,2}. The thermal effect is manifested as a rise in temperature of the irradiated system and is accompanied by physiological responses depending on the intensity and duration of the field. Non-thermal effects are manifested as changes in cellular metabolism caused by both resonance absorption and induced EMFs and, when neural structures are involved, are often accompanied by a specific behavioural response. An important difference between thermal and non-thermal effects is in the matter of time scale. Chickens exposed to a "slightly thermal" microwave field (20-50 mW/cm2) respond with an escape or avoidance reaction within a few seconds of the onset of radiation3.

The rapidity and compelling nature of this reaction led us to investigate microwaves in relation to the bird strike problem which is now reaching serious proportions in airline operations. Experiments are being conducted to determine the effectiveness of microwave radiation as a means of deterring birds from feeding in the vicinity of airport runways and of dispersing birds from commercial flight lanes.

The physiological correlates of this behavioural pattern have been studied to develop the escape reaction further and to determine the most effective form of microwave field. Experiments were conducted on three species of chickens using a 9.3 Gc/s microwave generator pulsed at 416 p.p.s. with a pulse width of 2.3 usec. Peak power into the horn antenna is 94 kW and the average power is correspondingly 90 W. The field intensity in the calibrated

test cage at a level 6 in. above the floor is set at 46 mW/cm² (average value).

The general pattern of behaviour of both young and fully grown chickens in the test cage is as follows. At the onset of radiation the wing outside the field of radiation becomes collapsed and the opposite wing is extended. A

similar phenomenon is observed with the legs. chickens incline their heads so that the eye closest to the field of radiation is oriented to the field and the sagittal axis of the head is kept in line with the appropriate axis of the body. The bird turns down to the outside of the field following this axis of the body. It is apparent that in this turning reaction the outer side of the animal is paralysed and on reaching the floor of the cage the reaction is manifested by increased extensor reaction of the inner (field) side and the head is turned to face the centre of the field.

On occasions this pattern of behaviour has not occurred and the following observations have been made. It seems that some animals present a hyperactive side. When these animals are radiated with the weak side facing the centre of the field they rapidly orient themselves to present the strong or hyperactive side to the field. The previously described behavioural pattern then ensues. Some birds are excited at or before the time of radiation because of outside influences, for example, handling, change of cage. These animals continue to be excited and at the onset of radiation move along the field so that one side of the head and body will be alternately outside and inside the field. Chickens in these conditions either reach a terminal state in the experiment by remaining quiet for a few seconds and then collapsing to the corresponding side, or in their agitation initiate flight.

During all these experimental procedures we have observed that different reactions can be obtained from a bird depending on which surface is irradiated. little or no reaction is detected if the bird is irradiated from below, thereby affecting its ventral surface. When irradiated from above, however, a clear distinction has been observed between the distal dorsal and tail regions of the animal and the head, and the neck to proximal dorsal regions. In the first case, an excitatory effect is obtained which can lead to the animal changing position or flying. The second case usually ends in a collapsing action as previously described.

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Bacteria-free Culture of Oyster Larvae

LARVAL cultures of many marine bivalve molluses have been maintained through stages leading up to metamorphosis and the principal requirements are now known, particularly for the European oyster Ostrea edulis L., the American oyster Crassostrea virginica (Gmelin) and the American clam Mercenaria mercenaria (L.). It is common experience that cultures may fail unexpectedly and there is evidence that bacterial contamination is often the cause^{1,2}. In this laboratory we have also had inconsistent results and sudden mortalities, and have found that bacterial counts vary greatly and are often high, even when antibiotics are used. Although antibiotics reduce the percentage of failures, Hidu and Tubiash² have shown that streptomycin, which is commonly used for this purpose, can increase the total bacterial number; its beneficial effect is to limit harmful bacteria and to increase useful types. The use of antibiotics leads to levels of success that would be acceptable in hatchery work, but it

is evident that critical experiments in larval nutrition, in genetic and environmental effects on growth and other aspects of larval biology, will depend on the elimination of the variable factor represented by the presence of bacteria. We have therefore investigated methods of growing the larvae of O. edulis in the absence of bacteria.

Hidu and Tubiash obtained bacteria-free fertilized eggs of M. mercenaria by repeatedly washing them in sterile sea water and the larvae were then maintained in aseptic conditions. In the absence of added algal food, however, there was little growth. O. edulis is larviparous, and in order to purify the larvae, which have a functional gut probably containing bacteria, we used strong antibiotic solutions as suggested by Droop⁴. One of several successful methods which we used was to allow larvae to swim for 48 h in a mixture of 670 µg/ml. of penicillin, 50 µg/ml. of streptomycin and 5 µg/ml. of chloramphenicol, made up in sterile sea water. The larvae were then washed for 1-2 h in sterile sea water to remove the antibiotics. Larvae were transferred to a series of test tubes each containing 25 ml. of autoclaved sea water and sufficient sterile culture of the flagellate Monochrysis lutheri Droop, to give a final concentration of about one hundred cells/mm³. The tubes were plugged and maintained at 20° C. Algal food was added aseptically to the tubes after 6 or 7 days. Tubes were finally opened in succession and sterility tests were made by incubating nutrient media which had been inoculated with material from the test tubes. Larvae were then measured.

Thirteen out of seventeen tubes thus treated were sterile. The fastest growing larvae increased in length by about 10 µ/day and after 22 days had eyes, indicating that development was complete. After 22 days, five out of ten sterile tubes contained larvae which had eyes and the highest percentage in a tube of larvae with eyes was 20 per cent. Few larvae died, and the total mortality was 0.6 per cent. In all tubes there was a wide range of larval sizes and this is a feature also found with the more usual method of culture. growth rates in our experiments were somewhat less than the fastest recorded rates in routine contaminated cultures which had been given frequent changes of water and carefully controlled concentrations of mixed algal diets. In our experiments, food concentrations were probably far from optimal; larval densities were sometimes too high and it is possible that accumulated waste products also retarded growth.

This work has established that oyster larvae can be rendered free from bacteria and can subsequently be grown to settling size on a pure algal diet of *Monochrysis lutheri* and do not need bacteria. With this aseptic technique, it should be possible to control much more closely the conditions in experiments with oyster larvae.

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Growth and Partial Metamorphosis of Imaginal Disks of the Greater Wax Moth, Galleria mellonella, in vitro

The most important of the problems of insect metamorphosis is the nature of the action of ecdysone. There is a particular lack of knowledge of the stages between the initiation of metamorphosis by ecdysone and the final

differentiation into the adult. Experiments on the action of pure ecdysone *in vitro* have been conducted with systems which respond in a limited way to ecdysone but do not grow or metamorphose^{1,2}. This communication reports the initiation of metamorphosis in an insect tissue by pure ecdysone *in vitro*.

Encouraged by the successful culture of eye-antennal disks of *Drosophila melanogaster in vitro*³, we cultured wing disks of *Galleria mellonella* in the hope that they would also grow and differentiate. This organism was chosen because it is easily reared in the laboratory; because the wing disks are large in comparison with the imaginal disks of *Drosophila* (giving promise of biochemical analysis); and because a great deal of attention has already been paid to the endocrinology of the lepidoptera.

In our conditions for growth, larvae spun eocoons 7-8 days after the last larval moult4. Donor larvae were washed in 70 per cent ethanol for 1 min, and rinsed twice in sterile insect Ringer⁵. The disks were dissected out in Ringer solution and transferred to Grace's tissue culture medium (Grand Island Biological Co.). After two rinses in sterile medium the disks were cultured in sterile plastic Petri dishes $(35 \times 10 \text{ mm}, \text{Falcon Plastics})$ which contained 0.5 or 0.7 ml. of Grace's medium plus 5 per cent lobster haemolymph (Grand Island Biological Co.). In several cases the haemolymph was left out of the medium with no apparent effect on the cultures. Most cultures were kept for 4 weeks, during which the medium was not replaced. The following results are based on observations of 145 cultured wing disks. No distinction was made between first and second wing disks in these experiments.

Wing disks from larvae which weighed between 0.25 and 0.30 g (within 1 day of cocoon spinning) were cultured if their tracheae had begun to spread out from the localized tracheal mass characteristic of younger undifferentiated disks. Twenty-seven out of thirty-one such wing disks exhibited continued tracheal spreading and growth after only 24 h in vitro. The length of these disks increased from about 1 mm to 2 mm within 2 weeks, but did not increase after that (length measured with an ocular micrometer was used as a convenient parameter of growth in these experiments). Furthermore, the disks became more wing-like and frequently formed vesicular outgrowths, which are characteristic of insect organ cultures. By contrast only four out of forty-five disks from larvae which weighed from 0.1 to 0.2 g had initiated metamorphosis even after 1 month of culture.

If ecdysone is the only extrinsic agent necessary for metamorphosis, then its addition to cultures of disks which would not otherwise grow should cause them to grow and metamorphose. To test this, ecdysone (1 µg/ 10 μl. of 10 per cent ethanol) was added to 0.7 ml. of medium in Petri dishes. The following doses were used with equal success: 1·5 μg, 2 μg, 5 μg and 10 μg. An equal amount of alcohol was added to control cultures. (Ecdysone (2 µg) from the same source was sufficient to initiate metamorphosis in brainless pupae of $Hyalophora\ cecropia^7$.) Disks were taken from larvae weighing 1.5-3.0 g. Those disks which had already initiated metamorphosis were discarded. Each group of four disks from a single larva was divided equally between control and experimental cultures. Between 3 and 10 days after the addition of ecdysone twenty-six out of thirty-five disks had initiated metamorphosis, while in the control group without ecdysone only three out of thirty-four had done so. Vesicles were found infrequently in the disks treated with ecdysone, although they were common in disks which grew without ecdysone in the medium.

Thus we have found that wing disks of Galleria mellonella will grow and partially metamorphose in vitro if metamorphosis has already begun in vivo, but will not do so otherwise unless ecdysone is present in the culture medium. Clearly, ecdysone alone is responsible for the initiation of metamorphosis in these disks in vitro. Why the wing disks do not complete their growth and dif-

ferentiation in vitro, and why ecdysone takes several days to produce a visible change in the cultures, is not yet known. Nevertheless, we hope that this system will be useful in investigating the action of ecdysone in vitro.

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Short Term Cultivation of Trypanosoma brucei in vitro at 37° C

STUDIES on the African pathogenic trypanosomes have been severely hindered by the failure of the infectious forms of these organisms to grow in vitro. All previous reports of the cultivation of the African trypanosomes refer to non-infectious forms resembling those found during the multiplication of the flagellates in the insect vector (Glossina sp.). Occasional infectivity of such cultures has been reported¹⁻³. This communication cultures has been reported1-3. describes the occurrence of a short term multiplication which occurs when trypanosomes are separated from infected blood and maintained at 37° C in the supernatant fluid over a monolayer of L-cells.

Tissue cultures of the mouse L-cell line were prepared in 8 oz. screw-top medical flat bottles. L-cells were inoculated into 5 ml. of medium and the cultures were incubated at 36°-37° C until confluent monolayers were established. The medium used for cell and trypanosome/cell cultures was NCTC 109 (Difco Laboratories) containing in addition 10 per cent v/v pre-sucking calf serum, 50 μ ml. penicillin and 50 μ g/ml. dihydrostreptomycin. NCTC 109 was used empirically. The gaseous phase consisted of air in some experiments and of 5 per cent CO_2 , 10 per cent O_2 and 85 per cent N_2 in others⁴. Ninety-five per cent O2 with 5 per cent CO2 was harmful to trypanosomes.

A Parkes strain female mouse was inoculated with the Lister strain 427 of T. brucei. Approximately 105 infectious organisms were inoculated into the peritoneal cavity of the mouse so that between 10⁷ and 10⁸ organisms were present for each ml. of blood after 48 h. The mouse was bled from the heart under light ether anaesthesia and the blood was diluted four-fold with warm medium ($30^{\circ}-35^{\circ}$ C) and defibrinated by rotation with glass beads. The blood

was transferred to a centrifuge tube and further diluted two-fold with more medium. The blood cells were deposited by centrifugation at 250g for 4 min and the supernatant containing trypanosomes was removed. The number of trypanosomes was adjusted to 2-6 × 105/ml. of medium and 5 ml. of this mixture was inoculated into each tissue culture bottle from which the medium had been decanted just before use. Aseptic precautions were observed throughout the procedure. Samples were removed at intervals for counting without dilution in a Neubauer haemocytometer. Organisms prepared in this way multiplied about threefold in vitro with a generation time of 6-9 h. Significant increase was never observed in control bottles without L-cells, but dividing forms were often present. The success of any culture varied in particular with the origin of the organisms in relation to the phase of growth in the donor animal, the serum component of the medium and the pH of the medium. Organisms taken from near the peak of parasitaemia in the donor animal were much less consistent in multiplying in vitro. It is not certain whether this result was a simple function of the stage of the growth cycle because the use of organisms from late in the growth cycle involved greater dilution of the infected blood and increase in numbers was not consistent when organisms were washed by centrifugation The pre-sucking calf serum was and resuspension. superior to normal rabbit serum, but not all samples of calf serum were equally satisfactory and selection of a suitable serum was initially necessary. Precise experiments on the effect of pH were not possible in the system described, but values below pH 7.2 or above 7.6 inhibited multiplication. Animals could be infected regularly with organisms from 24 to 36 h cultures and occasionally up to 70 h. Multiplication usually ceased after 20-30 h and attempts to subculture the organisms before this time gave most variable results. Even the division of the supernatant fluids into two and their replenishment with fresh medium often inhibited all further increase in numbers. The organisms present after 24 h or more were more easily damaged by centrifugation or pipetting than the original inoculum. Multiplication after subculture was observed after 12 and 24 h in a few instances when the culture bottles were tilted slightly, allowed to remain like this for 2 h and the top 3 ml. of medium removed and replenished. This contained only a third of the total number of organisms present. Preconditioning of medium in the presence of L-cells did not favour further multi-The supplementation of the medium with plication. washed mouse red cells, mouse or rabbit red blood cell lysate, or with haemin in varying concentrations down to μg/ml., inhibited or severely reduced multiplication.

Table 1 and Fig. 1 show the results of an experiment in which five-fold multiplication was observed. In a series of twenty similar trials the mean multiplication observed in the presence of L-cells varied from none (four experiments) to five-fold (three experiments), with the remainder showing a regular three-fold increase. In the absence of the L-cells, the trypanosomes frequently declined in number rapidly from the beginning of the experiment.

A preliminary study of the incorporation in vitro of radioactive leucine, thymidine and uracil into trypano-

Table 1. RESULT OF EXPLANTING TRYPANOSOMES FROM ONE MOUSE INTO THE MEDIUM DESCRIBED IN THE PRESENCE AND ABSENCE OF L-CELLS

Culture No.	0	6	13.5	Hours in culture 20	36	44	68
1 + L-cells 2 + L-cells 3 + L-cells Mean count/ml, $+ L$ -cells	$222*/<10\uparrow/4\ddagger$ 224/<10/4 210/<10/4 5.5×10^{5}	221/30/2 197/41/2 203/52/2 1·0×10 ⁶	237/67/1 303/100/2 352/92/2 1·8 × 10*	266/86/1 352/201/1 222/100/1 2·8 × 10 ⁶	415/119/2 305/128/2 211/57/1 1·8 × 10°	287/24/8 320/46/2 249/61/2 7·1 × 10 ⁵	§ 128/24/8 125/32/4 c. 2 × 10 ³
4 - L-cells 5 - L-cells Mean count/ml L-cells	224/<10/4 219/<10/4 5.5×10 ⁵	225/30/4 215/28/4 5·5×10 ⁵	227/59/4 218/44/4 5·5 × 10°	214/72/4 226/55/4 5·5 × 10 ⁵	132/87/4 94/20/4 2·8×10 ⁵	Not counted Not counted	Not counted Not counted

^{*} Number of organisms counted. Samples of the culture supernatants were placed without dilution in an improved Neubauer haemocytometer. Counts were performed using phase contrast illumination, magnification × 200.

† Number of dividing forms in first count. Based on the observation of organisms in which division of the cell body and flagellum was apparent. All dividing forms, including multiple dividing forms, were counted as single organisms in first count.

‡ Number of "squares" of the haemocytometer from which counts were made. 1 "square" = 0-1 mm³.

§ Contaminated with bacteria.

somal protein and nucleic acids, carried out in conjunction with Dr B. A. Newton, showed that linear uptake of these substances occurred over the 6 h period studied. There were no significant differences between the incorporation rates in the presence and absence of L-cells. Test and control cultures could readily be distinguished at 18 h, however, by the superior survival and normal appearance of the organisms in the L-cell cultures as compared with the controls. The lack of measurable differences in the incorporation rates may have resulted from the high cell densities used in these studies, which effectively prevented multiplication. L-cell cultures were chilled at 4° C for I h before use in order to reduce the synthesis of DNA in these cells. Supernatant fluids were free of detectable L-cells, provided newly confluent monolayers were hearr

It is evident that these results represent only a very limited success in cultivating trypanosomes because the multiplication seems to be as dependent on the use and preparation of a suitable trypanosome population as on the conditions of the culture. It is not, however, strain dependent, because all the four strains of T. brucei tested seemed to react similarly. Although complex, the system seems to offer the opportunity of examining the nutrient requirements of trypanosomes in conditions in which the base line for comparison represents an increase rather than a decrease in numbers. No conclusions can be drawn from these experiments about the nature of the dependence of the multiplication observed on the presence of the L-cells, but this might be further investigated by the pre-treatment of the L-cells with metabolic inhibitors affecting pathways absent from the trypanosomes, or by the irradiation of the L-cells with X-rays or ultraviolet light. The finding that multiplication could be obtained but not indefinitely prolonged might suggest that in addition to the dependence of the multiplication on the L-cells the explanted organisms contain a pool of an essential metabolite sufficient for a limited number of cell divisions and lacking from the medium used. Several other explanations are, however, possible. The fact that all these experiments were carried out at 37° C, that there was no observable lag phase at the start of the cultures and that significant (more than two-fold but less than three-fold) multiplication of an akinetoplastic strain of T. evansi could be observed in the same system supports the belief that these results represent multiplication of the blood form of a pathogenic trypanosome rather than the appearance in unusual circumstances of other stages of the life cycle. More precise studies on the nutritional requirements of these parasites are needed.

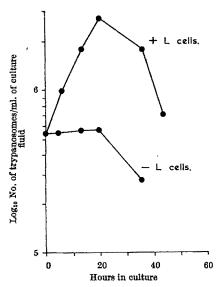


Fig. 1. Growth curve of T. brucei at 37° C. Data plotted from Table 1.

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Accumulation of Nitrite in Fresh Soils after Gamma Irradiation

Large increases of plant growth in irradiated soils have been attributed principally to increases in available ammonium- and nitrate-nitrogen1 although the accumulation of nitrate in fresh and dry soils has not always been observed^{2,3}. Experiments by Cawse⁴ have consistently shown substantial increases in nitrate when five fresh soils were incubated after treatment with 1.5-200 × 10³ rads.

To establish the point at which oxidation of ammonium to nitrate begins to be diminished by radiation, the soils previously described4 were irradiated with increasing doses up to 1 × 106 rads; during the course of this work it was noted that nitrite, as well as ammonium and nitrate-nitrogen, accumulated rapidly after one soil (Broad Series) had received 5×10^5 rads. A similar organic calcareous soil (pH 7·4 in 0·01 molar calcium chloride) was obtained from the Icknield Series⁵ for irradiation, and it, too, accumulated nitrite. Only ammonium- and nitratenitrogen showed increases in the other four soils, even when the dose was extended to 2.5 × 10° rads; data for Grove Series are representative of this group.

The fresh soils were passed through a 2 mm sieve, and water was added to obtain a moisture level between 60 and 70 per cent of field capacity. After 2 days of equilibration, 70 g samples were irradiated in a source of Sample containers were cobalt-60 $(6.8 \times 10^5 \text{ rads/h})$. incubated at 25° C, and were never opened before analysis except for leaching tests described later. Exchangeable ammonium- and nitrate-nitrogen were extracted with acidified N-potassium chloride, and the filtrate was analysed. Nitrite was extracted with water at neutral pH, and determined by a modified Griess-Ilosvay technique. All results are means of single determinations on duplicate samples.

The response of nitrogen mineralization in Broad, Icknield and Grove soils 7 days after treatment for the range of doses $5 \times 10^4 - 2.5 \times 10^6$ rads is shown in Fig. 1. In the first two soils nitrite accumulates as soon as nitrate falls with increasing radiation, and two principal mechanisms were considered possible; first, a blockage of the nitrite oxidation process in Nitrobacter spp., or second, the reduction of nitrate.

The rate of nitrite formation in Broad soil was examined by treating 10 g samples at 62 per cent of field capacity with 8×10^5 rads (at 9.0×10^5 rads/h); extractions were made after a series of incubation times up to 128 h after irradiation (Table 1).

Nitrite formed very rapidly, and the instantaneous extraction indicates that it increased during the irradiation period, similarly to nitrate4. Nitrite in the filtered extracts was completely stable up to 5 h at 20° C.

Irradiation of dry, medium and wet Broad soil showed that high soil moisture greatly enhanced nitrite accumulation (Table 2).

To examine whether the accumulated ammonium- or nitrate-nitrogen was the source of nitrite, 10 g samples of

Table 1. RAPIDITY OF THE INCREASE IN NITRITE IN BROAD SERIES SOIL AFTER 8×10^6 RADS

***************************************	*****
Extraction time (h after irradiation)	Mean p.p.m. of nitrite-nitrogen
Instantaneous	4.3
0.5	5.5
1	5.8
2	6.8
4	10.1
8	13.4
	20-4
16 32	24.3
64	29-9
128	26-4
Control (pre-irradiation)	1.0

Table 2. EFFECT OF MOISTURE ON NITRITE ACCUMULATION IN BROAD SERIES

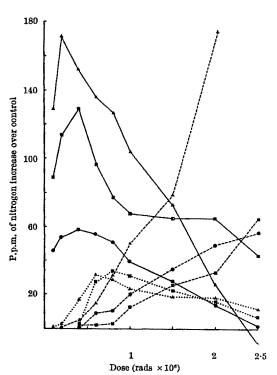
,	Treatment	Moisture % of	Mean p.p.m.	of nitrite-nitrogen day 7	at
		field capacity	Control	8×10 rads	
	Dry	7	2.2	1.2	
	Medium	48	1.4	26.1	
	Wet	8 8	2.0	44.8	

Table 3. ORIGIN OF THE INCREASE IN NITRITE AFTER IRRADIATION

	Mean p.p.m. nitrite-nitrogen 4 h after irradiation			
Leaching solution	Broad soil	Icknield soil		
Water 100 p.p.m. NH ₄ -N 100 p.p.m. NO ₃ -N None	1·4 2·0 9·7 10·1	1·7 0·7 8·9 7·5		

Broad and Icknield soils moistened to 60-70 per cent of field capacity were irradiated in 3.5×10 cm filter tubes with 8 and 6 × 10⁵ rads, respectively. Immediately afterwards they were leached with 100 ml. of water, or ammonium sulphate (100 p.p.m. NH₄-N), or potassium nitrate (100 p.p.m. NO₃-N), all adjusted to pH 7 with 0·1 normal potassium hydroxide. Nitrite was determined after incubation for 4 h (Table 3), and it only appeared in amounts almost identical to irradiated unleached soils after displacement of the soil solution with nitrate.

It was concluded that nitrate reduction was the principal cause of nitrite formation observed in these experiments, and further confirmation with nitrogen-15 will be attempted. It is well known that nitrate is used by micro-organisms instead of oxygen in completely or



Increases in the concentrations of ammonium (---), nitrite
 , and nitrate (——) in Broad (■), Icknield (▲), and Grove (●) soils after irradiation and 7 days of incubation at 25° C.

partly anaerobic conditions and nitrite accumulates in the medium. Pseudomonas and Bacillus spp. isolated from waterlogged soil have been shown to accumulate large amounts of nitrite in pure culture7.

There are reports of toxicity of nitrite to plants⁸, and because soil sterilized and partly sterilized by radiation is often used or advocated for agricultural research, the growth of lettuce (Lactuca sativa variety 'Cheshunt Early Giant') was examined in pots of Broad soil treated with 8×10^5 rads. A fallowed treatment was analysed during the growing period, and proved the persistence of nitrite after 60 days. Toxicity symptoms8 did not appear, and yields after 60 days of growth exceeded control, as in previous trials with other soils1.

One explanation of nitrite accumulation may be that in some soils there is greater radiation damage to nitrification compared with nitrate reduction, and the latter becomes the dominant process before it, in turn, is inhibited by increasing dose (Fig. 1). Campbell and Lees¹⁰ point out that if aerobic organisms reducing nitrate to nitrite were operative in soil, their activities would be difficult to detect where Nitrobacter is active. Cooper and Smith¹¹ reported nitrite accumulation by nitrate reduction in some alkaline soils, and nitrite reduction was the rate limiting process for denitrification. Allison12 has proposed the formation and decomposition of ammonium nitrite as a mechanism in gaseous nitrogen losses from soil, and it is notable that conditions in the irradiated soils would favour the reaction by ammonia release and partial inhibition of nitrite oxidation.

It is improbable that proliferating organisms are involved in the nitrogen transformations above 5×10^5 rads, but bacterial enzymes are very radioresistant18 and can remain active in non-proliferating cells¹⁴. A residual soil enzyme fraction may even be responsible, of comparable existence to soil phosphatase15.

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MICROBIOLOGY

Selective Feeding of Tubificids on Bacteria

THE characteristic abundance of tubificid worms in the benthic coze of sewage outfalls, their curious up-ended posture and sedentary feeding habit suggest a saprobic, non-selective type of nutrition. Feeding has been inter-

preted as a continuous process of indiscriminate ingestion and pumping of an endless column of sapropel through the digestive tract1. We have gathered evidence, however, which does not support this generalization, but indicates selective feeding.

Homogenetes of mixed populations of tubificids (Limnodrilus sp., Tubifex sp. and Peloscolex sp.) were analysed by gas-liquid chromatography for the presence of an organophosphate pesticide (diazinon) incorporated by bacteria on which the worms were fed. It was thus possible to establish, on the basis of diazinon intake, the ingestion of the corresponding bacterium. The bacteria were incubated for 3 days on a mineral salts medium (MSM) which supports a wide range of soil organisms2. Diazinon was substituted for glucose as the carbon source. The cultures labelled with diazinon were then centrifuged and washed three times to remove extraneous diazinon and transferred to aerated cylinders. Each cylinder contained some twenty tubificids previously conditioned for 2 days on 1 per cent sodium substituted MSM with no carbon source. (Tubificids exhibit a marked sensitivity to potassium3.) After 5 days the worms were washed, homogenized in acetonitrile, extracted in petroleum ether and injected into an 'Aerograph' gas chromatograph with an electron capture detector. Diazinon was identified in worms exposed to Escherichia coli, Sphaerotilus natans and Aerobacter aerogenes, but not Arthrobacter sp., Micrococcus flavus and Chromobacterium sp. Behavioural studies had previously indicated no response by the tubificids to the presence of diazinon alone, though the worms readily assimilated the compound.

To determine the nature of the basis for discrimination, the microbial cultures were incubated for 2 days on BHI agar and transferred on 3 in. cubes to 6 in. Petri dishes lined with filter paper and containing § in. of 1 per cent sodium substituted MSM. The response of the worms to different combinations of bacteria was tested by placing three cubes (one control) at equal intervals at the perimeter of the dish and with each respective colony well above the surface of the water (Fig. 1). A 1 in. clump of worms was placed central to, but not touching, each cube. Two forms of avoidance responses were observed: either a clump pulled away by the more distal worms; or when water was too shallow (< 15 in.) to permit the clump response, a streaming behaviour by individual

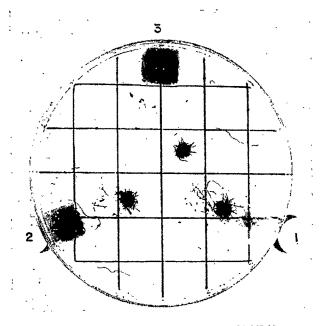


Fig. 1. Avoidance of bacteria by clump migration of tubifield worms.

1. Uninoculated agar control; 2, agar inoculated with Micrococcus flavus; and 3, agar inoculated with Chromobacterium sp.

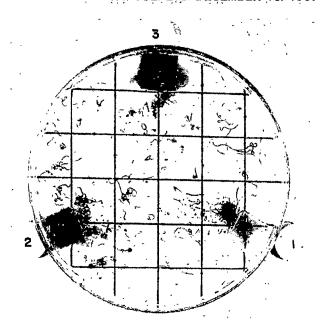


Fig. 2. Avoidance of bacteria by streaming action of tubificid worms.
 1, Uninoculated agar control; 2, agar inoculated with Micrococcus flavus; and 3, agar inoculated with Chromobacterium sp.

worms was seen (Fig. 2). Avoidance responses were elicited, in order of decreasing intensity, by Micrococcus flavus, Arthrobacter sp. and Chromobacterium sp. Positive or neutral responses (no avoidance) were demonstrated, in order of decreasing intensity, by S. natans, E. coli, A. Aerogenes and the control. Because avoidance was observed within 0.5 h of initiating the test, the presence of one or more readily diffusible water soluble metabolites is suggested. The identification of the active principle

presently being undertaken.

The results of the chromatographic and behavioural studies agree well. Diazinon was detected in those worms demonstrating a tolerance to the bacteria and not identified where avoidance was elicited. There has been little success in correlating habitat selection with environmental factors^{4,5}. If this laboratory model does reflect field conditions, and food preferences do have relevance in determining population distributions, the resistance of Arthrobacter sp. to ingestion may explain the ubiquity of its distribution: on the other hand, the amenability of E. coli to ingestion may provide a basis for its rapid disappearance from water and soil. In any event, this observation provides a model for further studies in population dynamics.

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BOOK REVIEWS

ORIGINS OF THE GENETIC CODE

The Genetic Code

The Molecular Basis for Genetic Expression. (Modern Perspectives in Biology.) By Carl R. Woese. Pp. viii+200. (New York and London: Harper and Row, Publishers, 1967.) n.p.

THE very wide interest in the genetic code which has occurred during the past 6 years stems from the fundamental nature of the relationship of DNA to proteins. The major break-through came in 1961. Then, Nirenberg and Matthaei showed that a codon for the amino-acid phenylalanine was composed of a series of uridylic acid residues, and Crick, Barnett, Brenner and Watts-Tobin showed that the genetic code was a triplet code, and thus a codon for phenylalanine was UUU. Since then the meanings of the sixty-four codons have been elucidated.

Before 1961 much speculative theoretical work, starting with Gamow's diamond code in 1954, laid down the basic ideas of what properties the genetic code might have. These ideas helped in the interpretation of the plethora of experimental results which appeared in the early 1960s. Dr Woese is one of the few people who have devoted much serious thought to the genetic code and it is therefore particularly appropriate that he should have decided to write this book. The first two chapters introduce the reader to the fascinating historical background which the discoveries of 1961 were to obscure so quickly. Chapter 3 covers the experimental determination of which codons code for which amino-acids—the dictionary of the genetic Chapter 4 includes a section on punctuation signals and a thumbnail sketch of control mechanisms, which an uninitiated student might find tough going. The next chapter concludes our knowledge which bears on the genetic code with a standard description of the translation process, including a section on ribosomes, the structure of transfer RNA molecules and Crick's wobble hypothesis.

These first five chapters are all orientated to help the reader consider what the author sees as the next phase in the genetic code: why does the code have the structure it has and, what must necessarily be considered together with this, how did the code and the translation apparatus evolve? This highly speculative field is covered in his two final chapters and it is here that this book distinguishes itself from many others which discuss the more familiar aspects of the genetic code. Basically Dr Woese considers two extreme models of how the code might have "Stochastic" models suppose that the allocation of codons to the twenty amino-acids occurred essentially by a haphazard process and that the order seen in the code today reflects Nature's attempt to minimize the effects of errors made in replication or some step of the translation process. "Mechanistic" models suppose that there was (or is) some structural relationship between a codon and the amino-acid for which it codes, and that therefore phenylalanine was more likely to be coded by UUU than by AAA. Dr Woese tends to favour partly mechanistic models, for he and his collaborators find some correlation between the physical properties of amino-acids and the structure of their codons. The conceptually difficult problem in this field is how a completely selfreplicating unit might have evolved, and what did it contain. Are transfer RNA molecules very primitive structures? Are they as large as they are because the most primitive translating system had no ribosomal

structure and the adaptors had to be big enough to "stick" to one another?

This book presents a particularly personal approach to the genetic code as seen by a theoretician. This has its merits in that the reader is unlikely to lose his path in a maze of biochemistry. It is unfortunate, however, that an excess of new jargon has appeared (such as "AERSs" for the activating enzyme recognition sites of transfer RNA molecules), that most molecules have become "tapes" or "tape-readers" and that several needless and trivial errors occur (the structure of uridylic acid in Table 1-2 has a gratuitous "+CO₂"). In parts the style is over-dramatized, but apart from these criticisms this book is a stimulating and valuable contribution to those wishing to understand how the code was solved and what its solution means in terms of the origins of life.

MARK S. BRETSCHER

LAKE DISTRICT LANDSCAPES

A History of Man in the Lake District By William Rollinson. Pp. xii + 162 + 15 plates. (London: J. M. Dent and Sons, Ltd., 1967.) 42s. net.

FEW regions in the British Isles have been as fortunate as the English Lake District in the quality of the descriptive writings they have inspired. Thomas West wrote his Guide in 1778 and William Wordsworth wrote in 1810. The poet envisaged the area "as a sort of national property" and since 1951 its 900 square miles have been one of Britain's most popular National Parks. It was here, too. that the National Trust had its home and became possessed of its first property in 1902. Scientific survey, however. has not kept pace with romantic conservancy, although W. G. Collingwood showed the way in 1925. Since then many geological, ecological and economic studies have been made, but no comprehensive survey of the evolution of the cultural landscape has appeared. This book partly fills the gap, though it is aimed at the interested layman and makes no claim to be a work of detailed scholarship. The author's statement that the fascination of the post-Roman period lies in the fact that there can be few certainties about it clearly puts him on the side of the romantics rather than the scientists.

The approach is systematic, but the first chapters on geology, glaciology and archaeology are sketchy and uncritical and the information often out of date. The interest of the writer, and therefore of the reader, quickens as the historic periods are reached, and the best sections are those dealing with agricultural and industrial development. The final chapter is a reasoned statement of the problems of combining conservation with maximum use of the region's resources. The photographs and the line drawings are attractive, in particular the distribution maps, but scientific accuracy is sacrificed by the use of picture symbols which may occupy more than a square mile of ground. The book is marred by innumerable disrupting footnotes which should have been incorporated in the text, and has other marks of the beginner. The author is clearly deeply attached to the Lake District and knows it well, yet the full flavour of field work is missing. Having cut his teeth on the area, he should go on to bite E. E. EVANS deeper into it.

DEVELOPING TRADE

The Growth of the British Cotton Trade 1780-1815. By Michael M. Edwards. Pp. viii+276. (Manchester: Manchester University Press, 1967.) 45s.

THE British cotton industry was the first major industry to be completely mechanized and converted to the factory system: it has been termed the "leading industry" in the first Industrial Revolution. It has fascinated many observers, and a small library has been written about it. Yet, surprisingly, numerous gaps in our knowledge about it remain to be filled, and this volume fills one of them with great skill and perspicacity. The author, in fact, treats his topic with remarkable mastery and maturity, and only the unnecessary length and detail of the book betray its origin as a doctoral thesis.

The period chosen is dominated by the war against France, and it is useful to be reminded how much the major wars of the period up to 1815 did influence the course of British industrialization. The author, with his detailed, almost microscopic knowledge of the period, discerns the alternation of years of heavy investment and expansion, 1780–1792 and 1798–1803, and years of over-production, loss of markets and stagnation, 1793–1797, and summer 1803–1815. Although the case for writing off the whole of that last period is not altogether convincing and is contradicted by some of the evidence, the division is useful, and it is salutary for our understanding of the age to be made aware that high profits, even in cotton spinning, were not automatic and not continuous.

There are two main themes covered by this study. One is the organization of the trade, the merchants. brokers, commission agents and factors who imported and distributed the raw cotton, the yarn and the finished cloth; the other is the method of financing specialization and skill, with the growth in the size of the industry in those years; and both show the remarkable flexibility and inventiveness of the age in organizing its buying, selling and financing. The extent to which the industry bent financial institutions to meet its needs is again confirmed, and so is the relative unimportance of fixed capital, and the weight of circulating capital, like work in progress and mutual indebtedness, in which the banks, despite the conventional picture of aloofness from industrial entanglements, played a principal part. Although there were, as there always have been, price fixing agreements by the big units, there was no monopoly and the environment was basically highly competitive. In these conditions the drive for more cotton and for more money was like a flood, breaking down its obstacles or, where it could not entirely sweep them away, seeping through the cracks, swirling around the blockages and undermining the opposition. In the end, nothing could hold up the use of the new power.

It is, perhaps, a pity that economic science took this mobile, flexible, inventive and competitive world as its model, holding other conditions to be deviations or falls from grace. The conditions in the cotton industry in its first generation of the factory system were exceptional, perhaps even unique. It was probably the only period in history where all the initiative came from below, where ability was more important than money in the rise of the individual, and where greed and ruthlessness became a force which imparted a mighty turn to the wheels of human progress.

FRAMEWORK OF GEOLOGICAL DATA

The Stratigraphy of the British Isles
By Dorothy H. Rayner. Pp. x+453+8 plates. (London: Cambridge University Press, 1967.) 70s. net; \$12.50.

This is a welcome and, by any standards, remarkable book. Dr Rayner, senior lecturer in geology in the University of Leeds, has made a synthesis, broad but well founded in details, of the succession and occurrence of rocks in the British Isles. Ireland is at long last accorded its rightful place in the scheme of things. Such a synthesis, which has not appeared for several decades, will be seized on by famished college and university teachers for use in their more advanced courses. The British Isles are

geologically highly complex, and the literature on the succession of deposits must run into tens of thousands of items, controversial issues abounding. Though rarely demonstrated explicitly in the text, Dr Rayner has clearly brought herself to master the detail as well as the essence of these various publications, a selection of which is listed. Works dated 1967 are included in the book and there are footnotes dealing with some very recent discoveries. The assimilation of all this information has in no way detracted from Dr Rayner's clear and unobtrusive style of writing.

The author's method is simple. The geological systems are treated chronologically, one to a chapter, except for the Ordovician and Silurian which are treated together in one, and the Carboniferous which is split between two. Each chapter begins with a general statement which includes a list of the principal divisions of the system together with a map of present outcrops and the chief physiographic elements of the time. There follows an expansion of this in terms of either geographical regions or geological units, as the circumstances of outcrop and knowledge dictate. There is usually a terminal summary in which difficulties are high-lighted. Two chapters are not concerned with a system. The first chapter, intended to remind the student of what he has learned elsewhere, is a review of stratigraphical concepts and methods, and a general account of the tectonic setting of the British Isles. The final chapter, called an epilogue, reviews briefly the kinds of data on which palaeogeographies have recently been based; some attention is given to the hypothesis of continental drift as affecting the North Atlantic border-

The Pre-Cambrian rocks of Britain are highly complex, for they exist in scattered outcrops greatly diversified as to scale and succession. The principal exposures, in Scotland, are associated still with major controversies. Dr Rayner's view is that the Moine rocks are probably equivalent to the Torridonian. Unfortunately, the section on the Torridonian itself already needs modification in the light of recent work which has demonstrated the existence of an unconformity and large time-gap within the series.

Wisely, the author groups the Dalradian rocks of Scotland and Northern Ireland with beds elsewhere classified as Cambrian. The implications of drift are accepted and the scene is set for the protracted events of Lower and early-Upper Palaeozoic time. The British Isles are seen as definitely peripheral, in Cambrian times, to the North American shield; a structurally stable area is glimpsed in north-west Scotland, and to the south-west of this lie the Ecoambrian Dalradian trough and somewhat younger basins of Wales and Ireland.

The Ordovician and Silurian rocks of Ireland are, generally speaking, poorly known compared with the mainland successions. However this may be, the integration of data from the two islands leads to a convincing picture of the distribution and character of the several basins and swells distinguishable in the beds. The pattern of distribution could, however, have been demonstrated more clearly if more attention had been given to maps of sedimentary facies and sediment dispersal. Data of these kinds are central to a grasp of the roles played by the different structural units.

In the British Isles the Devonian is represented by rocks chiefly of the Old Red Sandstone facies. These beds straddle in time a major tectonic event, for the Lower Old Red Sandstone is essentially "Lower Palaeozoic" in distribution and relationships, while the Upper Old Red Sandstone broadly exemplifies Upper Palaeozoic patterns. It is in this chapter on the Devonian that one particularly misses in Dr Rayner's treatment the link between sedimentation and tectonics. Her account of the succession of rocks is exemplary, but what may lie concealed in the unconformities discussed receives scarcely a mention. These hidden events are not altogether speculative, however. On a minor point, one cannot agree with Dr Rayner over

her interpretation of the Middle Old Red Sandstone break in Ireland, or with her over-simplified account of Upper Devonian events in South Wales.

No system in the British Isles has been so intensively studied as the Carboniferous, and the account of this is a model of concision. Comment is required only on minor points. The author should have made it clear that some of the Namurian Grits of the Pennine area have been derived from the south, off St. Georges Land. The relationship of the succession in the South Wales Coalfield to the Culm of Devon and Cornwall is not clarified. The knowledge that a part of the Coalfield succession is of southerly origin should have invited a closer analysis.

In Germany and the Netherlands there are Permian and Triassic standards of great importance to an understanding of the British succession. Dr Rayner refers to these standards, but is hampered in her application of them to Britain by the difficulties of dating the British rocks. The depositional break at the base of the British Keuper is mentioned but is accorded no particular significance. It now seems likely, from palynological and other work done on the mainland and in connexion with North Sea exploration, that the Keuper Sandstone is of late Bunter age. The whole early "Keuper" sequence may reveal in Britain events closely connected in Germany and the Netherlands with the Muschelkalk transgression. Other kinds of environment seem preferable to Dr Rayner's Keuper "plains and lakes".

We find the author on firmer ground when it comes to the Jurassic, Cretaceous and Cainozoic rocks, all of which have been much studied. The account of the Jurassic System is unexceptionable and mercifully brief. Only one comment is due as regards the Cretaceous. Dr Rayner assigns the final submergence of the London Platform to Gault times; the sedimentological evidence, however, points to a connexion by at the latest the end of the Lower Albian. A stronger exception must be taken to the interpretation placed on the Reading Beds which begin the Cainozoic in the area west of London. At least the basal gravel of this formation is fully marine, and there is evidence that the higher rocks may be of littoral origin. The Palaeocene transgression was probably as widespread as the later, though more substantial, Ypresian one.

Too often the British geologist dismisses Plio-Pleistocene deposits as a subject of little concern. This is an unfortunate view, for herein is to be found a greater range of geological phenomena than in perhaps any older beds. Moreover, the late Pleistocene deposits afford a natural laboratory in which process and geological resultant can be closely matched. The task of outlining the Plio-Pleistocene of Britain has not been shirked, but the complexity of the subject finally defeats Dr Rayner. Once the Crags have been dealt with, the treatment falls below the high standard set in earlier chapters.

It would be ungracious to deny Dr Rayner the praise due to an important work, yet false to suppose that other interpretations of the subject of stratigraphy did not exist. It is fair to conclude that Dr Rayner aligns herself perhaps more with the "classical" than with some modern conceptions of the subject. This viewpoint invites a number of questions which all geologists should debate with themselves from time to time. Can there be a stratigraphy of the British Isles, or is this yet another conceit of the island race? What of the geological units—especially the Caledonides and Hercynides—of which the British Isles once formed a part? Should a stratigraphy be developed for these units rather than geographical or political ones? What place should be given to the relationship between sedimentation and tectonics? If the connexion is recognized—and this seems unavoidable—then unconformities should be explored in as much detail as sedimentary formations. Further, isopachous, facies and sedimentdispersal maps would have an important place in any stratigraphical account. Can a rehearsal of outcrop, zone and lithology be considered enough? J. R. L. ALLEN

ATTRACTION OF EARTH

The Gravity Field of the Earth from Classical and Modern Methods

(International Geophysics Series, Vol. 10.) By Michele Caputo. Pp. xiv+202. (New York: Academic Press. Inc.; London: Academic Press, Inc. (London). Ltd., 1967.) 78s.

In this book Dr Caputo provides a useful exposition of the framework of mathematical theory needed in studies of terrestrial gravity and the external gravitational field. The theory expounded in the first part of the book is applicable mainly to measurements made on the Earth's surface, by the traditional methods of gravity survey and geometrical geodesy. The theory is developed in terms of ellipsoidal co-ordinates, and gives in closed form the potential of a rotating planet whose gravity field has an ellipsoid of revolution as an equipotential surface. The practical problems to which the theory applies include the international gravity formula and hydrostatic equilibrium figures. In the remaining seventy pages of the book the theory of Earth satellite orbits is developed. generally on quite conventional lines, by expanding the gravitational potential in Legendre polynomials. section ends with a review of recent determinations of the geoid by analysis of satellite observations.

The book is written in a pleasant style and the virtues of the "ellipsoidal approach" are well brought out. Certainly this approach has advantages, but it also has deficiencies, as the author tacitly admits by not adopting it when he comes to treat satellites. The book is well produced, and expensive; misprints are fairly frequent, but usually trivial.

D. G. King-Hele

MODELS IN STRUCTURAL GEOLOGY

Gravity, Deformation and the Earth's Crust As Studied by Centrifuged Models. By Hans Ramberg. Pp. ix+214. (London: Academic Press, Inc. (London). Ltd.; New York: Academic Press, Inc., 1967.) 57s. 6d.: \$11.

THE use of scale models to investigate the mechanical processes in the Earth's crust has had limited success. This is because of the well known difficulty of scaling down the dimensions, the time scale, the forces and the mechanical properties in the same model. One of the greatest hindrances has been the lack of a sufficiently large body force in the model to represent gravity. This has been a serious difficulty, for gravity has a profound influence on the formation of most geological structures and it is the controlling factor in some of them.

Ramberg has made an important contribution to structural geology by introducing the centrifuge technique into scale model investigations. This enables centrifugal accelerations of up to 3,000g to be applied, which can provide at last a realistic representation of gravitational body forces in the crust. The central theme of the book is the application of this technique to problems such as the rise of salt domes (so important in the North Sea oil hunt) and the uplift of mountain ranges. Some noncentrifuged models are also included, and the experimental work is backed up by theoretical calculations which are mainly based on Newtonian viscosity.

The book contains much important information for the structural geologist. One can mention the demonstration by theory and experiment that the downbuckling mechanism of geosyncline subsidence cannot work, and the beautiful scale model experiments of salt dome rise. There is also controversial matter, such as Ramberg's preference for vertical body forces to horizontal compression as the primary mechanism for mountain building.

Professor Ramberg is to be congratulated on his important contribution to geology. The same cannot be said. however, for the production of the book. It makes slow and often uninteresting reading despite the exciting subject matter. Perhaps someone ought to read the article by the Editor of *Nature*, "Is the Literature Dead or Alive?"

M. H. P. Bott

QUANTUM MECHANICS

The Mathematical Principles of Quantum Mechanics By Derek F. Lawden. Pp. xiv + 280. (London: Methuen and Co., Ltd., 1967.) 50s. net.

DURING the past twenty years or so, a scarcity of books on quantum mechanics has been happily transformed into a situation where there now exists an embarrassingly wide selection of good books on this fundamental subject, so that the need for new books has considerably diminished unless they possess some original feature.

As its title suggests, the volume under review sets out to emphasize the mathematical aspects of quantum mechanics while mostly disregarding the physics of the subject. The book begins with a chapter on the representation of states by vectors in a multi-dimensional vector space. Then comes a chapter on spin operators followed by chapters on operators possessing continuous eigenvalue spectra, on the variation of state vectors with time, on angular momentum operators, on perturbation theory and on Dirac's relativistic theory of the electron.

The presentation of the subject matter is quite straightforward. The operator approach to quantum mechanics is chosen as the basis of the subject and the Schrödinger equation arises in the development as the eigenvalue equation for the Hamiltonian operator in the co-ordinate representation. Regrettably there is virtually no discussion of the relationship between the Schrödinger equation and classical mechanics: my belief is that this connexion is an extremely important one, for it leads to considerable clarification of the position of quantum mechanics in the general field of physics, and indeed was the original approach used by Schrödinger.

This book by Lawson also omits discussion of variational principles and methods, and it only takes a cursory glance at the theory of collisions as an application of perturbation theory. Nevertheless it can be recommended to a student whose primary interest is in the mathematical aspects of quantum mechanics, for it presents a lot of valuable material very clearly, but for a fuller understanding of the subject it would need to be supplemented by other books.

B. L. Moiseiwitsch

NEW ASPECTS OF REPRODUCTION

Advances in Reproductive Physiology

Vol. 2. Edited by Anne McLaren. Pp. 347. (London: Logos Press, Ltd., in association with Elek Books, Ltd., 1967. Distributed by Academic Press, New York and London.) 75s.

It can be argued with some justification that there are already too many books on fertility and reproduction. Certainly, there is a wealth of review on the subject and it is depressing, not to say painful, to compare the volume of relevant literature with its substance. As a discipline, the biology of reproduction seems to be sorely in need of a major breakthrough, and a constant flow of monographs, in which authors repeatedly ask the same questions instead of getting on with answering them and saying something new, can be extremely tedious. Moreover, there is always the danger that the "multiple author" type of book merely serves as a platform for certain individuals to parade as experts in their field. A book intended to be purposeful can thus be marred. Such judgments should, of course, be tempered by the fact

that monographs involve an enormous amount of difficult work, and if they are done properly they should be and are of interest and great value.

Anne McLaren clearly has a flair for editing, for she avoids almost all the best known pitfalls. It is claimed in the editor's foreword that the volume is "...neither balanced or integrated", and it would seem largely because of this that the book is by no means a run of the mill text on reproduction.

The first two chapters are about behaviour. This alone makes the book "different" and is an advance on volume 1 which covered more conventional topics. The first chapter of volume 2, by Margaret Bastock, is particularly effective in that it contains a nice attempt to integrate behaviour and physiological mechanisms, and although in the second chapter M. P. M. Richards does not try to do this, he succeeds in making maternal behaviour in rodents and lagomorphs a fascinating subject.

A. B. Gilbert writes the third chapter on egg formation in the chicken. This is a novel and absorbing part of the book and its inclusion is most refreshing. Lightheartedly, however, I cannot resist mention of the banality of the opening sentence in this chapter which is only outshone by the opening phrase of the next chapter. Chapter 4 is written by B. J. Restall and is an attempt to survey the biochemical and physiological relationships between the gametes and the female reproductive tract. To try to do this in twenty pages is to attempt the impossible and, needless to say, the impossible has not been achieved. Nevertheless, the twelve pages of references are a useful supplement to a brave if superficial approach to the problem.

Chapters 5 and 6, respectively, on oestrogens and implantation, are erudite, but less appropriate to this volume than most of the other chapters. This in no way reflects on such distinguished authors as Professors Emmens and Psychoyos; indeed, chapter 5 is probably the best written chapter in the whole book. But the subjects are too topical to be in context. Could it also be that in these two chapters much is assumed for the uninitiated, and little new is added for the expert? It will be for each sort of reader to make a personal decision on this.

A chapter by Peter Gruenwald on growth in the human feetus is a valuable contribution, and the closing chapter by J. M. Tanner on puberty is scholarly and affords some intriguing information, even though it is perhaps a bit light on purely physiological aspects of the problem.

This book should have wide appeal, and it can certainly be recommended to reproductive physiologists and other biologists, not because it is just another book on reproduction, but because it is an unusual and informative book on reproduction. The title of the book bears hardly any relation to its contents, but this is of little import here, and if anything it enhances the interest.

T. D. GLOVER

Appointments

REAR-ADMIRAL F. Dossor has been appointed director of hovercraft at the Ministry of Technology.

DR R. B. BUZZARD, at present research director of the National Institute of Industrial Psychology, has been appointed director of the institute in succession to Dr C. B. Frisby.

Announcements

C. H. Van de Hulst, professor of theoretical astronomy at Leiden, has been elected to succeed Dr A. Hocker as chairman of the council of ESRO for 1968; Professor R. Lüst, director of the Max-Planck-Institut für Extraterrestrische Forschung, Munich, and chairman of the Launching Programmes Advisory Committee of ESRO, and Dr J. Stiernstedt, head of the Department of

the Royal Ministry of Education and chairman of the Swedish Committee for ESRO, have been elected vice-chairmen.

PROFESSOR IGOR TAMM, of the Rockefeller University, has been awarded the Alfred Benzon Prize for 1967 for his achievements in virus research. The award was made in Copenhagen and Dr Tamm became the first American to receive the award of \$3,600 and to deliver the Alfred Benzon Lecture.

ERRATUM. In the News and Views note "Royal Society Blooming" (Nature, 216, 841; 1967), the study group under the chairmanship of Dr H. A. Cole should be described as being concerned with marine pollution.

CORRESPONDENCE

The Brain Drain

SIR,—The emigration from Britain of trained scientists and engineers is a matter on which expatriate Britons also reflect. I came to the United States 15 years ago, before the brain drain became fashionable, and have observed the drain mainly from the American end, though I have kept in touch with the situation at home through my annual visits to Britain. Because my experience has lain principally in British and American universities, I am better acquainted with the flow of scientists and engineers from and to these institutions than I am with conditions in industry. But I assume that a reversal of the brain drain in the educational and research fields would not be frowned on in Britain.

It is of advantage to Britain that its young scientists and engineers should spend two or three years in a new environment learning new methods. The only real problem is to get them back again after they have spent some time in North America.

The British Government's Working Group on Migration and the leading article in Nature of October 14, 1967, wish to attribute the brain drain mainly to the higher dollar salaries current in North America. This idea must be based on arithmetic alone: £5,000 converted at 2.8 yields \$14,000 (\$12,000 since November 18) and therefore \$14,000 presumably enables a man and his family to live in North America in the way he could live in Britain if he earned £5,000. An illusion such as this can only be dispelled by living for a time on this side of the Atlantic as an ordinary resident and not as a much-fêted temporary Before I came to the United States I was a department head in the University of London and I am now one in my present post. On our visits to Britain, my wife and I have noticed that our friends who are department heads and their families live precisely the kind of lives which we would choose to live were we back at home, and which we do live in the United States as far as circumstances permit. Our friends have pleasant houses, they own cars, they travel in Europe for their holidays, they send their children to schools of their choice, and so on. Yet the salaries of these men, multiplied by 2.8, let alone by 2.4, yield sums in dollars that seem pathetically small compared with my salary at the University of Illinois. The arithmetic, however, takes no account of the inescapable circumstances of North American life such as the rigorous climate or the sheer size of the country, to name but two. To keep warm in the bitter cold of winter and cool in the suffocating heat of summer is costly; the relatively thin spread of the population over this vast continent means heavy expenditures on transportation. Undoubtedly a scientist or engineer, whether he works in the United States or Britain, would like to have, and deserves, a good salary. He does not want to see his family in straitened circumstances. But what constitutes a good salary in either country is not merely a matter of arithmetic and of cost of living indices. It is most easily arrived at by living in each country in turn and is largely conditioned by the individual's idea of the kind of life he and his family want to live.

The Working Group on Migration is quoted in the New York Times of October 11 as stating that "the brain drain is essentially a consequence of a national attitude [in Britain]". This appears to me to be a far more pertinent remark than the pre-occupation with salaries converted from pounds to dollars at the official rate. Too often, as the British scene is viewed from a distance, the conclusion is reached that a man there regards his work as a boring nuisance which is to be got through with the least effort on his part and in any slipshod fashion. Against this, a man's income is sacrosanct and is owed to him by society irrespective of the quality of his work. Some years ago, in a large Glasgow factory, two or three workers were dismissed for slovenly work. All the workers in the factory immediately went on strike: in their minds apparently performance and wages were unconnected. attitude is the antithesis of that of the scientist and, I believe, of the engineer as well. They would not be what they are if they did not regard their occupations as absorbingly interesting in themselves and if they were not striving to do the best job they could. Moreover, they need the help of technicians if they are to operate successfully. If the technicians were to adopt the attitude of the Glasgow factory workers, scientists and engineers would have to look forward to much time spent in a state of frustration.

It is also sometimes said that the brain drain is partly caused by the emphasis in Britain on "hierarchy and status". But this is no more than a minor irritant. The veneration accorded in Britain to membership in the Royal Society certainly looks faintly ridiculous from this side of the Atlantic. Over here, as far as I can judge, it is recognized that scientific genius is not the only passport to entry into academies like the US National Academy of Sciences or the British Royal Society.

Neither salaries nor these British attitudes are insuperable obstacles. Every society, the American included. has its disadvantages, and scientists and engineers are sufficiently intelligent people to realize this. As is reported in The Observer of October 15, it did not take Mr Philip Burnford, of Management Selection International, long to discover, after he had set up his office in New York. that many expatriate scientists and engineers wanted to go back to Britain if they could find out how to do it. But, apart from the question of method, I think that many of them doubt that they are wanted back at all. Most scientists are made to feel that their work has some value when colleagues in other institutions ask them to visit and lecture on the matters on which they are engaged. During the 15 years that I have been here I have often been asked to lecture at other institutions in this continent. I have also been invited to speak more than once at institutions in Germany, France and Portugal, countries that are not markedly richer than Britain. Yet it is only this year that, for the first time, an invitation of this kind has come from Britain. Hitherto, I had supposed that the work I was doing was of no interest to British scientists. An important result of my visits to Britain this year has been that I have been able to see the quite remarkable facilities available in a number of British universities. I am now able to encourage young expatriates to return in a way that was impossible to me before. Those scientists who remain in Britain know what the expatriates are doing because scientists at home presumably read the technical American scientific journals. Graduate associations also keep addresses of expatriates and can say where a man is located. A little money must be spent if expatriates are to be invited to address their colleagues in Britain. But the cost is not as large as might be thought, because the trip across the Atlantic may well be combined

with one financed by the expatriate's US institution, for some other purpose, or because he, in any case, has to return for some family reason. Even an invitation to give a lecture, which cannot be accepted for financial reasons, would serve to encourage an eventual return home. It does at least indicate to the expatriate that he has not been written off as dead.

I have had some indications that expatriates who wished to return have sometimes felt that they had received a somewhat cold reception from the British interviewing authorities in Washington. It is, of course, true that the Joint Interviewing Board, contrary to popular mythology, does not have the reversal of the brain drain as its primary function. Two students of mine returned a few years ago from one of these interviews in a very despondent frame of mind because they thought they had been confronted by a take it or leave it attitude. Their comments were not as caustic and unflattering as those of eight returned brain drainees, now working for Imperial Chemical Industries, which are quoted in an article by Kay Evans in The Observer of October 22. Had there not been an overriding reason which made a return to Britain imperative, my students might well have stayed in the United States or Canada and might have refused the job eventually offered them by the JIB. I believe that efforts are being made to remedy this situation. I have before me an advertisement in an American scientific journal issued by the British recruiting authorities in Washington and Ottawa. It is informative and business-like, but again I miss any sign of enthusiasm for the recovery of the lost sheep. The expatriate must regard nimsen only as a "candidate" for a post at home. He may well ask himself The expatriate must regard himself only as a why he should trouble to do so if the Americans are asking him to stay on with them. The advertisement may well attract a response only from those who have found themselves to be misfits over here.

My proposal to those at home, if they want to reverse the brain drain, is: Show an interest in the expatriate scientist or engineer, tell him that he would be welcomed back and ask him to return! You may well be surprised at the response you will receive. This conclusion I had reached some years ago. I was delighted to see, again in the October 22 article in *The Observer*, that Imperial Chemical Industries had been successfully practising the method for some time.

G. C. MOVITTIE

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Illinois, USA.

Organization at Ministry of Technology

SIR,—Your issue of November 25 reported a speech by Sir Richard Clarke, Permanent Secretary, Ministry of Technology, to a joint meeting of the Royal Institute of Public Administration and the British Institute of Management (Nature, 216, 745; 1967). Sir Richard compared the chances of able administrators, engineers, scientists and other specialists, getting to the top posts in his ministry relatively young, and concluded that the prospects were probably the same on all ladders. This is a seriously misleading statement. The fact is that in the Ministry of Technology able engineers and scientists have markedly inferior prospects to those of administrators of getting to the top. In this respect, this ministry is no different from the rest of the Civil Service.

There are 150 administrators in the Ministry of Technology of whom 22 are on salaries of £5,000 or more, that is 14.7 per cent. There are 1,985 scientific officers of whom 40 are on salaries at this level, that is 2.02 per cent. The prospects for professional engineers are much worse; there are about 1,100 qualified professional engineers of whom only 3 are at this salary level, which is much less than 1 per cent.

For the Civil Service as a whole, the comparative percentages of those on £5,000 or more are: administrators; 15·3 per cent; scientists, 2·3 per cent; professional engineers, 0·2 per cent.

The explanation is simple. At the highest levels in the Civil Service, and in spite of the changing role of government, management and administration are still almost exclusively the monopoly of the administrative class. Although departments differ in size, function and complexity, one thing remains constant: an administrative Permanent Secretary is at the head and he has one or more deputy secretaries, according to the size of the department, and a varying number of under secretaries, all of whom are members of the lay administrative class. Out of 36 posts at Permanent Secretary level, 33 are filled by members of the administrative class. This class is relatively small and consequently the career pattern referred to above is produced. As long as advice to Ministers, policy determination, and the control of the governmental machine are vested in the administrative class, the opportunities of advancement for scientists and engineers to the very top posts will be seriously restricted.

The Jones Committee on the brain drain said that last year Britain suffered a net loss of 1,870 engineers and technologists, aged chiefly between 25 and 35, which is a third of our average annual output, and that the trend was increasing. It forecast disastrous consequences for British industry and the economy within ten to twenty years if it were to continue at the present rate.

The Prime Minister has laid the chief blame on the British industrial system, which, he has said, did not put a proper valuation on such people. The Treasury has overall responsibility for career management in the Civil Service, but, in spite of all that has been said by its political masters about the need to improve both the status and the responsibilities of engineers and scientists, it regards comparisons between administrators on the one hand and professional engineers and scientists on the other as having neither validity nor relevance. Institution has tried repeatedly to persuade the Civil Service to remedy this situation but without avail. Statements and pledges have been made by various Governments from time to time which indicate, in the clearest possible way, the need for major reform. clearest pledge on the question of parity as between the administrative and the professional civil servant was given by Lord Cherwell, speaking in the House of Lords on behalf of the Government, as far back as 1943. He said: "I must frankly admit that the Civil Service has not hitherto shown due regard for the contribution which scientists are making to the nation's welfare. I am glad to be able to say in answer to Lord McGowan's question that this matter has now been reviewed, and that an investigation has been in progress to make sure that the conditions of service, pay and prospects of Government scientific employees compare favourably with those on the administrative side of Government work, so that, among other things, interchange may in suitable cases be made easier. I hope that a definite announcement that these reforms are to be put into effect may be made before long." There is great rigidity in the nineteenth century class "caste" system in the Civil Service, which cannot be modernized to fit purposive government without causing trouble. I leave it to your readers to judge for themselves the extent to which the Civil Service has implemented the long outstanding Government pledge which Î have quoted.

Yours faithfully,

T. H. Profitt Deputy General Secretary.

The Institution of Professional Civil Servants, Northumberland Street, London, WC2. Ţ.,

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 18

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, SW1), at 3.30 p.m.—Meeting on "New Insecticides and Fungicides".

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Cabling for Difficult Industrial Environments", opened by Mr G. L. Leighton, Mr D. Balk, Mr D. J. Mills and Mr T. B. Rolls.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Recent Developments of Inductive Radio Standards", opened by Mr J. J. Hill.

Tuesday, December 19

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2) at 5.30 p.m.—Dr F. E. Jones: "The Management of Men and Money in an Electronics Company".

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Kitchen Car Equipment".

Wednesday, December 20

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, SW1), at 2.15 p.m.—Mr A. P. W. Cane, Mr J. W. Blanchard and Captain F. Ormonroyd: "Instrument Approach Criteria".

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W1), at 5.15 p.m.—Dr T. D. Whittet: "Pepperers, Spicers and Grocers, Forerunners of the Apothecaries".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Mr J. W. Belth: "Selling of Engineering Producta" (Thomas Hawksley Lecture).

Thursday, December 21

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W1), at 4 p.m.—Scientific Papers.

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, surlington House, Piccadilly, London, W1), at 5 p.m.—Professor J. R. ingrose: "Derivations and Automorphisms of Operator Algebras".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

HEAD (physicist with an established reputation of elementary particle physics research) of the Bubble Chamber Research Group, to be responsible both for the research programme of this group and also for the collaboration with all the University Bubble Chamber Groups who use NIMROD—The Director, Rutherford High Energy Laboratory, Chilton, Didcot, Berkshire (December 22),

LECTURERS (2) in the DEPARTMENT OF CHEMICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (December 23).

LECTURER IN DENTAL BIOCHEMISTRY—The Secretary of the University Court. The University, Glasgow (December 29).

DEMONSTRATOR In the DEPARTMENT OF GEOGRAPHY, to demonstrate in map laboratories and to engage in tutorial and field work—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (December 30).

map laboratories and to engage in tutorial and field work—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (December 30).

LECTURER and an ASSISTANT LECTURER IN PHYSICAL CHEMISTRY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W8 (December 30).

LECTURER (with a special interest in the application of quantitative techniques to regionalism, or urban studies or land use) in the DEPARTMENT OF GEOGRAPHY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (December 30).

LECTURER IN ENGINEERING MATHEMATICS—The Registrar, The University, Newcastle upon Tyne (December 31).

SENIOR RESEARCH ASSISTANT (with an honours degree, preferably the chemistry) to assist Professor N. Shappard with the research work of his group in the School of Chemical Sciences, and duties will include work in connexion with research grants and publications, literature and surveys and reviews, and liaison with individual research workers—Dean of the School of Chemical Sciences, University of East Anglia, University Plain, Norwich, NOR 85C (January 1).

PROFESSOR OF ZOOLOGY in the DEPARTMENT OF BIOLOGICAL SCIENCES, University of the West Indies, Trinidad—The Inter-University Council, 33 Bedford Place, London, WCI (January 4).

HEAD OF THE DEPARTMENT OF PHARMOV—The Principal, College of Technology, Belfast, Northern Ireland (January 5).

LECTURER IN ENGINEERING MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (January 5).

PROFESSOR OF BIOCHEMISTRY in the FAULLTY OF MEDICINE, University of the West Indies, Jamaica—The Inter-University Council, 33 Bedford Place, London, WCI (January 10).

LECTURER (SENIOR LECTURER in PHYSICS at the University of Queensland, Australia—The Association of Commonwealth University of Queensland, Australia—The Association of Commonwealth University of Sydney, Australia, Chair of Chemical Engineering at the University of Sydney, Australia

CHAIR OF CHEMICAL ENGINEERING at the University of Sydney, Australia
—The Secretary-General, Association of Commonwealth Universities (Branch
Office), Marlborough House, Pall Mall, London, SW1 (Australia and London,

fanuary 31).
OFFICIAL FELLOW OF LECTURER IN ORGANIC CHEMISTRY—The Secretary,

Solliof College, Oxford (February 1).
UNIVERSITY NUCLEAR PHYSICS RESEARCH FELLOWS (with a Ph.D. and a pecial aptitude for research in high or low energy nuclear physics)—The legistrar, The University, Liverpool, quoting Ref. RV/286/N (March 1).

Animal Technician for the College Animal House—Mr L. Foster, Chelsea College of Science and Technology, Animal House, Manresa Road, London,

ASSISTANT CYTOLOGIST (science graduate, preferably with experience in human chromosome cytology and cell culture) in the HUMAN CYTOGENETIOS LABORATORY—The Group Medical Superintendent, Royal Hospital for Sick Children, Court

human chromosome cytology and cell culture) in the HUMAN CYTOGENETICS LABORATORY—The Group Medical Superintendent, Royal Hospital for Sick Children, Glasgow, C4.

ASSISTANT EXPERIMENTAL OFFICER (with qualifications in physics or metallurgy or electrical engineering) for the ELECTRON MICROSCOPE LABORATORY—Head of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, SW7.

POSTDOOTORAL BIOCHEMIST (preferably with experience in protein and nucleic acid biochemistry) in the DEPARTMENT OF IMMUNOLOGY to investigate mechanisms of cellular hypersensitivity—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W2.

POSTDOOTORAL RESEARCH SCIENTIST to Join a team engaged in applying biophysical techniques to problems of developmental biology; the research will involve studies of interaction phenomena between macromolecules and tissues, and of factors concerned in the control of macromolecular synthesis—The Director, Strangeways Research Laboratory, Cambridge.

RESEARCH ASSISTANT (graduate biochemist, preferably with experience in hormonal control of mineral metabolism) at the Nuffield Institute of Comparative Medicine, to undertake metabolic studies in relation to bone diseases in primates—The Establishments Officer, Zoological Society of London, Regent's Park, London, NW1.

RESEARCH ASSISTANT (with a degree in biochemistry or physiology) for work in zonal centrifugation of kidney cell fractions—Dr D. Robinson, Queen Elizabeth College (University of London), Campden Hill Road, London, W8.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 777, Vol. 252 (14 September, 1967): A Discussion on the Terrestrial Antarctic Ecosystem. Organized by J. E. Smith, F.R.S. Pp. 167-392. (London: Royal Society, 1967.) 80s.; \$13.50. [149 At the Farmer's Service, 1967/68: a Handy Reference to Various Services available to Farmers in England and Wales. Pp. 119. (Pinner, Middlesex: Ministry of Agriculture, Fisheries and Food, 1967.) [159 The Edinburgh School of Agriculture. Experimental Work, 1966.) Pp. viii+114. (Edinburgh: The Edinburgh School of Agriculture, 1967.) 7s. 6d. [159 Careers in Chemical Engineering Processing Pr

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Ministry of Labour. Prevention of Explosions of Water Heating Systems in Launderettes. Pp. 5. (London: Ministry of Labour, 1967.) [219]

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Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences. No. 778, Vol. 252 (21 September, 1967): An Environmental Study of the Upper Domerian and Lower Toarcian in Great Britain. By A. Hallam. Pp. 393-445+plate 20. 21s.; \$3.15. No. 779, Vol. 252 (22 September, 1967): The Regional Anatomy of the Human Intergment with special reference to the distribution of Hair Follicles, Sweat Glands and Melanocytes. By G. Szab. Pp. 447-485+plates 21-26. 21s., \$3.15. (London: The European Economic Community. By Geoffrey Denton. (European Series, No. 5.) Pp. 54. (London: Political and Economic Polity Programme of the European Economic Community. By Geoffrey Denton. (European Series, No. 5.) Pp. 54. (London: Political and Economic Planning, 1967.) 7s. 6d.

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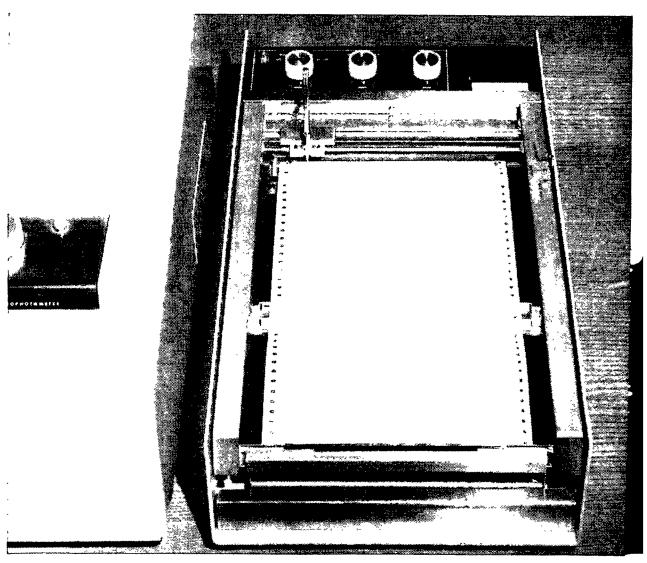
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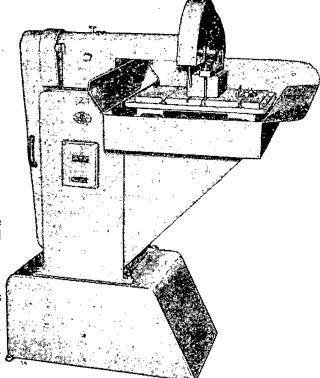
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NATURE Volume 216 DECEMBER 23, 1967

Put out no Flags

GREAT aircraft have something in common with great ships, and some of this was clearly apparent last week on the occasion when the first prototype of the Concorde aircraft emerged for the first time from its hangar at There were flags, military bands and speeches, some pompous and some silly. For a time, at least, even those who are accustomed to ask whether aircraft like the Concorde can ever be commercially successful may have found themselves catching their breath with the majesty of everything. Will that aircraft really be the first commercial supersonic transport? Will it earn valuable foreign exchange in huge amounts for two European countries much in need of that commodity? Or, even if it turns out to be a commercial flop, will it somehow earn reputation instead? A tear-jerking roll-out, the anti-climactic aeronautical equivalent of the launching of a ship, is hardly the time to be asking questions like these in anything but a respectful tone of voice. But that brave day is now well past, and it also happens that there are now urgent reasons why the British Government for one should make a careful appraisal of what the Concorde project is likely to achieve. Not even the most devout aircraft modeller should be surprised if there has recently been another flood of rumours that the British Government may now, at last, withdraw from a project which it has regarded with distaste ever since 1964. As it happens, however, the set of questions which the first appearance of the Concorde has provoked are a small part of the general problem of how intermediate nations such as Britain should somehow seek to manage their technology.

For intermediate nations, the case of the Concorde is an object lesson in the difficulty of knowing just what an intermediate nation should do with its technical resources. For one thing, the project is a great technical adventure which has done much to stimulate industry in Britain and France and which may yet win modest profits as well. But the project is also a great gamble. Anything may go wrong between now and 1971, when the first aircraft are due to enter service with the airlines. The steady erosion of performance which has marked the past year's experience may, for example, continue and even accelerate. Already the fully laden weight has increased from 250,000 pounds to 376,000 pounds, while the number

of passengers and the cruising speed have been marginally reduced. Given that the aircraft will not leave the runway at Toulouse for another six months, is it any wonder that people are asking what kind of a performance will be left by 1971? The fact that the Boeing Company seems also to be having difficulties in the design in the United States of the supersonic transport due to enter service in 1975 may be a comfort to those directly engaged in the Concorde project, but it serves only to emphasize to other people the hazards of this novel technological field. On technical grounds, the Concorde must be something of a gamble.

Even if the Concorde is an outstanding success as a flying machine, however, there can be no assurance that it will prove to be commercially successful. Indeed, it seems to be quite openly acknowledged that the cost of research and development will not be recovered by the sales of completed aircraft. So far, the British share of the development programme has worked out at less than 40 per cent of the £250 million contracted for under the agreement with the French Government. But the number of aircraft on order is still less than eighty, while even the most optimistic of the Concorde salesmen are not at this stage allowing themselves to dream of selling more than 200 aircraft. Evidently there is not much scope for recovering initial costs of between £4 million and £8 million an aircraft without pricing the Concorde right out of the market in which it makes most sense—the busy long haul routes such as those across the Atlantic. This is why a good deal of argument in favour of the Concorde, in Britain at least, now centres on the view that even if the financing of Concorde is enough to make accountants' hair stand on end, the aircraft (if successful) would bring in lots of foreign exchange. Specifically, and from the point of view of Britain alone, the expenditure of £250 million on research and development may—with luck enable the aircraft industry to earn up to £800 million or so in foreign exchange spread over the decade of the seventies. To begin with, it is only proper to acknowledge that this is tantamount to a form of devaluation—goods are being sold abroad at less than their true cost. More important, the potential benefits seem hardly worth the expenditure. Where else could governments afford to pay export subsidies of 30 per cent or so to particular industries? And are there any circumstances in which such a course would be considered when the chances of winning a reasonable return on the funds laid out are as hard to calculate as

The article on SI units advertised last week will now appear on Dec. 30.

with Concorde? In reality, of course, there are many safer and more profitable ways in which public expenditure could bring in foreign currency in large amounts.

In these circumstances, it is not surprising that the British Government is continually looking quizzically at the Concorde project. And it is natural that these inclinations should be particularly noticeable at times of economic stringency, when the hunt is on for almost any way of reducing the scale of public expenditure. In the present climate, it is extremely hard to see how the Concorde can survive for long. There is no need to say hard things about the aircraft, or about the difficulties of knowing how it could be made to fly over populated territories without causing an intolerable nuisance, to know that the game is hardly likely to be worth the candle. No doubt it will be sensible to wait a few months, until there is some experience of how the aircraft behaves in flight but before the heavy costs of an extensive testing programme have begun to mount up. By the summer of 1968, however, it would be entirely sensible if the British Government had found some graceful way out of what it has considered for three years to be an uncovenanted albatross around its neck. By that time, no doubt, there will be so many cancelled projects of one kind or another that the mere cancellation of Concorde may be a comparatively unspectacular affair.

But if, in the months ahead, it comes to seem comparatively easy for the British Government to withdraw from the building of the Concorde, there will remain the long-standing problem of how to avoid similar entanglements in the future. The international character of the venture is not by itself discouraging, for it has yet to be demonstrated that the costs of Anglo-French collaboration have been greater than would have been the costs of strictly national programmes for the development of supersonic aircraft. The real trouble is that projects like Concorde are at once too speculative and too inflexible for nations such as Britain prudently to undertake. So much is clear from the way in which the Anglo-French consortium has had to put all its efforts into a Mach 2 aircraft, and in the process deny itself the chance of rewards if the American choice of Mach 3 or thereabouts turns out to be more fortunate. In other words, the collaboration on Concorde is a gamble in which the participants are denied an opportunity to hedge their bets. But the gamble also has too much the sense of being an all-or-nothing choice. There is very little profit in stopping half-way.

These awkward circumstances should be a clear indication of the directions in which intermediate nations should seek to edge their policies on technology. Quite apart from calculations of the profitability or otherwise of particular ventures, there are also important strategic advantages to be had from development projects which are flexible in scale and which are also so well within the compass of the nation concerned that it can reasonably hope to spread some of the risks which inevitably attend advanced development of any kind. By this test, there is clearly much that is dubious about the British Government's hope to see the emergence of a British computer industry able to service the whole of Europe in competition with companies from the United States and from France. That, too, is a gamble, though by now so much money and prestige has been committed to it that it would be a pity to see it fail. The development of hovercraft is similarly a case in which too many eggs may rest in the same basket. This is the kind of project in which it would have been more sensible to develop some other form of fast sea propulsion in parallel with hovercraft. All these, however, are projects in which everything hangs on the problematical outcome of a long programme of research and development. cleverly arrangements may be made to see that all likely successes are well supported, rewards do not come until a long programme of work has been completed. It would be much safer, and in the long run more profitable, if more could be done to support ventures in which even small expenditures could bring rewards. This is why there is a case for asking that governments in intermediate nations should be more assiduous in the encouragement of comparatively humdrum activities such as the improvement of electrical generating machinery, and less anxious to keep up with more powerful competitors in the development of the most radical innovations. It is true that there is always a chance that these projects may become enormously rewarding, but this is always something of a gamble.

More Ructions about the Library

THE members of the House of Lords are fond of saying that, even if they have long since lost most of their pretence to be political powers in the land, they are at least the best debating club in Britain. Unfortunately, the debate on December 13 about the issue of the library at the British Museum was almost by itself a proof that even this conceit is unjustified. The debate was sadly one-sided and unconstructive. It provided an opportunity for several of the trustees of the British Museum to let off the head of steam which has been building up in the weeks since the Secretary of State for Education and Science, Mr Patrick Gordon Walker, casually put an end to the plans which the trustees

have been nourishing all these years, but that is not the kind of debating society which the House of Lords would like to be. To be sure, it is asking almost too much of flesh and blood to hope that Lord Radcliffe, the chairman of the trustees, would fail to take this opportunity to give the Government a verbal trouncing, but was it really sensible of the other trustees to spend so much time on expressions of defiance and, comparatively, so little on asking what should happen now? It may even have been a mistake to let it be so widely known that the trustees as a whole will probably resign if the British Government seeks to separate the library from the British Museum and then to use it as

the nucleus for a national library away from Bloomsbury. Certainly it was a mistake for Lord Eccles, once a Minister of Education, to make the absurd declaration that "Under the existing British Museum Act, no minister has any power to move a single volume from Bloomsbury to Bayswater, or from either place to some other place". New legislation is easier to come by than are good ideas.

In the event, of course, everything will depend on what the committee under Dr F. S. Dainton recommends, and one of the useful products of the debate in the House of Lords was the announcement by the Government of the four members of the committee who will share the unenviable task of deciding what should happen now. They are all distinguished men, although none of them is a librarian. Their biggest difficulty in the six months or so ahead will be somehow to pick out solid ground among the mass of contradictory opinions which must surely assail them. The trustees of the museum would be well advised if they could somehow bury their chagrin at how the Government has dealt with them, for, if they fail to present a rational case for the preservation of their own version of the status quo, the chances are that the decision will go in favour of a separation between the museum and the library. Indeed, whatever strength there may be in the case the trustees have hiding up their sleeves, the advantages of a properly unified library will outweigh the advantages of a combined museum and library. In other words, there is a danger that the trustees' bluster at the way in which the Government has behaved itself will very soon seem to be bluster at the awkward implications of logic.

Small Cheer for Non-proliferators

It is something of a puzzle to know whether to be cheerful or otherwise about the results of the long session of the United Nations Committee on Disarmament which adjourned its meetings in Geneva last week for the Christmas holidays. For the past few weeks the chief participants at Geneva—the United States and the Soviet Union—have been suggesting that agreement on a non-proliferation treaty might have been close at hand. But it seemed to have become plain, by the end of the session, that the more cheerful predictions were based on the assumption, or perhaps the hope, that serious problems would somehow vanish. Yet there seems no real prospect of a rapid solution to the problem which dominated the last few meetings of the committee—the issue of whether the Euratom countries should be allowed to substitute to some degree inspection of nuclear installations by Euratom itself for the inspection by the International Atomic Energy Agency that will apply to everybody else. France is not, of course, represented on the committee, but it seems that the Federal Republic of Germany has not been the only sponsor of the view that Euratom inspection should be allowable. The Italian delegation at Geneva seems also to have been working for this -objective.

Perhaps the most alarming feature of this development is that the European advocacy of Euratom

inspection has come as such a surprise to the nuclear powers. It is now roughly a year since the argument was first raised outside the meetings of the committee on disarmament. The fact that Euratom itself seems to be in a sickly condition as a means of prosecuting research and development in nuclear energy is neither here nor there—the Euratom safeguards system is still a going concern. But the Soviet Union is unlikely to be able to agree to much less than full-blooded inspection by the International Atomic Energy Agency in Vienna. Evidently the British and American governments were hoping to take some of the edge off these proposals by announcing that civil nuclear plants in Britain and the United States would be subjected to inspection by the international agency even though nuclear powers would not formally be subjected to inspection of any kind under the terms of the treaty which have so far been negotiated. The trouble, of course, is that even such a gesture of voluntary submission may cut both ways. Although nations in Europe concerned about the supposed potential loss of commercial secrets might benefit from knowing that the United States and Britain would run similar risks, the Soviet Union is likely only to be embarrassed by this precedent, and there is no suggestion that France would be prepared to follow suit. In all these circumstances, it would not be entirely surprising if the next meeting of the disarmament subcommittee, provisionally set for January 19, 1968, is eventually postponed.

Less for United States Science

THE war in Vietnam and the need for economic restraint at home have combined to squeeze United States spending on research and development quite drastically. Although spending in other directions has also been cut, research and development seem to have been hardest hit. In spite of a request for \$5,100 million for NASA, for example, Congress approved only \$4,588 million, the deepest cut it has ever made in the United States space effort. And the Department of Defense, although it emerges with a total budget at about the same level as last year, has been told to cut its support for basic research from \$399 million to \$361 million, more than 10 per cent. This was part of a much more drastic cut demanded for the whole Defense Department—it was asked to cut some \$2,000 million from its total budget, but only in activities unconnected with Vietnam.

Other agencies were treated more gently. Atomic Energy Commission got an allocation of \$2,509 million, a 14 per cent increase over last year, although less than the original budget request. High energy physics research showed a \$10 million increase, and support for controlled thermonuclear fusion research went up from \$22.6 to \$26.2 million. Reactor development was increased by about 10 per cent, to \$507 million, and the Plowshare programme, which seeks to devise peaceful uses for nuclear weapons, went up from \$13.3 million to \$20.7 million. In other departments, the rule of thumb seems to have been to cut down programmes to a size midway between the actual figures for last year and the Administration's suggestions for this. Exceptions were the individual institutes within the National Institutes of Health, which each got exactly what had been requested. The

National Bureau of Standards, with \$32.5 million, is marginally worse off than last year. The National Science Foundation, on the other hand, has done rather better this year than last. Its budget will be \$495 million, an increase of \$15 million over last year. Elementary and secondary education shows an increase of over \$200 million, to \$1,667 million, but higher education has been cut by \$20 million, to \$1,158 million.

In many cases, further cuts are threatened by a plan recently announced by the Administration. intended to force agencies to cut their expenditure below the levels proposed to Congress by the President. Under the formula, some programmes are to be cut by 10 per cent of programme costs and 2 per cent of payroll costs. The idea behind the reductions was to try to persuade the House Ways and Means Committee to look more kindly at the Administration proposal for a 10 per cent tax increase. The committee has so far remained unconvinced, preferring to cut public expenditure rather than raise taxes. Much will depend on whether the projected budget deficit for this year is greater than that for last year or not. In some cases the cuts imposed by the Administration will be less severe because of the reductions imposed by Congress, but the AEC, for one, seems likely to be a sufferer if the plan goes ahead.

The effects of these cuts will not be fully appreciated in the universities until the spring, when arrangements for the academic year beginning in September 1968 will have to be made. Administrators seem to be most uncomfortable about the possibility that smaller research budgets will make it much more difficult to retain new graduates as assistants, who shoulder collectively a good deal of the teaching burden at a great many universities. But there is also talk of how laboratories dependent on the mission-oriented agencies for finance may have drastically to alter plans for the years ahead. In the circumstances, it will not be much of a surprise if many organizations find themselves running for cover at the National Science Foundation.

British Computers

For some months, the leading British computer companies, International Computers and Tabulators and English Electric/Elliott Automation, have been coyly denying rumours of a liaison. But now the secret is out—at the annual general meeting of ICT on December 13, the company chairman, Colonel A. T. Maxwell, admitted that discussions were in progress. The matchmaker has been the Ministry of Technology, which has never hidden the fact that it is interested in a rationalization of the British computer industry. So far, the Ministry says, only ICT, English Electric and itself are talking, but it is probable that other British companies are taking more than a passing interest. The talks, which have been in progress for a few months, are likely to be concluded within "months, rather than weeks".

The argument used in favour of the merger, if it comes about, will be that the UK market is too small to support two companies competing across the board. Too much duplication of research and production, it is said, is more than Britain can afford in a market still dominated by United States companies. As well as the production of central processors, both firms make a wide range of peripheral devices. Although ICT estim-

ates that the British market is going to show a growth rate of around 15 per cent for the next few years, American competition, chiefly in the form of IBM, is likely to swallow up about half of that. ICT says that it has one-third of the British market, the same proportion as that taken by IBM. If this sounds like drastic American penetration of the British market, it is worth remembering that IBM has something between 70 and 80 per cent of the world market; judged on this scale, the performance of the British companies in recent years has been quite respectable.

English Electric, although it is still making a few Leo and KD machines, is concentrating now on its System 4 range. There are several models in this ambitious range, the first to use wholly integrated circuitry, and they range from the 430, which costs about £100,000, up to the 475, a multi-access computer costing around the £1 million mark. English Electric says that it has had orders for the range worth £25 million. The plans for a big computer, originally proposed by Elliott Automation before it was taken over by English Electric, now seem to be in the melting pot, and a joint planning group has been set up to The integration of decide what should be done. English Electric and Elliott Automation is now going ahead quite quickly, but there is still work to be done, including most probably the unravelling of an arrangement negotiated by Elliott's with National Cash Register.

ICT also makes a full range of computers, less ambitious technically than the System 4, but successful commercially. At the annual general meeting last week, Colonel Maxwell was able to announce cheerful increases in profits, up from £2.22 to £2.87 million.

ICT, then, is in better shape than before, while English Electric is still struggling. No amount of confident talk can conceal the fact that substantial production difficulties have arisen with System 4. În the circumstances, ICT sometimes looks as if it is waiting for English Electric's position to get weaker still before it steps in with a firm merger offer. This may be the reason why Colonel Maxwell assured his shareholders that ICT would be the largest single component in a combined company. Quite clearly neither company is as keen on merger as the Government, and it is probable that some "lubrication", in the form of cash from the Industrial Reorganization Corporation, will be needed before terms are finally agreed. But the fact that the two companies market directly competitive ranges means that no easy merger is likely—in this case there can be no talk of the two ranges being complementary. The chances are that one would have to go.

Proton Accelerator Delayed

The meeting of the council of Cern held at Geneva a week ago served principally to confirm what has been clear for some weeks now—that the 13 members of Cern are not yet able to commit themselves to the project for building a 300 GeV proton accelerator. That said, it does appear that the tone of the meeting was cheerful and even constructive, so that even though the deadline originally set for a decision about the machine has now been passed, the hiatus in the planning is more accurately described as a postponement than an abandonment.

Several developments have helped to interrupt the smooth attainment of the timetable spelled out a year ago by the council of Cern. For one thing, there has been the devaluation of sterling, which explains why the United Kingdom and Spain were not able, at the council meeting last week, even to approve the budgets for 1968—197.5 million Swiss francs for the basic costs of the Meyrin laboratory and 28.43 million Swiss francs for the programme connected with the Intersecting Storage Rings. The British Government is particularly concerned with the international consequences of devaluation, for something like £5 million a year out of the annual budget of £33 million of the Science Research Council is at present allocated to overseas expenditure. The question which has to be settled before it is possible for the Science Research Council to participate even in the ordinary activities of Cern is whether such a large and uncovenanted increase of real expenditure is possible without harming the rest of the council's work.

The 300 GeV project seems also to have been held up by doubts in West Germany about the desirability of the particular design worked out by the European Committee for Future Accelerators and published in Especially since details of the design of the United States 200 GeV accelerator at Weston, Illinois, have been made known in the past few months, there have been some suggestions that Europe might hope to obtain better value for money by building a somewhat simpler machine. The meeting of the Cern council last week seems to have reaffirmed the earlier view that a European machine should be generously provided with experimental facilities of all kinds, but at the same time it seems to have been acknowledged that the studies of the 300 GeV problem will be continued at Cern, so that the design already published must be regarded more as a feasibility study than as the detailed prospectus which it seemed to be a few months ago. To this end, the council last week did approve an allocation of \$4 million for the continuing programme of planning and design. Although it is theoretically possible that a final decision on the accelerator could be taken at any one of the four council meetings due to be held in the year ahead, the optimists who hope that everything will be settled by June are likely to be disappointed.

More about Stansted

London's third airport was discussed at Westminster again last week, this time in the Lords. Lord Macpherson of Drumochter initiated the debate, the first in the House of Lords on the subject since the White Paper was published in May with the decision to build at Stansted. In the eight hour debate only five of the thirty-one speakers supported the government's decision. The feeling among the rest was not that Stansted was necessarily the wrong site for the airport, but that public opinion had not been satisfied that the choice was the best. The facts behind the present situation were put forward—the Interdepartmental Committee report of 1963, calling for an inquiry; the inquiry inspector calling for a further review of the problem; the government review held in private coming out in favour of Stansted. Doubt about the decision to build arose because the final review was held in private, particularly as the reasons for this privacy were inconsistent. The White Paper stated

that the urgency of the problem precluded a public inquiry, which anyway would produce no additional facts. In the debate, however, Lord Kennet said, for the government, that the proper discussion of defence issues could not be carried out in public. Viscount Dilhorne submitted that because a considerable number of new facts had come to light since the original inquiry, it should be re-opened in public, in the manner recommended by the Franks Committee. Only in this way would "broad justice to those affected" be done and be seen to be done.

The White Paper suggested that a second pair of runways would be added to the two originally planned for Stansted. This proposal was not considered at the original inquiry, and would significantly alter effects on the area. Silverstone received a passing mention as an alternative, but most attention was focused on Foulness. Included in the cost of an airport at Foulness was £25 million (a much questioned figure) for moving the Shoeburyness firing range. Beswick, putting the government's case, admitted he had "never treated this one very seriously". His main objection to the building of an airport on land reclaimed from the sea was based on the length of time it would take, quite apart from the time involved in holding a public inquiry. Lord Beswick believed the loss of food production from building an airport on good Stansted land, estimated at £1 million a year by Lord Butler, would be far outweighed by the foreign currency gained from a third airport built in the early seventies. If there was no such airport by 1977, on British Airports Authority estimates, nearly 3 million overseas visitors would have to be turned away, losing Britain an estimated £36 million in foreign currency from landing fees, spending money and airline earnings.

Lord Plowden described the White Paper as "full of statements presented as facts which the public are expected to accept". He remarked on the paucity of information on cost-benefit analysis. The social costs involved might be so heavy that the country could well afford the time to investigate the situation again. The theory that Whitehall is always right was questioned by Lord Balfour, who quoted as an example the speech he himself made as Minister of Aviation in 1939, which was full of miscalculation about airports.

The belief of the Lords was that there is still time to reconsider the Stansted decision. The most important product of the whole debate was the announcement by Lord Kennet that the government is intending to improve the planning laws for projects on the scale of airports. Under the present system planning permission is sought for one specific site only, but the proposed Town and Country Planning Bill would allow alternative sites to be listed in the request for planning permission. An inquiry would then determine which of the alternatives was most suitable before the present procedure is gone through.

Changing Units

A COMMITTEE appointed by the Ministry of Technology to investigate the adoption of metric units has reported that, unless steps are taken now, an important sector of the economy may be slow to accept the new units. The committee, formed by the CBI at the request of the Standing Joint Committee on Metrication, a

Ministry of Technology body, has investigated preparations being made in the non-industrial sector of the economy, which includes bottling and packaging, the retail trade and transport. Because this sector is not the direct responsibility of any government department, the CBI has concluded that the change to SI units is not being properly organized. The CBI is alarmed that the advantages to industry of the change to the new units will be wasted because the packaging industry, and the retail outlets, will still be using Imperial units.

In fact the change to SI units is happening more rapidly than most people suspect. As well as the encouragement given to the scientific journals by the Royal Society (Nature, last week), the Department of Education and Science and the Confederation of British Industry are well aware of the implications of the change. Abroad, the change is going even faster—by 1975, SI enthusiasts say, only the United States and Canada will be using Imperial units, and they can be expected to change rapidly as soon as they begin to feel isolated. In Britain, the timetable for the change was set out in a parliamentary answer by Mr Douglas Jay when he was President of the Board of Trade. He said that by 1975 the majority of British industry should have moved over to SI units. Unfortunately, he said nothing about the non-industrial sector of the economy, and no government department has taken it under its wing. For this reason, the CBI has been driven to suggest the establishment of a Metrication Board, rather similar in structure to the Decimal Currency Board. The board would have as its responsibility the co-ordination of metrication in the various parts of the economy which have so far been lagging. For one thing, the SI system proposes a standardized set of package sizes, but the packaging industry is not yet planning effectively for the change. another, all the road signs in the British Isles will need to be changed from miles per hour into kilometres per hour. Some observers, alarmed at the slow progress, are inclined to point out that when Sweden changed from the left to the right side of the road it was after four years of the most intensive planning. Similar planning, they suggest, will be needed for the successful adoption of the metric system.

The proposed Metrication Board, the CBI says, should not be a part-time body. What is needed is a body representing both government departments and the interests concerned, with a full-time chairman and secretariat. And, if the progress towards metrication is not to be slowed down, the board should be set up as a matter of urgency.

Instruments for Technology

THE Scientific Instrument Research Association (SIRA) spends the £0.5 million it receives each year from its members and from the Ministry of Technology on trying to promote measurement and control technology in science and industry. But the association suffers because most of the industrial companies working with anything like advanced technology are able to look after their own measurement and control systems. The SIRA laboratories are on the whole left with the small fry—the craft industries. For them SIRA does a good job. It has, for example, taught British biscuit manufacturers how to measure the water content of

biscuits by measuring colour. It has also devised ways of testing coin blanks before minting and has used a laser for micro-machining.

Given the dominance of the German and Japanese optical industries, it is perhaps surprising that one of the outstanding achievements of SIRA has been the development of two very sophisticated machines for testing lens performance. This work began 9 years ago with a contract from the then Ministry of Aviation, but now receives international sponsorship. One of the machines, now commercially available and slowly finding a world market, measures the optical transform function of a lens so that the quality of the image can be specified objectively. The other, a pupil scanning lens tester, measures the extent to which the wave front emerging from a lens deviates from being spherical. A computer attachment can then calculate the various aberrations present in the lens.

SIRA hopes that this work will eventually lead to a much needed international agreement to specify lens performance objectively, in terms of optical transform functions.

No Buns for the Elephants

THE decision of the council of the Zoological Society to ban all feeding by visitors at London Zoo and Whipsnade Park from the beginning of 1968 is the final stage of the policy adopted in 1965 in the interests of the health and general welfare of the animals. The policy of no feeding has been introduced gradually as new animal houses have been opened, and now the ban is to apply to the remaining animals, the most important of which are the monkeys and apes, some of the bears, the elephants at London and the herd animals at Whipsnade.

Visitors to the zoos will find with their guide books and pamphlets a leaflet telling them not to feed the animals and explaining why the ban is considered necessary. The two zoos have veterinary and supplies officers who know the nutritive requirements of the animals and who try to provide them with a balanced diet. When the visitors feed the animals, unbalanced diets are unavoidable, and these are not good for the animals, who may do themselves harm, sometimes serious, by over-eating or eating proffered food which is quite unsuitable for them. There may also be accidents, not always as serious as the death of Dicksie the elephant this year, when she fell into the ditch around the enclosure while stretching out for food.

The visitors too will be safer when the ban is enforced; during one year at London 180 people have been bitten by monkeys, parrots, cockatoos, ponies and other animals. During the same year elephants at London seized fourteen coats, twelve handbags, ten cameras, eight gloves and six return tickets to Leicester, damaging them all beyond repair.

The council of the Zoological Society also says that the control of feeding in zoos is necessary to create conditions in which the animals may breed and rare species may be conserved. Some animals are now so rare that they are only available from surpluses bred in other zoos. These include the orang-utan, the European bison and Père David's deer. The last two are now extinct in the wild, and the Zoo is determined that unbalanced feeding should not be allowed to make them extinct in zoos as well.

This policy brings London and Whipsnade into line with almost all continental zoos, which have already banned feeding by visitors. Chester and Edinburgh also enforce the ban, and Bristol will be doing so at the beginning of next year. Zoos which have already adopted the policy have found that animals are much fitter and that the volume of deposited litter has decreased considerably. The problem of educating the public to resist throwing buns, nuts and other food to the animals is considerable. As well as the leaflets there will be more "no feeding" notices, and, although nobody will be turned out of the zoo for feeding the animals, offenders will be asked to stop by watchful keepers. Nevertheless, London Zoo expects it to be three years before its annual total of two million visitors are all persuaded to observe the ban.

Inexpensive Technology

A British group set up to promote technological development in backward parts of the world has just published an unusual report. Instead of familiar and extravagant declarations of what could be done by setting up large nuclear power stations linked to desalination plants, or by harvesting the sea, the report concentrates on much more mundane pieces of equip-The group is the Intermediate Technology Development Group Ltd, and the report—Tools for Progress—lists agricultural and industrial equipment which can be bought for modest amounts, within the budgets of developing countries. The director of the group, Dr E. F. Schumacher, says in the introduction to the report that "It is easy to think of the progress that would be possible if the majority of the world's peasants had the means of making full use of modern science, and the necessary knowledge and method to boot. But this means solving the problem by assuming it away. The crucial question is rather how to help the majority to help themselves.'

The group has set out to do this by making people aware that cheap methods, tools and equipment do exist, and that these can be made available where they are needed. Tools for Progress is the result of a survey made of British manufacturers, and includes short descriptions and pictures of most of the tools which fall into the low cost category. The guide also lists the manufacturing agents in developing countries, and British organizations concerned with development problems. Three thousand copies of the booklet are to be distributed to banks, co-operatives, government officials and the like in developing countries, and further copies can be had for 15s. each.

The guide is a cheerful mixture of products, some of them looking rather old-fashioned. But all look purposeful and sturdy, and where prices are quoted (it is a pity they are not given throughout) they seem reasonable. It is possible, for example, to buy a windmill pump capable of pumping 1,130 gallons per hour for £120, from H. J. Godwin Ltd. A Kentish foot loom can be bought from Douglas Andrew Ltd for £80. The group plans to produce revised editions of the guide periodically, and would welcome helpful suggestions.

Investigating the Earth

THE International Upper Mantle Project, begun in 1963 to co-ordinate international research into the

geophysics of the region just below the Earth's crust, is now well under way. Among the techniques used have been the measurement of heat in deep bore holes, aeromagnetics, and the measurement of gravity from orbiting satellites, the last of these particularly requiring the co-operation of many countries. Large numbers of research workers have converged, naturally enough, on the East African rift system and the Mid-Atlantic Ridge, but geophysical understanding of great areas of the world has already been increased. The interim report on the Canadian effort, for example, is able to announce that: "Canadian scientists can begin to talk knowledgeably of the geological and geophysical properties of the entire Canadian crust".

It would, perhaps, be invidious to mention any individual piece of research completed so far, but as far as Britain is concerned, there are a number of projects, either approved or under consideration, which promise to be of great interest. One of these is the planned aeromagnetic survey of the rift valley in the Red Sea and Ethiopia. This is a region peculiarly unsuited to ground observation but in which there may well be magnetic anomalies similar to those discovered in the Mid-Atlantic Ridge, where it was found that the magnetic irregularities were mirror-images of each other on either side of the ridge. The Natural Environment Research Council has given a grant to support this work. There is also a plan, not yet approved by NERC, to take measurements across the rift valley further south over a period of years, and so detect earth movements, a technique which has provided encouraging results in Iceland. experiment on the rift would be carried out by associate teams from some British universities, including Birmingham and Durham. This plan is to investigate the geology of Lake Victoria by analysing the seismic effects of explosives dropped into the lake.

National Parks

THE National Parks Commission has in the past few months produced reports on the Brecon Beacons and the coasts of Hampshire and the Isle of Wight. Both documents are concerned with conservation and each is one of a series on similar subjects. The detailed guide to the Brecon Beacons is the fifth in a series of ten on the National Parks, while the report of the Regional Coastal Conference on Hampshire and Isle of Wight Coasts is the second of a series on coastal preservation and development. National Park guides are designed for the visitor, to enable him to understand and appreciate the countryside better. Coastal conferences, on the other hand, are designed to produce a comprehensive survey of the whole coastline so that a co-ordinated policy can be developed.

The National Park in the Brecon Beacons, with more sheep than people in the upland regions, is unindustrialized and largely unspoilt. The authors of the guide hope that the visitors who come will keep it that way, but endeavour to increase appreciation of the area with this detailed description of things to see, whether living or historical, and things to do, in the way of pony trekking or caving. The Palaeozoic rocks of the area are described; many of the 200 kinds of birds and their localities are listed, as are animals, insects and flora. Scheduled ancient monuments, places of interest and Welsh names in the park are

also given. The guide is an interesting and comprehensive account of the area.

The coastal conferences were designed with a more definite aim in view. Studies of coastal areas by the local planning authorities were first called for in 1963 by the Ministry of Housing and Local Government. Development plans were to be considered in relation to conservation of natural attractions, places and nature of amenities for holidaymakers and areas of special scientific interest. The ministry asked the National Parks Commission to arrange conferences to coordinate the plans of the various local authorities. In the meantime, additional studies are in motion, investigating aspects of the problem of coastal development in terms of social, economic and recreational factors. Places of scientific interest are being identified and classified by the Nature Conservancy. The conference on Hampshire and the Isle of Wight, which was held in Southampton in June 1966, began with a reminder that industrial requirements must be considered alongside any developments for holidaymakers. The conference proceedings form the first part of the report, the rest being given to written reports from planning officers in the area, statistics of land use of the 133 miles of coastline, and the movement of retired people to the area, with accompanying social problems. A map summarizes the development and protected areas along this section of the coast, with figures for visitors in main resorts. Coastal eyesores such as wartime remains, rubbish tips and abandoned vessels have also been listed. These are encouraging steps in coastal planning and improvement. While waiting for the results of all the conferences, the ministry has called for a "holding operation" so that the coasts are protected until a definite policy has been worked out.

Research in Education

THE twenty-first annual report of the National Foundation for Educational Research in England and Wales has recently been published. During the year the foundation transformed itself into an incorporated organization (a company limited by guarantee). The purpose of this change was to give the foundation a separate legal existence. Sir Francis Hill, chairman of the Executive Committee, retired and was replaced by Sir Alan Lubbock.

According to the report, these changes in the foundation's financial and administrative structures have been matched by a continuing process of change in the whole field of research in education. Between 1966 and 1967, two bodies—the National Advisory Council for Educational Technology and the Committee on Research and Development in Modern Languageswere set up in addition to the Schools Council. Each of these has considerable government finance behind it and is likely to undertake or commission research work which will involve the schools. It has also been suggested that something similar should be set up for teacher education and training. Another new development is the setting up of a Planning Branch by the Department of Education and Science to undertake research in education through its own staff and through staff specifically employed for particular pro-This new department, it is stated, will concentrate on relatively short-term studies mainly in the field of economic research.

Three new developments which reached an advanced stage during the year were: the first issue of the foundation's newsletter, *Educational Research News*; the development of research paperbacks which are now in the press; and the production of pamphlets, setting out the practical applications in schools of the foundation's recent researches, which will be ready in the new year.

The foundation has supported mathematics research, reading research and research on the teacher's work and training. In addition, research projects in technical and further education have been continued. A Constructive Education Project, sponsored by the Home Office and the Department of Education and Science, is investigating the influence of schools on children with particular reference to their achievements, attitudes and behaviour. The project, which was started in 1964, will continue for eight years.

Over the two years from 1965 to 1967, sponsors have increased finance for projects by 125 per cent and this now accounts for a considerable portion of the foundation's finances. Generally, finances for the work of the foundation are drawn from two main sources, subscription income and contract finance, both of which are controlled by the board.

During the year the basic subscription income increased slightly to £64,133 and income from publications rose significantly to £8,600. Work on sponsored projects increased by approximately 23 per cent over the previous year while grant-in-aid from the Department of Education and Science remained at £20,000.

Currents in the Atlantic

THE research vessel Discovery was brought back to her home port, Plymouth, on Monday this week after a two month cruise in the east Atlantic under the leadership of Dr J. C. Swallow of the National Institute of Oceanography. Most of this cruise was concerned with taking an initial look at deep current patterns over a reasonably extended period, now that the techniques for doing this are becoming available. The basic current meter used on this cruise is a device made in the United States, but the ancillary equipment which has enabled the maximum information to be derived from it has been specially developed at the National Institute of Oceanography. Anchored deep current buoys were put down on the flat "bottom plain" at the mouth of the Bay of Biscay. These were positioned about 30 miles from each other and made measurements at three levels—400 m, 1,200 m and at bottom water below 2,500 m. The deepest position was at 4,800 m where a buoy remained for 5 weeks. The others were in position for shorter periods but still of the order of weeks.

Because so little is known of the long term fluctuations of deep currents, three drifting floats given neutral buoyancy to remain at similar depths were used as a check for the buoy measurements. The floats contained an acoustic beacon with a range of 4–5 miles to show the way the instruments drifted in the current. Dr Swallow said he was surprised that there were not more fluctuations in the currents measured. Findings from the floats alone indicate that the deeper level currents tend to move faster, but from this cruise it was found that there was always a relationship of general direction between the three currents when

calculated over a period of several days. Current speeds were found to vary between 2 and 20 cm/sec. Subtler data from the continuous readings of the anchored buoys have yet to be analysed at Wormley. The hope is that from this initial experience a programme for immediately reducing data from the buoy recorders on board Discovery can be ready in time for the installation of a computer on board. This, it is hoped, will be working when the next deep current cruise takes place in about a year's time.



Launching a deep-sea buoy for current measurement from RRS Discovery (National Institute of Oceanography).

The ultimate object of this work is to investigate the relationship between atmospheric phenomena and deep water currents. It is expected that this interaction will be expressed as a time variation with a period of several weeks. Studies like these could eventually help with long-range weather forecasting.

High off the Ground

When Concorde made its first appearance at Toulouse on December 11, reporters and officials were dwarfed by the undercarriage, especially at the nose where the aircraft is 15.5 ft. off the tarmac. There are two reasons for this. Because of the delta design, the Concorde has a high landing angle, about 10° nose high. And because of this high nose angle, the nose has to droop 17.5° for the pilot to be able to see out properly when landing or taxi-ing. The design of this undercarriage, however, has not presented many problems to Messier and Hispano-Suiza, who were responsible for the nose landing gear and the main landing gear respectively, as the Concorde lands at 153 knots, not much faster than the normal landing speed of subsonic jet aircraft.

Concorde's drooping nose seems to be a common factor with all supersonic airliners. The winning US design, the Boeing 2707, has one, as did the Lockheed design. The Russian TU-144, another challenger to Concorde, also has the droop nose. The Concorde project is costing the French and British governments £250 million each. Each aircraft is expected to cost £7.5 million, including initial spares.

Report Justified

Nor many compilers of annual reports seriously consider whether their labour is worthwhile. Dr S. G. Burgess, scientific adviser to the Greater London Council's Scientific Branch, has at least tried to justify his annual report for 1966. He says it is only by collecting results of scientific studies and presenting a summary that it can be assessed whether the scientific branch is worth its keep. The report is published by the Greater London Council,

price 5s.

According to the report, some 124,672 samples were examined during 1966 by the scientific branch. These included asphalts, tarmacadams, plasters, plastics and putties; butter, blancmange powders and suet; insecticides, paints and rubber. Items which receive most attention in the report are: water pollution and sewage treatment, building materials for construction and maintenance, environmental studies, and general supplies and services. W. B. Chapman, the public analyst, has also included a report within the main one which refers to 3,727 samples received under the Food and Drugs Act. Dr Burgess has also indicated probable lines of progress for the following year.

Generating Neutrons

In an admirably clear-headed report, the Science Council of Canada has now recommended in principle the building of an intense neutron generator. A preliminary survey of the project, costing \$7.5 million, is to proceed forthwith. If this is favourable, present estimates call for a total of \$155 million to be spent

by 1973 in building the generator.

The intense neutron generator will be based on a 1 GeV proton linear accelerator. In 1952, W. B. Lewis of the Canadian Chalk River Nuclear Laboratories realized the significance of certain experiments carried out with high energy protons from the cyclotron at Berkeley. These experiments implied that, in a thick target of heavy elements such as bismuth, each proton would generate many neutrons. The neutrons could be used to generate fissionable material by neutron capture in elements such as thorium. The uranium-233 thus produced, when burned in a reactor, might lead to the production of more electrical energy than was used originally to accelerate the electrons.

Apart from its possible relevance to low cost power production, the generator will be a valuable research tool in its own right. As the source of intense fluxes of thermal neutrons, it is expected to have applications in solid state physics, nuclear physics and materials science. It will also provide intense beams of neutrons, muons and pions for use in nuclear probes and elementary particle studies. An important industrial application will be the production of high-grade radioactive materials of commercial value. A breakdown of the various uses and purposes of the ING attributes about 60 per cent of its probable value to basic research and 40 per cent to applied research.

In making its recommendation, the Science Council of Canada has also taken explicit account of the nonscientific benefits to be expected from the generator.

It points out that, for a country of Canada's size, a degree of specialization is essential if high standards are to be achieved. Standards of excellence in given areas will not only encourage scientists and engineers to stay in Canada but also constitute the only sure way of achieving results which are internationally significant and competitive. The ING project is related to an area in which Canada already has an important investment and an international stature. It will have an encouraging effect on those concerned with the basic research and engineering objectives of the project, and it is expected to have a substantial industrial fall-out. It does not duplicate research abroad, and should attract foreign scientists to Canada as well as placing Canada in a good position for exchanging research information with other countries.

Control of Malaria

THE World Health Organization has been so successful in eradicating malaria from certain parts of the world (particularly in Europe, where the number of malarial cases fell from more than four million to less than seven thousand in the first fifteen years of WHO's existence) that it can now turn its attention to the problem of keeping these areas free of the disease. Unfortunately, laboratory tests for detecting potential malarial carriers are not completely effective, and a person infected by a mosquito may remain a carrier for a very long period of time. Permanent immigrants are more likely to re-introduce the disease than tourists, and the WHO technical report devoted to the problem stresses the necessity of co-operation between government authorities concerned with health and immigration. But it is possible to contract malaria during a short stay in an infected region, and the report recommends that shipping companies be aware of the problem, and that passengers and crews should be instructed in the use of prophylactics. A new difficulty besetting health workers has been the increase in the number of Plasmodia resistant to 4-aminoquinolines. Fortunately, however, few resistant strains have reached malaria-free countries; WHO hopes shortly resume publication of its reports on the spread of the strains.

The measures Britain takes to keep out malaria are hardly exemplary. No checks for the disease are made on passengers coming from abroad—though the Board of Trade does issue advisory notices to shipping companies on the diagnosis and cure of the disease. Yet Britain has not been troubled by a malaria epidemic since 1921, and during the past ten years no cases have been reported. The reasons for this are partly fortuitous—Plasmodium falciparum does not usually survive in the mosquito long enough to be transferred to the next human host. This is not true of Plasmodium vivax, the malarial parasite endemic in Britain in the past but now imported by immigrants from India and Pakistan. Indian immigrants, however, tend to live in cities where the appropriate mosquito species are rare. In any case it appears that the mosquito population as a whole has been greatly diminished by farm insecticides—the use of which is not, of course, primarily directed against mosquitoes. Finally, as more and more people travel by air rather than by sea, the risk of passengers being infected with malaria en route and entering Britain as innocent carriers of the disease has

lessened. Not only do air passengers spend less time in infected areas, but aeroplanes and airports are rather easier to spray than ships and harbours.

Tissues in Store

THE problem of a Christmas present for the biologist who has everything might be solved by a glance at the catalogue of the Exotic Animal Tissue Bank. This is a collection of tissues which was established in September 1967 at the St. Louis Zoological Park, in St. Louis, Missouri, in co-operation with the Center for the Biology of Natural Systems at Washington University.

The tissues come from animals which have died in the zoo—the service operates as part of the zoo's research programme. There are now more than 1,000 specimens available to research workers, teachers and anyone else who would like them. Prices range from \$2.00 for 1–10 g to a minimum of \$25.00 for amounts greater than 1 g. Prices for whole organs weighing less than 10 g will be quoted on application. With each specimen comes a brief history, including the species, age, sex, treatment given and cause of death. Many specimens have already been distributed to workers in a wide range of disciplines.

Specimens are fixed in 10 per cent buffered formalin, or frozen or made into slides, and it is hoped to expand the service to include fixation for electron microscopy. Anyone wanting to look at such things as the aorta of the sand boa constrictor, the spleen of the African lion, the rumen contents of the onyx, or the uterus of the agouti will now know where to apply.

New Look for Classics

"An Approach through Classics" is a report prepared for the Schools Council on a possible course in the humanities for children between 11 and 14. In these three years, the children are to be given an account of events, myths, discoveries, literature, art, politics and personalities from the Egyptians and Sumerians to the Renaissance. Those who jib at the superficiality of learning implied by this syllabus could well reflect on how few "educated" people could give an account that was more than superficial on any topic within this huge range. What most people learn vaguely and by diffusion it is proposed to teach rather less vaguely, though necessarily with very little detail. But it is, perhaps, a pity that the syllabus makes no mention of any specialization, not because it would be good to induce in children the notion that human development has been a series of "set periods", but so as to point the dangers of generalization which such a course is prone to.

The course involves no study of any particular language nor—a more serious omission—any teaching of elementary linguistics. No one could expect fourteen year olds to have enough Latin to be useful in understanding Roman life, but it might well have been profitable to make mention of language as a repository of culture. The use of translations in the study of classical literature, much emphasized in the report, will also bring problems. "Pupils very soon absorb Homeric style and diction, his similes, stock epithets, etc."—but is it useful to have children emulate cliches, even Homeric ones? The report quotes a splendid

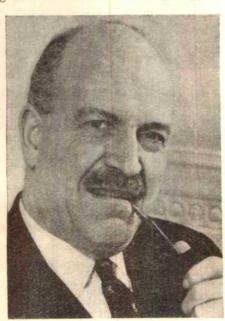
pastiche of nineteenth century Homeric translation,

written by a boy of eleven.

These are, however, minor points. Perhaps the most serious difficulty that would arise if the syllabus came into wide service would be the shortage of teachers knowledgeable enough to take the course.

New Man for Porton

In March 1968, Mr Gordon Neville Gadsby will take over as director of the Chemical Defence Experimental Establishment at Porton, near Salisbury. He will succeed Mr E. E. Haddon, who is retiring. Mr Gadsby, who is the present deputy chief scientist (Army), was an honours graduate in chemistry and a Cadbury Prize winner at Birmingham University and before the war was senior chemistry master at Waverley High School in Birmingham. After service in the Army and as a lecturer at the Royal Military College of Science, he moved into operational research. In 1960 he became director of operational science and research and, later, director of the Army Operational Research Establishment. In 1965 he became director of biological and chemical defence (Army).



Despite its fearsome image, the establishment at Porton is responsible for a great deal of useful work. Mr Gadsby is not yet ready to suggest any changes which he may favour when he becomes director, but makes it clear that he does not want to give the impression of being a new broom.

Improving Traffic Flow

TRAFFIC delays throughout Britain are estimated to cost £750 million a year. This figure is clearly one reason why the Road Research Laboratory is spending £0.5 million on a large scale experiment in traffic control by computer. The experiment, taking place in Glasgow, is described in a recent report from the laboratory.

A similar experiment carried out in West London was devised to solve a particular problem, but the Glasgow experiment is designed to test and compare

eight different systems of traffic control. The experiment started on May 10, and results so far are said to be encouraging. The experiment is controlled by a Marconi Myriad computer which has been used to link 80 traffic signals controlling a square mile in the centre of Glasgow. The systems under test do a variety of things, from minimizing the delay in networks controlled by fixed time signals to arranging for equal saturation on all branches of a crossroad. Each system is assessed by measuring the average journey time over standard routes with instrumented cars. Five of the systems under test have been tried previously, either in this country, Europe or the United States; the other three have been newly devised. As far as the RRL knows, this is the first time that a fully comparative experiment has been conducted. Traffic delays in central Glasgow, the RRL estimates, cost £2 million a year-if only 5 per cent of this can be saved, a reasonable estimate, the experiment would pay for itself in five years.

Following Parasites

The Ministry of Overseas Development has made a grant of £45,000 to establish a new headquarters for what is called—somewhat alarmingly—the Blood Meals Service. This is a unit financed by the government to analyse the stomach contents of blood-sucking insects carrying malarial and other parasites. Smears of the stomach contents are sent from abroad, and their analysis provides clues to the feeding habits of vectors, and other information of importance to foreign workers and authorities such as the World Health Organization. Until now this has been done at the Lister Institute of Preventive Medicine, Elstree, but work has now begun on the new centre at Silwood Park, near Ascot, the field station of Imperial College, and it is hoped that the service will be able to move there early next year.

The Ministry's grant also covers the cost of the service for the next three years, and the new director of the unit, Mr Peter Boreham, is hoping to be able to carry out research on the pathogenesis of trypanosomiasis as well as operating and improving the service itself. The unit will be an important addition to the research teams at Silwood under the direction of Professor T. R. E. Southwood. The Blood Meals Service was founded in 1948 by Professor Bernard Weitz, who continued as director until this September, when he left to direct the National Institute for Research in Dairying at Shinfield, Berkshire.

European Forestry

Forestry cannot keep pace with the marked growth of industrial production. This is one of the important findings of a report called *Cost Studies in European Forestry*, published by the Royal College of Forestry in Stockholm.

Conditions in forests and forest industries are considered in detail for each country, and the results include the share of forestry in the gross national Product, total roundwood balances, external trade in forest products and wage development. Data for Sweden served as a standard for the work in other countries and analysis of material was carried out at the Royal College of Forestry in Sweden. For the purpose of the study, Austria, Finland, Norway and

Sweden were considered to be timber exporting countries, while Denmark, Germany and Switzerland were classified as timber producing and timber importing countries. Britain was considered to be the sole

timber importer.

The report shows that the share of forestry in the gross national product between 1953 and 1962 was highest for Sweden and Finland, but that a declining trend is evident everywhere. Despite this, net production value of forestry in absolute figures has remained approximately constant. In some cases, declining timber prices have been compensated by increased timber output. Germany is the largest consumer of forest products and in 1960 use of forest products in Britain amounted to only half the consumption in Germany. Wood consumption per head has increased in Sweden, Austria, Germany and Switzerland and decreased for Finland and Norway.

One of the most important purposes of the study was to compare the prices of roundwood in the different countries, because these prices determine the revenue which the forest owners receive from timber sales: the distance which the saw logs have to be transported to the consuming centres seems to have a decisive influence. Prices for saw logs are highest in Switzerland and the highest prices for pulpwood are also paid in

Switzerland.

Administrative or overhead costs in forestry are highest in Germany and lowest in Norwegian crown forests. And the report suggests that a systematic levelling of the standard of living of the workers is taking place. Finally, the report concludes that Switzerland makes the biggest profits from forestry.

MRC Appointment

SIR HAROLD HIMSWORTH, who has been secretary of the Medical Research Council since 1949, is to retire at the end of September or earlier. His successor will be Dr J. A. B. Gray. When Dr Gray, previously professor of physiology at University College, London, was appointed second secretary of the council in May 1966, it was widely understood that he would succeed Sir Harold.

Sir Harold Himsworth became secretary at the age of 44. After a distinguished academic career, also at University College, he was appointed professor of medicine at London University and director of the University College Medical Unit. He was knighted in 1952 and elected to the Royal Society in 1955. Under his aegis the budget of the MRC has grown from £1.6 million in 1949 to £14.2 million in 1967–68. During the same period, the number of staff has increased from 1,401 to 3,529.

Parliament in Britain

Nuclear Stations

The Minister of Technology, Mr A. Wedgwood Benn, put the total expenditure on the development of the Magnox system for civil use at about £20 million, just over half relating to the development of fuel elements, in respect of which appropriate surcharges were included in fuel prices. If charges were imposed, sufficient when discounted to 1967 to cover the remaining cost of development of the reactor system itself, the average cost of generation from Magnox stations

would rise by about 0.007d. per unit, assuming 20 years' life and 75 per cent load factors. The expenditure to March 31, 1967, on the development of the Advanced Gas-Cooled Reactor system amounted to £89 million. Whether a royalty of 0.014d. per unit would recover these costs in full depended on how many such stations were built and how much electricity they generated. (Written answers, December 11 and 12.)

Students

MRS S. WILLIAMS, Minister of State, Department of Education and Science, estimated that the annual cost to public funds of a student who completed his course at a college of education was about £785, and that the estimated annual cost to the Exchequer was about £510. Non-graduate entrants to courses of initial teacher training numbered 29,058 in 1965 and 33,378 in 1966; the provisional figure for 1967 Most of these students are following was 36,500. 3 year courses; of the students who entered in 1961, 1962 and 1963, about 90 per cent completed their courses satisfactorily. About 4 per cent of entrants did not continue in training beyond the first year For 1966-67 the technical colleges of the course. admitted to advanced courses some 27,000 full time and sandwich students, 36,000 part-time day and 28,000 "evenings only" students. It is estimated that in the academic year 1971-72 total recurrent expenditure by universities (other than expenditure on research contracts and other self-balancing items and local authority rates) will be about £200 million with student numbers between 220,000 and 225,000, giving an average expenditure per full-time student of about £925, compared with £835 in the academic year 1966-67. The fourth annual report of the Universities Central Council on Admissions gives a total of 13,535 admissions by universities in Great Britain to first degree and first diploma courses in pure science starting in October 1966, and estimates that places could have been found for about another 1,000 if more suitably qualified candidates had come forward. (Written answer, December 14.)

Oceanography

In an adjournment debate in the House of Commons on December 11, Mr T. Dalyell raised questions about the development of the marine sciences and environment and the general state of British oceanography. Replying for the Government, the joint Parliamentary Secretary, Minister of Technology, Dr J. Bray, concentrated on the technological rather than the scientific aspects. The integration of the Ministry of Aviation with the Ministry of Technology had facilitated the combination of those parts of the defence research problem and the civil research programme which fall Following the Harwell Conference a in this field. working party had been asked first to review research and development in progress on all aspects of marine science and technology in the United Kingdom, and secondly, to identify areas of marine science and technology which are likely to be the most profitable economically and to consider what further research and development would be necessary to exploit them. Thirdly, the working party was asked to advise on a programme of action and on means of co-ordinating the existing and proposed programmes of research and development.

NEWS AND VIEWS

Seal Hunting

In her article on page 1237, Mrs E. G. Simpson describes the efficient way in which, under United States administration, the Fur Seal of the Pribilof Islands, Alaska, is hunted and killed. These observations are a happier story than the previous report (Nature, 214, 1274; 1967) of the killing of the Harp Seal in the Gulf of St. Lawrence, when Mrs Simpson concluded that "a large percentage of the hunted animals die in a manner which is of doubtful humanity". At the Pribilof Islands the United States government is itself responsible for the killing of seals, whereas in the Gulf of St. Lawrence the hunting of seals is the prerogative of commercial organizations which are supervised, as far as possible, by government inspectors.

Humanitarian issues apart, the ecology of the two seal populations is also affected by the different government policies. At the Pribilof Islands, the stock of the Fur Seal has grown from an overhunted population of about 200,000 at the beginning of the century to its present value of one million. This seems to be the optimum population size, above which the natural mortality rate rises. The control and exploitation of the Fur Seal is recognized as a classic example of how an animal population may be conserved with maximum economic value and minimum ecological damage. In contrast, the Harp Seal population of the Gulf of St. Lawrence is under no such rigorous control. Its numbers are estimated to have fallen from about 3 million in 1950 to 1 million ten years later. Since 1965 the Canadian Government has fixed a quota of 50,000 on the number of young seals which may be taken in each year. This applies only to firms operating from ships and aircraft, and Mrs Simpson estimates that a further 30,000 seals may be taken by firms operating from the land. The Canadian Department of Fisheries has carried out population surveys and believes that at its present numbers the Harp Seal is in no danger of extinction. But the level at which an animal population ceases to be self-sustaining is difficult to determine, and not all zoologists share the Canadian Government's optimism. The USSR, for example, has prohibited the hunting of the Harp Seal in the White Sea since 1930, when numbers had declined to dangerously low levels.

The argument is often put forward, both by seal hunters and fisheries, that because seals are voracious predators of fish, the severe culling of their numbers enables fish populations to increase so that more fish can be caught. There is no evidence, however, that seals materially affect the stocks of fish in the sea, which are subject to large fluctuations caused by several factors of which the size of the seal population is only one. Harp Seals are also vectors of the codworm nematode in one stage of its life cycle, but here again the infor-

mation upon which an assessment of economic damage might be made is not forthcoming.

In fairness to the Canadian Government it should perhaps be emphasized that the hunting of the Harp Seal is much more difficult to control than that of the Fur Seal in the Pribilofs. Only the young Harp Seal, the fur of which is still white, has economic value, although a small but unrestricted number of adult pelts are also taken for leather. The young seals are distributed over a large area of the ice and cannot be herded together as can the bachelor males of the Fur Seal. But under these circumstances, it might be argued, ecological studies should have been seen to be all the more necessary.

Chromosome Deletion in Action

A TEAM under the leadership of Professor W. M. Davidson of King's College Hospital, London, has produced what may be the first pictures of somatic autosomal deletion in man not induced by mutagens. viruses or irradiation. (Day, E. J., Marshall, R., MacDonald, P. A. C., and Davidson, W. M., The Lancet (i), 1307; 1967.) A child was admitted to hospital with multiple somatic anomalies and died within three months. Lymphocyte cultures from both father and child showed partial deletion of the long arm of chromosome 18, the mother's karyotype being normal. Both father and mother are phenotypically normal, so that it is to be expected that the father has a balanced translocation, though no normally large chromosomes could be detected. At the same time one fifth of the cells of the father's lymphocyte culture showed progressive fragmentation of the short arm of chromosome This chromosome is known to be polymorphic. the sizes of its arms varying between individuals and even in the same tissues of the same individual, but fragmentation of this kind has not previously been noted. The extent of the abnormality varied from cell to cell in such a way as to suggest that deletion was in process of developing.

The team hints at the possibility that some process which first affected chromosome 18 may now be starting to act on chromosome 16. It is also possible that the abnormality is hereditary, but there is as yet no evidence to support this. The team is now working on cell cultures of the skin of the father. If chromosome 16 is found to be normal in these and other cultures of the father's tissue, then it is unlikely that the man inherited the defect from his parents; the hypothesis that the chromosome fragmentation in the lymphocyte culture represents the beginning of deletion will therefore be greatly strengthened.

Stellar Diameters Observed

The first results have now been published (Monthly Notices of the Royal Astronomical Society, 137, No. 4: 1967) of the measurements of stellar diameters carried

out with the apparatus constructed by Dr R. Hanbury Brown and his colleagues. The intention is to correlate in time the intensities of the light signals received at two mirrors separated by a distance of some tens of metres. Hanbury Brown and Twiss first showed in 1956 that such a correlation should exist between two coherent beams of light, but the realization of their plans has been an arduous undertaking. The construction at Narrabri Observatory, 300 miles north of Sydney, consists of a circular railway track 188 metres in diameter on which are mounted two composite mirrors 6.5 metres in diameter. Time correlations are based on periods of observation which may amount to more than 40 hours. So far, the apparatus has been used to measure the diameters of fifteen stars, most of them young O and B stars. Angular diameters of about one-thousandth of a second of arc have been obtained in this way. One valuable by-product of the work so far is a more accurate estimate of some surface temperatures.

Keep on the Grass

by Mary Lindley

THREE members of the Nuffield Unit of Tropical Animal Ecology described some of their work in the Queen Elizabeth Park—which surrounds Lake Edward and Lake George in western Uganda—on the habits of the larger mammals at the scientific meeting of the Zoological Society of London on December 12.

Mr C. Field of the school of agriculture in Cambridge has studied grazing habits using wild and tame animals. The hippopotamus which grazes at night and spends the day in the water has caused some serious erosion, and cropping began in 1958. About 10,000 animals have been shot since then with the aim of reaching an optimum density of twenty hippos to a square mile. Mr Field has been able to make use of this cropping programme to examine stomach contents, and in four years he examined between two and three thousand hippos. The preferences of the animals were found by identifying the inflorescences in their mouths as they grazed. Mr Field also had tame animals, like the buffalo in the photograph, which were taken into the field and watched while they grazed.

The first results of this work have shown that the hippo prefers the creeping grasses, that the antelope prefers the more erect cauline leaf grasses and that the buffalo prefers the tussock grasses. Leaves of the cauline species have a high protein content, but the buffalo has a large mouth and cannot separate them from the stems. The antelope, however, can separate the stem and leaves and will eat these grasses. The hippo is the dominant species and it acts as a nutrient sink by grazing heavily and defecating into the water. Heavy fishing means that these nutrients are lost to the environment. Hence the need for cropping, and there are signs that the same may have to be done to the elephants, which are increasing very rapidly.

Mr C. Spinach of the Wellcome Institute of Comparative Physiology has been studying the waterbuck, and he discussed some aspects of its territoriality and socialization. The male is weaned at six to eight months and joins a bachelor herd, usually about seven animals. At five or six, the bachelor settles down to establish a territory and by seven years is

completely territorial, occupying a territory from which he is unlikely to wander and which he will vigorously defend. The territories observed by Mr Spinach varied in size from 40 to 550 acres. The waterbuck may establish himself by joining an already established male in his territory, being allowed to stay if he holds his own in battle. A weak male may return to the herd and later establish his own small territory. Mr Spinach watched a male do this, and later return to the territory of his original choice when the established male was beginning to age. The average maximum life span is about eleven years, but at nine to ten years a male may be driven into an inferior territory, perhaps without water. This may in part account for the peak in deaths at this age.



Observing the feeding habits of a tame buffalo in the Queen Elizabeth Park, Uganda.

The female waterbuck wander through a home range which covers several male territories, but with increasing age she restricts herself more. She gives birth in isolation within the male's territory. Territoriality seems to ensure the maximum of reproduction with the minimum of aggressive contact, but Mr Spinach remarked that much more information is needed before the full reasons for territoriality are understood.

Mr J. Grimsdell of the Marshall Laboratories in Cambridge has studied the African buffalo, which is abundant in the lake regions of western Uganda. In Queen Elizabeth Park there are more than 16,000 buffalo in herds usually consisting of between twenty and forty animals. Members rarely leave the herd, in which there is linear ranking. Rank is related primarily to seniority; the number one bull is one of the oldest but not necessarily the oldest. Each animal appears to know its place in the ranking and there is little fighting.

Mating is carried out by the higher ranking bulls, and the peaks in conception and births, which follow after eleven months of gestation, occur towards the end of the rains. This is a time when the protein content of the grass is near the maximum. This seems to be a solution to the problem of providing extra nutritional requirements for the near term female and the newborn buffalo. Areas such as the Queen Elizabeth Park may soon have to pay their way, and the buffalo, if efficiently managed, could be a good source

of meat. With the buffaloes, as with the other animals, the Nuffield Unit, although engaged in a pure research project, is providing information which will be valuable to the management of the park in their work.

Inhibitor Inhibited

from our Molecular Biology Correspondent

THE trypsin inhibitors form an interesting class of low-molecular weight proteins of remarkable specificity. Several plant and animal trypsin inhibitors have been studied, and quite recently the full amino-acid sequence of the bovine pancreatic inhibitor (often known as Kunitz and Northrop's basic inhibitor) has been determined. Like other animal trypsin inhibitors, this protein has three disulphide bonds, and their relation to the reaction with trypsin has been investigated by Kress and M. Laskowski, sen. (J. Biol. Chem., 242, 4925; 1967).

The sequence and position of the disulphide bonds were both reported earlier by Laskowski and his group. It now turns out that one of the disulphide bonds (between residues 14 and 38) can be reduced without damage to the other two—a reaction which has been paralleled in several other proteins. Again, as in one or two of these cases, the product retains its activity. If, however, the new sulphydryl groups are carboxymethylated, activity is lost and, indeed, the protein becomes prone to hydrolysis by trypsin. The partially reduced inactivator reforms the broken disulphide bond under the usual conditions of reoxidation in air; in its complexes with trypsin, however, the reoxidation is almost wholly prevented.

The drastic effect produced by the quite modest steric disturbance of introducing the two carboxymethyl groups is in interesting contrast to the observation of Chauvet and Acher (J. Biol. Chem., 242, 4247; 1967) that the four lysines of the inhibitor may be guanidinated without detriment to the activity. This reaction amounts to a small displacement of the charge by effectively lengthening the lysine side chain. The overall charge, however, must be maintained, for acetylation of the lysines inactivates the inhibitor completely. It is also interesting to note that whereas all five amino groups (the four lysines and the N-terminus) are reactive in the free state, one of them (lysine-15) is shielded in the complex with trypsin, and may therefore form part of the active site.

Both trypsin and the inhibitor are quite small molecules; the latter indeed has only 58 residues, and is therefore one of the smallest globular proteins (excluding from this definition the polypeptide antibiotics). This therefore provides one of the very simplest systems available for the study of specific interactions between proteins.

Histone Synthesis in HeLa Cells

from our Cell Biology Correspondent

THE discovery that histones are uniquely associated with DNA in cell nuclei and do not occur in the cytoplasm provoked the hypothesis that histones function as repressors of gene transcription and has led to attempts to establish a temporal relationship between histone and DNA synthesis. It now seems fairly certain that histones do have a role in genetic regulation, but whether DNA replication and histone synthe-

sis are always synchronous is still an open question. No doubt much of the confusion about this relationship has arisen because different workers have used different methods to identify histones and synchronize cell populations and they have used different cell types. But Robbins and Borum (*Proc. US Nat. Acad. Sci.*, 57, 409; 1967) and Borum, Scharff and Robbins (*ibid.*, 58, 1977; 1967) seem to have established that, in HeLa cells at least, histone and DNA synthesis are synchronous and, more unexpectedly, that histones are synthesized in the cytoplasm.

Robbins and Borum used gel electrophoresis to identify two heterogeneous histone fractions in protein extracted from a synchronized population of HeLa cells with hydrochloric acid. With this method and using C^{14} lysine to label histones and C^{14} thymidine to label DNA, they found that lysine incorporation into histone is negligible during the G_1 phase of the cell cycle. But as the S phase begins and DNA synthesis starts, lysine incorporation into histones increases. The incorporation of lysine into non-histone protein stays constant, however. Thus the synthesis of histones is uniquely synchronized with DNA synthesis.

Where are the histones synthesized? To answer this, synchronized cells were given a 2 minute pulse of C¹⁴ tryptophan, to label non-histone protein and H³ lysine, and then chased in a medium containing a large excess of unlabelled lysine and tryptophan. After 2 minutes incorporation, 80 per cent of the lysine was in the cytoplasm and during a 2–12 minute chase the amount of incorporated lysine in the nucleus increases by 33 per cent. Lysine rich protein is therefore being transported into the nucleus. Tryptophan labelled protein, however, shows no such transfer. The clear implication is that the histones are synthesized in cytoplasm, and if so it should be possible to detect cytoplasmic polysomes synthesizing histone.

Robbins and Borum analysed the distribution of incorporated lysine and tryptophan among HeLa polysomes from cells at G_1 and S phases. At the S phase in the cell cycle more small polysomes are present than at G_1 and a significantly greater percentage of the lysine is associated with the small polysomes than with large polysomes. Furthermore, when cytosine arabinose, an inhibitor which stops DNA and histone synthesis, is given during the S phase, the lysine rich small polysomes disappear. They concluded that DNA and histone synthesis is synchronized and that the histones are made in the cytoplasm on small polysomes.

Borum, Scharff and Robbins, continuing the study, isolated the nascent polypeptides from the small cytoplasmic polysome fraction and found that they are lysine rich and migrate on gel electrophoresis like authentic histones from nuclei. A rapidly labelled RNA fraction isolated from the polysomes appears to be histone mRNA. This RNA first appears at the beginning of the S phase and its synthesis ceases about 2 hours before cell histone synthesis ceases at the end of the S phase. The histone mRNA has a short half life of 1-1.5 hours.

These results indicate that the principal control of histone synthesis is at the level of gene transcription, but this does not, of course, exclude some fine control at the translational level. It will be very interesting to see whether or not all these conclusions are valid for other cell types.

Thin-layer Chromatography

by
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A symposium on quantitative thin-layer and paper chromatography will be held on January 3 and 4, 1968, by the Pharmacological Society of Great Britain and the Society for Analytical Chemistry. What follows is a description of how this new technique has been developed in recent years.

In 1903, the Russian botanist, M. Tswett¹, working in Warsaw, separated the various components of chlorophyll by pouring a light petroleum extract of green leaves over calcium carbonate packed in a long glass tube and followed this by adding more of the solvent until he could see the different coloured bands separated in the column. Tswett named this technique "chromatography", but his work remained unnoticed until the early 1930s when the technique was used to separate the components of many complex mixtures, particularly pharmaceutical preparations, so that similar preparations could be distinguished. This was followed by the separation of mixtures of chemical substances, some of which were colourless so that special methods for detecting the substances after separation had to be devised. Since then there have been many and sometimes dramatic advances in methods of chromatographic separation so that the original definition of chromatography no longer applies.

Today, chromatography may be defined as those processes which permit resolution of mixtures by effecting separation of some or all of their components in concentration zones or in phases different from those in which they were initially present, irrespective of the nature of the force or forces causing the substances to move from

one phase to another.

Kinds of Chromatography

The scientific principles involved in chromatographic separation may be adsorption, partition or ion exchange, and gel filtration could also be included in the wider concept of chromatography. In any particular separation one, two or all of the first three processes may be involved although for historical reasons the names given to the various methods are based on the actual technique rather than the scientific principles involved—for example, column chromatography, paper chromatography and gas

chromatography.

The most recent development is thin-layer chromatography (TLC) which involves the use of a thin layer of a powdered stationary phase spread evenly over the surface of a suitable support such as a glass plate. The earliest attempts to use this "open column" method were made by two other Russian workers, Ismailov and Schraiber², in 1938, but little was done to exploit this technique because of the problems associated with loose powder and the difficulties of detecting colourless components. Meinhard and Hall³ in 1949 overcame the difficulties to some extent by mixing the powdered adsorbent with starch paste in order to make the material adhere to the plate. Others tried different binding agents, but it was not until 1956, when Dr Egon Stahl^{4,5}, professor of pharmacognosy, University of Saarbrücken, published details of standardized stationary phases, equipment and techniques which allowed more reproducible results, that thin-layer chromatography became a widely used separation technique.

Alumina and Silica Gel

In 1956 the stationary phases commonly used were activated aluminas and silica gel so that the separation was essentially by adsorption. As such, the technique was claimed to have many advantages over paper chromatography, particularly for hydrophobic substances, though the method could be used with advantage for hydrophilic substances. Today magnesium silicate, cellulose, ion exchange celluloses, impregnated kieselguhr, powdered nylon (polyamides) and gel filtration materials are also used so that all the four physico-chemical processes may be involved and the method is equally applicable to both hydrophobic and hydrophilic substances.

The original claims for the advantages of TLC over paper chromatography are fully justified. They are: (1) greater efficiency; (2) greater sensitivity; (3) greater speed; (4) more compact spots; and (5) the possibility of using more corrosive agents and higher temperatures for detecting the substances. TLC is much more selective because complete separation of very closely related compounds with only slight differences in structure or con-

figuration can be achieved.

The advantages of TLC over gas chromatography lie in the simplicity, cheapness and resolving power at room temperature so that it can be used for high melting point

substances without difficulty.

There are many variations in the design of equipment available for TLC, but the original and basic technique described by Stahl is as follows. Thin layers, 250-300µ thick, of a suitable adsorbent* are prepared by spreading thick aqueous suspensions of the adsorbent over the surface of glass plates which are then air dried to remove excess water and finally heated for a definite time at a given temperature. This is essential if adsorption is the main process for separation. The mixture of components to be resolved is applied in a suitable solvent as small spots 1 cm (or more) apart along a starting line 1.5 cm from one edge of the plate. The plate is then carefully placed in a separating chamber or tank containing sufficient solvent so that the plate stands to a depth of 0.5-1.0 cm and the tank is then closed with an airtight lid. The solvent rises by capillary action through the layer on the plate to a predetermined height (usually 10 cm), after which the plate is taken from the tank, the solvent removed by means of warm air and the plate examined for the positions of the separated substances. If the substances are colourless, examination in screened ultraviolet light at 254 mu and 365 mu may be useful, but

• The materials are referred to as "adsorbents" in the commercial literature, irrespective of the particular process for which they may be used.

it is usually necessary to use a specific spray reagent and possibly to heat the plate as well.

Absorbent Layers

A number of modifications and refinements of this technique can be used. The support for the stationary phase need not be glass, provided it has suitable properties, and the thickness of the layer may be varied from 100 to 500µ; if the thinner layers are used, smaller loads must be applied. The actual amounts which can be applied depend on the degree of resolution of the mixture and the ease with which the separated substances can be detected, but normally the range is 10-100 µg. Lack of uniformity in the thickness of the layers may affect the results, but this can be overcome by using the commercially available spread layers, either on glass or on plastic film (known as precoated plates or foils). Although the earlier adsorbents all contained gypsum as a binding agent, adsorbents and precoated plates and foils can now be obtained without binding agent.

Since the slurry of the stationary phase used for spreading the layers is usually made with an aqueous medium, buffer solutions may be used so that partition takes place at known pH values or other substances may be added which will facilitate separation or detection of the separated compounds. The inclusion of silver nitrate to give an impregnated layer has proved very successful for the separation of liquids, while the inclusion of sodium fluorescein facilitates detection of colourless unsaturated compounds if the developed plate is subsequently exposed to bromine vapour when the fluorescein is converted to eosin except in those parts occupied by the substances. Inorganic phosphors are also added so that substances can be detected by examining the developed plates in screened ultraviolet light of appropriate wavelength, the substances quenching the fluorescence caused by the phosphor.

Measurements of Diffusion

Although the primary use of TLC is to effect separation of mixtures of substances, it is desirable and frequently essential that the Rf values, which are defined as the ratio of the distance moved by the solute to the distance moved by the solvent front, obtained for the separated substances should be reproducible. Many factors are concerned with reproducibility of Rf values in TLC, but the most important is the nature of the stationary phase at the time of use. This involves to some extent the uniformity of the layer thickness-and in this respect the commercially precoated plates and foils may be an advantage-but chiefly the water content of the layer. The water content of the layer and thus the activity of the adsorbent can be preconditioned by the heating process after spreading, but, in order to retain this activity, it is essential that the plates are stored in an atmosphere of earefully controlled humidity until required for use. Dallas⁶, Geiss^{7,8} and others have shown that the variable humidities of different laboratories-or the same laboratory at different times—can affect the adsorptivity very considerably and even the breath of the worker on the layer while he is applying the substance can reduce the adsorptivity of alumina. If the Rf value is not vitally important these elaborate precautions are not necessary, but, even so, the plates should be stored in a desiccator or airtight cabinet until required and should not, even then, be stored for too long a period.

Another factor relating to the layer itself is the amount of binder which might be present. Calcium sulphate is itself a strong adsorbent and it is possible that variations in the amount present can affect the separation. Thus it is better to use adsorbents without binding agents if reproducibility of Rf value is required. Another important factor affecting reproducibility of Rf values is the state of the atmosphere on the tank during development. As with paper chromatography, the atmosphere in the tank should be saturated with the vapours of the solvent being used for the development and the layer, like paper, should be conditioned in this atmosphere before being developed. Unfortunately, the majority of commercially available tanks are not designed for this, and the only effective way of ensuring saturation is to line the inner sides of the tank with filter paper soaked in the solvent system. It might be noted, however, that the actual resolution of mixtures is frequently better in tanks that are not fully saturated.

Choice of Solvents

Separation of a mixture may not be complete after a 10 cm movement of the solvent, in which case the distance can be increased to 12 or 15 cm or modified techniques such as two-dimensional, multiple and gradient development or electrophoresis in association with TLC can be used. Reversed phase TLC can be employed by impregnating an adsorbent with a non-polar substance-for example, liquid paraffin—and selecting a suitable solvent system for development, while low temperature and gradient TLC are also modifications which have been used to bring about separation.

The choice of solvent system will depend on the nature of the stationary phase and on the type of substances that need separating. Several workers have indicated eluotropic series suitable for adsorption chromatography (starting with light petroleum or hexane as the nonpolar solvent and ending the series with methanol as the most polar solvent). With polyamides the eluotropic series starts with water and moves through ethyl acetate to less polar solvents. It may be necessary to use a mixture of two or more solvents to effect a good separation, but with adsorption chromatography a mixture of solvents may behave as independent solvents to give two or more solvent fronts and this would affect the movement of the substances. The solvents used in TLC should always be freed from impurities. Stahl's recommends that all solvents should be purified by distillation before being used.

The methods of detecting substances after separation are basically the same as those used for paper chromatography. As indicated previously, however, more corrosive substances, including sulphuric acid, can be used with the inorganic adsorbents. Sulphuric acid followed by heating which chars the organic substance to give brown spots is not specific, but there are many specific reagents available; for example, ferric chloride in perchloric acid followed by heating at 150° C for half an hour is specific for certain oxindole alkaloids but is unsuitable for paper chromato-

Recording Results

The method of recording results needs to be discussed because it is not convenient to keep the layer adhering to the glass plate in the same way as it is possible to keep paper chromatograms. If the Rf value is reliable this can be given together with details of the shape, colour, etc., of the spot. Otherwise a permanent record should be made, such as a photograph, tracing, etc. It is also possible to preserve the actual layer by spraying it with a solution of polyvinylpropionate which sets to a clear plastic film and which, together with the adhering layer, can easily be stripped from the glass plate under water. Precoated foils provide permanent chromatograms.

It is understandable that the separation of the components of a mixture by such a relatively simple technique should lead to the possibility of isolating the separated substances on a larger scale. By using larger plates and thicker layers, increased loads can be applied although the degree of resolution is reduced. Thus it is only possible

to use preparative thin-layer chromatography for isolating those compounds with Rf values which are significantly different from those of adjacent compounds when examined on the conventional layers. It is claimed that 500 mg can be applied to a layer 5 mm thick, although it is more common to apply 100 mg as a continuous band to a layer 1 mm thick, because the problems associated with the preparation of a layer 100 × 30 cm and 5 mm thick are very great. The position of the separated bands can be detected either by ultraviolet light or by spraying a narrow strip along each edge of the plate and the zone of adsorbent containing the substance can then be removed from the glass plate and the substance extracted with a suitable solvent. This method has considerable advantage over column chromatography because in order to separate large quantities of material wide columns are necessary with the subsequent loss of efficiency because of the heat of adsorption produced in the middle of the column.

Quantitative Methods

It was also inevitable that the separation of the components of a mixture by the technique should lead to the possibility of estimating the amount of substance present. As in paper chromatography, there are two possible procedures (after separation and detection). procedure is the determination of the quantity of substance after eluting the substance from the layer. requires a quantitative recovery of the substance from the adsorbent without the extraction of interfering substances. This latter condition is rarely achieved, however, and it is necessary to extract a similar amount of adsorbent taken from the same plate at a corresponding Rf value to use as a reference solution.

The second procedure is the determination of the quantity of substance directly on the layer. This presents a number of problems, not the least of which is the determination of the exact boundary of the spot and to distinguish it sufficiently from the adsorbent. This may The earliest be achieved by a suitable spray reagent. quantitative procedures were those by Seher, in 1960,

who plotted area of spot against weight of substance, and this was followed by Purdy and Truter10,11 in 1962, who established a linear relationship between the square root of the spot area and the logarithm of the weight of substance. A method of minimum spot visibility has also been devised whereby the substance is progressively diluted and chromatographed until no spot is visible, the results being compared with similar dilutions of a known quantity of the substance. The intensity of colour in spots, whether naturally coloured or as a result of spray reagents, can be measured with a densitometer and the method can be extended to the measurement of fluorescence or the quenching of fluorescence. (Spots which are radioactive may be quantitatively assessed by specially designed radio scanners.) Recently Stahl and Jork 12 have developed an ultraviolet spectrophotometer which can measure ultraviolet adsorption of the substance directly on the plates.

There is no doubt that TLC has developed very considerably during the 10 years or so that have elapsed since Stahl renewed interest in this very simple technique, and it must be one of the most commonly used separation techniques used today. It finds application in all branches of chemistry, in biochemistry, pharmacognosy and pharmacology, to name but a few of the sciences which have benefited considerably from the ability to separate and determine minute quantities of substances in admixture.

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Effects of Pollution from the Torrey Canyon on Littoral and Sublittoral Ecosystems

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Long term (chronic) pollution can drastically reduce both the floristic diversity and the net annual primary production of littoral and sublittoral ecosystems dominated by attached macrophytes¹.

Pollution from oil and detergent deposited on the beaches of Devon and Cornwall after the grounding of the Torrey Canyon on March 18, 1967, has altered the balance of littoral and sublittoral ecosystems at two sites. This effect is most marked on the littoral zone, and falls off below the low water mark.

> The Torrey Canyon disaster presented the opportunity of assessing the effects of short term (acute) pollution by crude oil and detergent. A phytosociological survey at twenty-nine sites along the coasts of Devon and Cornwall

Table 1. PHYTOSOCIOLOGICAL DATA FOR THE LITTORAL ZONES AT ALL SITES STUDIED

						100 PM	SCHOOL SEE TO SEE TO SEE THE SECOND S	
Name:	Unpo Porthallow	olluted Roskilly	Mullion	Polluted Porthtreath	Cape Cornwall	Polluted (li Lamorna	itoral zone mari Porthleven	(edly green) Sennen Cove
Grid ref.	SW 798233	SW 473273	SW 666178	SW 648457	SW 350317	SW 451240*	SW 627254	SW 849264
Year Species	66 67	66 67	66 67	66 67	66 67	66 67 *	3 66 67	66 87
Enteromorpha spp. † Fucus spiralis	+ 1 + 2 + 3 + 3	+ 1 + +	+ + + 1	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	+ 2 + 2	1 3 3 4	+ + 5 5 + 1 + +	+ + 5 5 + 1 + 1
F. vesciculosus	+ 3 + 3 + 2 + 3	+1 +1		+ + + + + + + 2 + 2	++++	+ + + + + + + + + + + + + + + + + + + +	+ + + +	+1 +1
Gigartina stellata Percentage devoid of	12 1 4	1 1 1 3	+ + + +	+ + + +	+ 2 + 1	1 1 + 3	1 3 1 2	+ 3 + 3
macrophytes :	20-40 20-40 40 40	50-70 50-70 23 24	70-90 70-90 29 29	50-70 50-70 43 47	$\begin{array}{ccc} 70-90 & 70-90 \\ 32 & 25 \end{array}$	60-80 40-60 29 30	70-90 5-10 * NR 30	70-90 5-10 49 50
Species lost ‡ Species gained§	5 5	4 5	6 6	4 8	12 5	3 4	No record	12 11

+ Signifies presence of species. * No record. † Braun Blanquet scale. ‡ Species lost, species which were recorded in 1966 but not found in 1967. § Species gained, species which were recorded in 1967 but not found in 1966.

in September 1966, by one of us (D. M. J.), gave a reference point for the post-disaster study.

Peak cropping methods used in the study of the primary production of kelp forest ecosystems have proved to be useful in comparative studies of the performance of species and species groups within such ecosystems in relation to the whole environment^{1,2}. Comparative studies of polluted and non-polluted ecosystems of this type within the disaster area should give corroborative evidence of damage if it has occurred.

All sites which had been surveyed in September 1966 were re-surveyed by exactly the same methods (those of the Zurich Montpellier school³) during the latter part of August 1967, some 5 months after the principal period of pollution by oil and detergent. The results are summarized in Tables 1 and 2.

Table 1 shows comparable phytosociological data for the littoral zones of eight sites. Cover values for the dominant species only are presented. The first figure in each case gives the cover value for the species for the whole littoral zone, the second is the highest cover value recorded for that species in any quarter square metre on the shore. Perhaps the most significant figures are those for the percentage of the rock surface between mean high water surface (MHWS) and mean low water surface (MLWS), which was devoid of attached macrophytes. At two sites, Sennen Cove and Porthleven, in August 1967. all stable rock surfaces were completely covered with a mat of green seaweed mainly Enteromorpha compressa4 and E. intestinalis. This is in sharp contrast to the situation at the same sites in 1966, and to the pre- and post-pollution data for all the other sites where the macrophyte cover was between 15 per cent and 70 per cent. Lamorna Cove deserves special mention; this site escaped during the principal phase of pollution, but was oiled and immediately cleaned with detergent in early summer. It is interesting that at this site three of the dominant species have increased in abundance. Inspection of the total phytosociological data (not presented here) shows no differential effect of loss or gain of the rarer species when comparing the polluted with the nonpolluted sites.

Study of the total phytosociological data for the sublittoral ecosystems shows that there has been no change in the cover abundance values for any dominant species. except Saccorhiza polyschides, which has shown a marked reduction at all sites, some far removed from the disaster area. Table 2 summarizes the floristic data for all sublittoral ecosystems studied. The loss and gain of species, all of which are in the category of lowest cover abundance. can be explained by natural loss or recruitment of species in mixed algal populations, or could be a result of the difficulties of sampling experienced by the diving phytosociologist. All that can be concluded from the data is that there has been no drastic effect on the species composition of the sublittoral ecosystems studied and that there appears to be no species which is particularly susceptible to this type of pollution.

At four sites, Roskilly, Hoe Point, Sennen Cove and Porthleven, more accurate studies of the overall cover values of the macrophytes were carried out. Table 3 gives the comparative data obtained from visual estimation of cover in twenty quarter metre square quadrats placed at random on solid rock surfaces at each site. If the very large cover values at both Sennen and Porthleven are attributable to pollution as indicated by the phytosociological data, then the effects of pollution are much less marked even in the immediate sublittoral.

Comparative studies of the structure and performance of kelp forest ecosystems at seven sites were carried out to investigate further any possible effects of pollution in the sublittoral.

Table 2. Numbers of algal species recorded in sublittoral ecosystems before and after pollution

Sites		No. of species recorded in 1966			No. of species recorded in 1967				Species	Species
	G	В	\mathbf{R}	Total	G	В	\mathbf{R}	Total	lost	gained
Unpolluted Polluted Polluted (littoral zone		10 19	29 48	$\frac{41}{72}$	$\frac{5}{7}$	$\frac{15}{23}$	29 59	49 89	20 20	13 11
markedly green)	4	12	32	48	7	13	37	57	15	õ
G. Green: B. brown	: 1	R. re	d al	zae.						

Laminaria hyperborea is a perennial algae, the stipe and hapteron growing for several years, a new lamina being produced each year from an intercalary meristems. Growth measurements of the perennial parts can be regarded as an integrated measure of performance in relation to the environment for the life span of the diplophase. Measurement of lamina production can be regarded as an assessment of performance in relation to the environmental conditions of the current growing season.

Table 3. MEAN PERCENTAGE ROCK SURFACE COVERED BY MACROPHYTES

	Unpolluted	Polluted	Polluted littoral zone markedly green			
	Roskilly	Hoe Point	Porthleven	Senner Cove		
Mid-littoral	21 ± 19	35 ± 17	* Horiz. 93 ± 4 * Vert. 99 ± 3	98 ± 4		
Lower littoral	36 ± 22	48 ± 15	* Horiz, 95 ± 4 * Vert, 97 ± 2	97 ± 6		
Immediate sublittoral	48 ± 22	56 ± 12	48±15	60 ± 18		
* Horizontal and ve	rtical rock fa	ices.				

Because of the great variability of the inshore marine environment over very short stretches of coast, it would be impossible to draw conclusions regarding the effect of pollution simply from a direct comparison of lamina performance unless the effect had been drastic. Using stipe performance at each site as a scaler for the effects of natural environmental conditions, it should be possible to arrange the sites in a decreasing order of "kelp potential". If lamina production has not been affected by pollution, then the sites should fall into the same order when arranged on the basis of lamina performance.

In Fig. 1 is shown the mean standing crop of both stipe and lamina plotted against age for kelp forests in shallow water where the greatest effects of pollution might be expected (0-6 m depths) at each site. Arrangement of the sites on the basis of integrated figures either

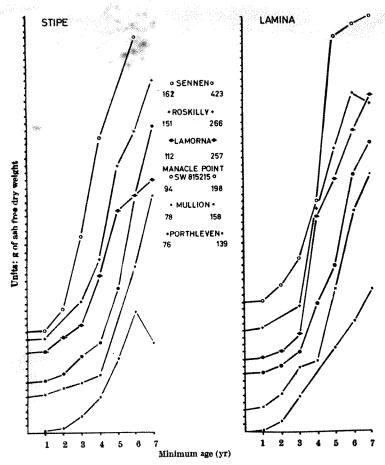


Fig. 1. Mean individual standing crop plotted against age. The origin of each curve is zero for that curve. Units were g of ash free dry weight; for stipe 1 unit = 1 g; for lamina 1 unit = 2 g. The "integrated performance figures" are corrected for this scale difference.

for stipe or for lamina production is the same. The figures which are simply the area beneath the growth curves in each case $\int_0^T w dt$ are shown beside the graphs.

Comparison of the age structure of all kelp forests studied reveals no striking differences between polluted and non-polluted sites except for a much larger number of first year plants at the two polluted sites, Porthleven and Sennen. Table 4 shows the comparative data. It is emphasized that the figures are derived only from the counts made as part of the production studies. They are not large enough to allow statistical treatment; however, the difference noted is so striking that it is felt it should be put on record.

Table 4. MEAN NUMBER OF FIRST YEAR KELP PLANTS/Ma

Depth below low water mark	Porthallow Manacle Rocks	Ros- killy	Mullion	Hoe Point	Lamorna	Porth- leven	Sennen Cove
0-3 m	4	4		4	2	36	28
3-7 m	õ	2	4	0	0	26	14
7-10 m	8		1	1	0		12
10-13 m	4		8	3			2

All the evidence gained from this study indicates that the effect of pollution from the Torrey Canyon disaster has been to alter the balance of littoral and sublittoral ecosystems dominated by attached macrophytes. This effect is most marked on the littoral zone and rapidly falls off with depth below low water mark.

The replacement of the normal littoral dominants by *Enteromorpha* spp. could be explained by alleviation of competition because of a reduction in the vigour of the former at the polluted sites. This seems an unlikely explanation when the low pre-pollution cover values are

taken into consideration. The replacement of these open mixed populations by a closed mat of Enteromorpha, which still harbours all the dominant species present in 1966, could best be explained by a drastic reduction in the populations of grazing organisms. The other possibility, a direct stimulation of the growth of Enteromorpha, seems unlikely. Georges, and O'Sullivan and Richardson', both report very high mortalities of littoral herbivores, especially limpets on shores treated with detergent. Southwards has shown by experiment that on exposed shores the low cover values of littoral macrophytes can be a result of high population densities of Patella. It might therefore be inferred that all sites showing this phenomenon (green sites) were badly affected by detergent. In many cases local enquiry did not clarify the exact details of the "clean up" operations. There is no doubt that the study areas of both Sennen Cove and Porthleven were treated by direct application of detergent at all states of the tide. Detergent was also sprayed on the study zone at Lamorna while the tide was out. The exact period of treatment of the other sites is not at all clear. We do, however, know that the littoral of all sites referred to as polluted was at some stage covered by a thick layer of oil and that detergent was used in the near vicinity.

Corroborative evidence was gained from a study of the 1967 limpet populations. Table 5 gives the results of a census of limpet populations (Patella vulgata, P. intermedia and P. aspera⁹) carried out at six sites. The figures recorded are simply the means derived from counts in twenty quarter metre square quadrats placed randomly within each zone. No limpets were recorded from the littoral zones at either Sennen Cove or Porthleven, in marked contrast to all other sites which had active

populations of limpets in August 1967.

Porth Mear is one of the most northerly sites affected by oil from the Torrey Canyon. Detailed records of the course of pollution and of the "clean up" operations were kept by one of us (T. D.) at this site.

Three adjacent areas of shore showed the full range of the effects of the disaster. Passing from south to north along the inner section of the bay, an overall distance of only 300 m, the first section was unpolluted, the next was oiled but was left to natural processes of cleansing, and the third section was oiled and cleansed with 2,000 gallons of detergent which was applied direct to the shore while the tide was out.

Table 5. LIMPET CENSUS RESULTS

	Porthallow	Ros- killy	Hoe Point SW 532278	Cape Cornwall	Porth- leven	Sennen Cove
Mid-littoral	48 ± 37	92 ± 56	20 ± 4	40 ± 25	* Vert. 0 * Horiz. 0	0
Lower littoral	28 ± 22	28 ± 23	8 ± 7	18 ± 21	• Vert. 0 • Horiz. 0	0

Figures are mean number of limpets/m².
* Rock face.

The littoral zones of the three sectors were studied using all the methods set out above. Sublittoral studies were confined to two areas, that directly below the artificially cleansed sector and that immediately south of the unpolluted sector, because it was felt that dispersion of the detergent could nullify any differential effects over a shorter distance. The results are summarized in Table 6, and it can be readily seen that they back up the findings of the main study in all details. The results also indicate that it might be safe to conclude that all "green shores" were the result of direct application of detergent during

Table 6. PORTH MEAR DATA, GRID, REF. SW 847716

Littoral dominants:	Site 1 unpolluted	Site 2 oil alone	Site 3 oil + detergent
Enteromorpha spp. Pelvetia canaliculata	1 3 + 1	1 3 + 1	5 5 + 1
Pucus spiralis F. vesiculosus F. serratus	1 3 1 3	† † 1 3 1 3	† † 1 3 1 3
Gigartina stellata Total cover of macrophytes Limpets/m*	23 ± 20 52 ± 31	$^{+}_{22\pm19}^{+}_{32\pm21}$	+ + 83 ± 3 3 ± 5
	Unpolluted	Polluted	Species common to both areas
Sublittoral: Greens Browns Reds Total	3 25 20 48	2 26 21 49	1 17 17 17 35
Number of first year kelp plants/m²: 3 m depth 8 m depth	4 12	36 20	
Kelp performance data $\int_{-T}^{T} w dt$			
2 m depth Stipe ⁰ Lamina	37 133	28 103	

periods of low water, and that all other polluted shores were not subject to high enough concentrations of detergent to produce this effect.

The following hypotheses are advanced to explain the results of these studies. (1) The effect of pollution from the Torrey Canyon disaster has been to alter the balance of ecosystems dominated by attached macrophytes by the destruction of the grazing organisms. (2) This effect is most marked in littoral ecosystems in which the herbivores play an important part in the maintenance of the balance of the primary producers. (3) The effect was caused by detergent or the detergent oil emulsion and not by the oil alone. (This is in marked contrast to the findings of the study of the Tampico wreck area10, where the populations of grazing organisms were decimated, although no detergent was used.) (4) The effect was most drastic at sites where detergent was applied direct to the littoral zone during periods of low water. It seems surprising then that any effect was found in the sublittoral, and it seems even more surprising that it was only found at the three "green sites", Sennen, Porthleven and Porth Mear 3, especially the latter where the volume of detergent used was comparatively small. If the high numbers of sporelings recorded at these sites

are any more than coincidence of quadrats selected, then an explanation for the phenomenon must be sought. It is suggested that the detergent or emulsion flowed down the shore and maintained a high enough concentration at the rock-water interface to do the necessary damage even at a depth of 7 m. This would corroborate the visual evidence of the death of all fauna in a shallow sublittoral gulley at Porthleven¹¹. At sites where detergent was added to oil floating on water dispersion and dilution of the emulsion through the water mass must have been sufficient to reduce its concentrations to levels which might only affect filter feeders or carnivores after concentration through the food chain. (5) If pollution had occurred later in the year (compare Lamorna) when all the algae would be in a more advanced state of development and grazing would have already taken its toll on juvenile stages, the overall effect would not have been so great. (6) There is no long term direct damage to the algae by either oil or detergent.

A brief visit to a number of the sites in October 1967 showed that changes were already taking place. The green carpet at Porthleven was variegated with patches of bare rock each supporting a new colony of limpets. The following limpet counts were obtained: Roskilly, mid-littoral $174 \pm 37/\text{m}^2$; Porth Mear 1, mid-littoral $81 \pm 20/\text{m}^2$; Porth Mear 3, mid-littoral $4 \pm 4/\text{m}^2$; Porthleven, mid-littoral vertical surfaces 0.15/m2; Sennen Cove. no limpets present.

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Effect of Humming on Vision

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Humming causes the eye to vibrate and this can produce a stroboscopic effect when a rotating black and white strobe disk is viewed in non-fluctuating light.

THE correct speed of a gramophone turn-table may be checked by viewing a strobe disk illuminated by electric light supplied by the a.c. mains. The strobe disk consists of alternating black and white sectors radiating from the centre of the turn-table, the angle between sectors being such that when the turn-table is rotating at the correct speed each white sector replaces its white neighbour in 0.01 sec. Because in Britain the a.c. produces a fluctuation in illumination 100 times/sec, the white strobe sectors will always be in a fixed position in space when the light is in its bright phase, and in the intermediate position at the dark phase. Thus, when the turn table rotates at the correct speed, the pattern of alternate lighter and darker

sectors will be seen stationary in space; but it will rotate slowly in the same direction as the turn-table (clockwise) if the speed is slightly too fast. So much is well known and

When the disk is viewed in non-fluctuating light (for example, daylight) naturally no strobe pattern is seen, but the observer may produce it by humming loudly. The note to be hummed for a stationary pattern is 100 c/s (bass Ab). If the note hummed is flat the pattern rotates clockwise; if sharp, counter-clockwise. When several observers hum at once, each sees the pattern moving in the direction appropriate to his own hum, independent of what others are humming and seeing.

Obviously humming interacts with vision in some periodic fashion, the period corresponding to the note hummed. This paper concerns the nature of the interaction.

The perception of visual detail results from a train of events. (a) Light is focused as a sharp image on the retina; it enters the foveal cones, is absorbed by the visual pigment contained in their outer segments and nerve signals result. (b) These signals are transmitted (with mutual interaction) so that they finally reach the brain and perception is a consequence of what they do there.

Impairment of Nerve Transmission

Perhaps humming affects, in some periodic manner, the transmission in visual nerves, for example, by interaction with auditory nerve impulses, shaking the nerve connexions in brain or retina, etc. If the nerve signals, on which the sense of brightness depends, fluctuated 100 times/sec because of such interaction, the effect would be as though the light itself were fluctuating in luminance at this frequency and thus we should see the same strobe pattern with humming as with a.c. lighting.

Light does not set up its nerve message instantly; there is a delay (latency) which depends on the light intensity. At moderate illumination a ten-fold increase in the light energy reduces the latency by 10 msec. This can be observed in electroretinograms resulting from light flashes of various intensities, but a more entertaining observation

is the Pulfrich pendulum1.

A pendulum swinging in a vertical plane is viewed binocularly by an observer with a dark glass over one (for example, the left) eye. He soon adapts to the darker view so that he sees nearly equally with both eyes, but the nerve message is set up with greater delay behind the -10 msec if the glass had 10 per cent transdark glassmission. Thus, in judging the momentary position of the pendulum, the unobstructed right eye sees "where it is", the left where it was 10 msec ago. The reader may verify at once that these two lines of sight intersect not in the plane of swing of the pendulum but behind it on the swing to the right, in front on the swing to the left. The pendulum, therefore, appears to execute an elliptical orbit from whose eccentricity (in conjunction with the geometry of viewing) we may measure the latency difference in the two eyes; it is found to be about 10 msec with a 10 per cent transmitting glass2.

We may now apply this result to the stationary strobe pattern produced by humming. In the same way that placing the dark glass before the eye made the sight of the pendulum refer to a moment 10 msec earlier, so with humming (if the appearance of dark sectors is caused by a periodic impact with nerve signals) the interposed dark glass would cause the dimming to refer to a moment 10 msec earlier. Thus reducing the light down to 10 per cent would cause a rotation of the stationary strobe

pattern by one white-to-white spacing.

I closed one eye and with the other looked at the strobe pattern through a photometric wedge of graded absorp tion from near transparency to 10 per cent transmission. I satisfied myself that I could hum sufficiently in tune with the proper note (provided by an oscillator) to keep the strobe pattern fixed. While in this condition I moved the wedge across the eye so as to change the light intensity rapidly back and forth some eight-fold. The pattern should have rotated back and forth nearly from one white sector to the next. But it remained practically stationary and such movement as occurred was clearly caused by slight variations in the pitch of my hum and was not correlated with the movement of the wedge. This makes it certain that humming interacts with vision before the place where latency develops. Category (b) is excluded; we must examine category (a).

Vibration

Because humming acts on the visual system before nerve messages have been developed to the stage recorded

by the electroretinogram, it looks as though it should act by vibrating the eye. In that case external vibration applied to the head should act similarly. I obtained a vibrator and drove it from an electric sine wave generator whose frequency and amplitude were under control. The same strobe pattern was seen in steady light when, instead of humming, the head was vibrated at the same frequency (for example, 100 times/sec). The vibrator acted well when applied to almost any bony part of the head; the cheek bone (zygoma) or chin was convenient. I could get stronger effects with the vibrator than with humming. When the vibrator was judged equal to humming in its effect on vision it also shook the head to about the same extent.

This was measured by attaching a dental impression to a flexible steel foot-rule held in a vice so that only horizontal vibration was permitted. The edge of the steel, close to the mouth, was brightly illuminated and some lun inous specks were observed in a microscope. When the subject bit on the impression and hummed, the horizontal vibration was communicated to the steel strip and the luminous specks were drawn out into luminous lines the length of which could be measured in the micrometer eyepiece of the microscope. Both humming and the equivalent applied vibration gave a peak-to-peak amplitude of 0·15 mm. With this head vibration the strobe pattern was very conspicuous, the hum was loud and, as the reader can verify, the vibration is easily felt with the flat of the hand on the head.

Impairment of Signal Generation

Perhaps humming or vibration affects in some periodic manner the efficiency with which quanta are caught by the cones or the catch turned into nerve signals. instance, if the turn-table rotates at correct speed and the hum or applied vibration is at 101 c/s, the strobe pattern slowly rotates counter-clockwise, bright sectors passing a fixed point once a second. According to the above explanation, as we gaze at the fixed point it will change from bright to dim and back once a second because the white sectors that are passing this point 100 times/sec are falling now in the efficient, now in the inefficient, phase of the 101 c/s vibration. If this is true, an expectation follows. The alternation of black and white produced by the rotating sectors may be presented without movement. It is only necessary to illuminate a white surface by a d.c. light interrupted at its focal point by a rotating sectored disk. With 50/50 on/off ratio at 100 flashes per sec, the white/black alternations of the strobe disk are imitated and with the head vibrating at 101 e/s we should see a 1 per sec fluctuation in brightness-a slow flicker whose temporal contrast should be about equal to the spatial contrast of the strobe pattern. When this expectation was tested, however, no fluctuation whatever could be detected either with humming or with external vibration of considerably greater amplitude. Evidently the fact of black-white alternation is insufficient as explanation; the way in which black replaces white is important.

Retinal Movement

A small front-surface mirror was set in a vertical radial plane just above the strobe disk. When viewed in a.c. lighting the radial strobe pattern could be seen reflected in the mirror, and when the speed of rotation was slightly changed, so that the sectors slowly advanced on the mirror (naturally) the image sectors advanced from behind the mirror to meet them, light meeting light and dark meeting dark. A less commonplace appearance resulted with humming or vibrating the head (viewing now with d.c. lighting). Again the strobe pattern was seen with sectors advancing on the mirror, and again image sectors advanced from behind to meet them, but now they have changed partners. Light sectors meet dark: dark meet light! If with the hum pattern stationary we point to a white sector, in the mirror we are seen pointing to a dark

sector. Wherein lies the asymmetry? There is none in the equipment, so it must lie in the eye.

The central retina is all moving in one direction at any given moment, but the image it receives is not. The disk and its mirror image turn in opposite senses and, when the retina marches with one, it will necessarily march against the other. Evidently the light/dark strobe pattern results from the superposition of rotation of the strobe on vibration of the retina.

Amplitude of Vibration of the Pattern on the Retina

If the head vibration simply translated all components of the eye equally, the movement of the image over the retina would be the same as if the eye was still and the vibration was applied to the turn-table. The head movement measured here was 0.15 mm. If this was applied to an object 85 cm away it would produce a fifty-fold reduction, that is, 3μ on the retina or 36 sec of arc in the visual field. With this vibration the strobe pattern was very conspicuous; a threshold vibration would be perhaps a third as great.

In order to measure more directly what amplitude of vibration made the strobe pattern detectable, the vibration was applied neither to head nor to object, but to a lens between them. The disk was viewed through a combination of two lenses, $+0.5\,\mathrm{D}$ and $-0.5\,\mathrm{D}$, close together so that zero convergence resulted and the disk was seen at its natural distance. One lens was fixed and the other could be vibrated. A displacement of x mm is easily seen to produce a deviation of $x/2,000\,\mathrm{rad}$ because the lens has 2,000 mm focal length.

When the strobe pattern was observed through the vibrating lens it had the same appearance as when vibration was applied to the head instead, except that the pattern was weak or absent along the diameter parallel with the direction of lens vibration. The amplitude was decreased to the point where the strobe pattern could only just be detected at the most favourable place. The amplitude of the lens movement was measured, as before, by viewing luminous specks on the lens surface in a microscope fitted with a micrometer eyepiece. The movement, x, was 0.09 mm giving 9 sec of arc, or 0.8µ on the retina. The distance between the centres of adjacent cones on the central fovea is 3µ; visual acuity is seldom better than 30 sec of arc. Thus the displacement of the image

across the retina is well below the limits of resolution, and in fact humming does nothing to blur the sharp view of a star. Our final question is "If the vibration is too small for detection when the object viewed is stationary, how is its effect so conspicuous when the object is rotating?"

Superposition of Movements

We have seen that the white sectors of the strobe pattern appear when the motion of the disk combines with one phase of vibration, the dark sectors with another phase. What are these phases? To answer this a 4 D lens was placed 500 mm above the turn-table so that a real life-size image was formed 500 mm above the lens. A beam splitter was placed just below this image plane. Thus when the lens was vibrated at proper speed the strobe pattern could be seen in the image planes by transmission or reflexion in the beam splitter. A fine slit was placed in the image plane above, aligned to coincide with the radiating lines of the strobe disk, and above the slit was a photocell which consequently received light either from the white or the black sectors as they rotated below. When the lens was not vibrating, white occupied one half of the cycle and black the other half, as the record Fig. 1(i) shows. The whole tracing occupies a little more than 1 cycle (horizontal) and the bright phase produces an upward deflexion, which lasts half the cycle.

When the lens was vibrating, the strobe pattern was seen reflected in the beam splitter, white and dark sectors slowly passing a thread placed to coincide with the image

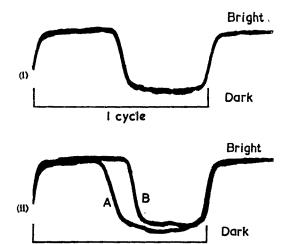


Fig. 1. The rotating strobe disk was focused on to a radial slit which admitted light to a photocell. (i) Photocell output showing one half cycle recording white sector, the other half recording the dark sector, (ii) Records when the focusing lens was vibrating once in a black-white-black cycle. A is when a dark strobe band lay on the slit, B when a bright band lay there.

I cycle

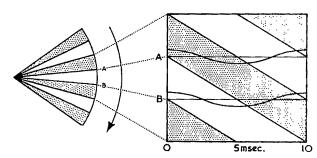


Fig. 2. Diagram to explain results of Fig. 1 (see text).

of the slit seen by reflexion in the beam splitter. Fig. 1(ii) shows two records taken in succession, A at a moment when a dark sector lay on the slit, B when a bright sector lay there. It is plain that in A the upward deflexion occupies less than half the cycle, in B more than half. Consequently the dark bands (A) are where we view an on/off flicker at 100 c/s with the light on less than 50 per cent of the time. This must give an average (Talbot-Plateau) illumination less than (B) where the light is on more than 50 per cent of the time.

Fig. 2 indicates how the records of Fig. 1 arise. At the left is represented the rotating strobe disk (with sector angles drawn four times too large). The vertical at 0 on the square graph represents (at time 0) the position of the rim AB and the adjacent dark segments. As the disk rotates, the white sector AB steadily descends. After 5 msec, A on the disk will have fallen to the initial level of B; after 10 msec the cycle is complete and a new white sector occupies the position AB on the retina. In the absence of vibration, the retinal points A and B remain fixed and follow the horizontal lines which clearly lie half in the white and half in the shade—as in Fig. 1(i). If, on the other hand, the retina is vibrating, retinal points A and B may follow the sinusoidal curves shown. These cause A to lie longer in the shade—as in Fig. 1(ii).

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Research Strategies in Schizophrenia

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A co-ordinated programme of research into schizophrenia is necessary in Britain.

Ir has recently been demonstrated beyond doubt that there is a genetic factor in schizophrenia in addition to the psychological and environmental factors involved (refs. 1-4 and personal communication from S. S. Kety et al.). This genetic factor must presumably be expressed by means of some biochemical mechanism. It would therefore seem to be a good moment to evaluate the current state of research into the biochemistry of schizophrenia.

The picture is not very bright. A great deal of work has been carried out in the past 50 years with few results. There have been three chief reasons for this: (1) the methodology has often been poor with inadequate controls; (2) the bulk of the work has been carried out in a purely empirical way without any directing hypothesis; (3) insufficient attention has been paid to certain methodological problems peculiar to this field which I shall attempt to outline here.

If we believe that there is a biochemical factor in schizophrenia, then, I would argue, the optimum strategy to follow in research would consist of four stages which must be tackled in order. This same strategy has been followed in the case of depression with notable success. These stages are as follows (with the details filled in for the case of depression).

Stage 1. The discovery of specific chemical agents that can induce the clinical syndrome (or an acceptable model) or alleviate it (for example, reserpine, α-methyl DOPA; monoamine oxidase inhibitors, imipramine). Stage 2. The discovery of the mode of action of these agents on the biochemical mechanisms of the brain (compare the vast literature extant on this subject: the key discovery here was the mode of action of reserpine). Stage 3. Testing the performance of the systems discovered in stage 2 in the patient. Stage 4. Trying to correct any lesion discovered in stage 3.

In depressive illness this programme is well into stage 3 in Edinburgh, Epsom and elsewhere. In schizophrenia research, however, we are still only in stage 1. What is now needed is to break into stage 2. We now have a number of drugs which in certain people produce a psychotic reaction markedly like schizophrenia, but we have very little idea how these drugs work. We also have a number of working hypotheses as to the nature of the biochemical lesion which may be involved in schizophrenia, but these are based only on stage 1 data.

Working Hypotheses

The first working hypothesis was put forward in 1952 by Osmond et al.5, and suggested that an aberration of methylation of catecholamines could lead to the production in the body of hallucinogenic relatives of mescaline. This was based on a comparison between the molecular structure of mescaline and adrenaline (that is, stage 1 data). In the next 5 years two developments occurred. (1) Harley-Mason⁵ had suggested for the first time in the literature that catecholamines might be O-methylated in the human organism: a prediction that adumbrated Axelrod's discovery in 1957 (unpublished work) that catecholamines are normally metabolized by 3-0-methylation by catechol-O-methyl-transferase (COMT). clearly strengthened our 1952 hypothesis, for the lesion postulated now became a small step away from a normal metabolic pathway. (2) Similar hypotheses were subsequently put forward with regard to serotonin and melatonin, both of which are close chemical relatives of hallucinogens (psilocyn and harmine, respectively).

It thus became clear that central transmitters (nor-adrenaline, 5-hydroxytryptamine and dopamine) have a number of psychotomimetic relatives and that the relationship in each case is the same; O- or N-methylation. This led to a complementary and more general hypothesis that schizophrenia is associated with some disorder in trans-methylation mechanisms.

The next stage, lasting from 1962 to the present, consisted chiefly of looking for mescaline-like compounds in the urine. This has still failed to yield conclusive results although most recent evidence suggests that these are various "pink spots", one of which may be tyramine, and another has finally definitely been identified as DMPE (ref. 6), but these may have no direct relevance to the illness. I suggest that a better policy to guide research in the next decade may be the intensive study of the mechanism of action of known chemical agents that can in certain normal individuals induce a psychosis remarkably similar to schizophrenia. The biochemical mechanisms underlying these forms of psychosis may in turn suggest hypotheses as to the type of biochemical lesion that is present in schizophrenia. That is to say, we must carry out stage 2 before we can hope to make any really useful hypotheses in stage 3. The tactics that it would be necessary to follow to carry out this strategy are presented in the form of three interlinked programmes.

Programme 1. When we come to consider how the mode of action of these drugs may be investigated-and why we still know so little in this field-we note at once an important difference between the hallucinogens (and other psychotropic drugs) and the other drugs studied in classical pharmacology. For if we apply the methods of classical biochemistry and biochemical pharmacology to these drugs we may well discover which enzymes, transport mechanisms, metabolic reactions and so on they affect, and details of their own metabolism. But there is no way of telling which (if any) of these properties are related to the effect of the drug on behaviour nor which metabolites are themselves hallucinogenic. In order to determine this we have to repeat these studies using close structural analogues of the drugs in question, some of which are hallucinogenic and some of which are not. Important studies in this field are those of LSD and BOL by the Sandoz group-which showed that the antiserotonin effect of LSD and its capacity for inhibiting cholinesterase are not relevant to its hallucinogenic effect—and the work of Freedman?, who showed that the capacity of LSD for increasing the rate of turnover of serotonin in the brain is so related. Thus the biochemical and pharmacological studies of hallucinogens cannot be properly evaluated without preceding structureactivity relationship (SAR) studies of these drugs and their analogues with respect to their effect on behaviour.

Programme 2. Thus a second programme analysing the interaction of hallucinogens and their analogues with behaviour is necessary. The Sandoz group have carried out extensive SAR work on LSD, but little work has yet been done on the much more interesting compounds mescaline and dimethyltryptamine. It is very difficult these days to give these drugs to humans, and so it has been necessary to develop animal tests that will enable us reliably to detect drugs that are human hallucinogens. These techniques are now available and have been used

in some of the SAR studies suggested^{9,10}. This programme may also be expected to yield new compounds of interest for study in programme 1, such as paramethoxyamphetamine, as well as new agents for exploring the biochemical mechanisms of the brain¹¹. These techniques have, however, additional uses, as follows.

Programme 3. (a) The study of the effect of manipulating brain amines and other brain chemicals on the behavioural effects of these drugs¹², and (b) the promising technique of pretreatment with inactive close structural analogues to see which can inhibit the active drug12. Clearly, here again we have to know which are the inactive analogues. It should be noted again that programme 1 cannot be evaluated, and programme 3b cannot be carried out, without programme 2, and it seems clear that a study in which all three programmes can be carried out in an integrated fashion would be desirable. They would require close collaboration between organic chemists, biochemists, enzymologists, pharmacologists and psychopharmacologists.

Probable Results

What results can be expected from these three interrelated programmes?

- (1) Programme 2 will add to our list of known hallucinogens. Were this all the programmes could do, they would not have much scientific merit and indeed might be less than helpful because hallucinogens are developing into social pests. We have, however, seen that programme 2 is also an essential prerequisite for programmes 1 and 3. Furthermore, the inactive analogues also discovered may be useful in treating addicts and we might avoid the situation of new hallucinogens catching the health authorities by surprise (as in the case of STP). This work might eventually lead to some answers to the problem of misuse of these drugs.
- (2) All these programmes should throw light on the biochemical mechanisms of action of the hallucinogens: programme 1 (+2) directly: programme 3a (+2) on modes of the interaction of hallucinogens and brain amines suggested by clinical data, by their chemical formulae and by Freedman's works; and programme 2 itself by means of possible deductions that could be made from the chemical formulae of active versus inactive hallucinogens, to possible biochemical mechanisms at their site of action. Studies with SAR are often disappointing in this field and it is rarely possible to argue from them directly to the possible molecular configuration of the receptor site, and, indeed, we may be "unable to make answers even to the simplest questions about the interaction of sympathetic amines and their receptors"13. It is sometimes possible, however, to construct working hypotheses about biochemical mechanisms involved which can be tested by experiment. In this case two such hypotheses have already been formulated9 and one of these is currently being tested directly. Furthermore, the technique described in programme 3b can often give further useful data for this purpose.
- (3) The data obtained by the processes described in the preceding paragraph may enable us to construct specific and testable working hypotheses as to the nature of the biochemical lesion in schizophrenia. The current hypotheses on this topic are based on a simple comparison of the molecular formulae of the hallucinogens and the central transmitting agents and therefore a knowledge of their mode of action may be expected to generate better working hypotheses. It seems likely that there are no short cuts here and much more basic work needs to be done before we can arrive at telling hypotheses in this field: the recent confusion over the "pink spot" indicates this clearly enough.

Criticism of the General Hypothesis

Finally, the general hypothesis outlined here has been criticized (largely under the influence of the dis-

appointment that the "pink spot" might be only a red herring) as having yielded only meagre results in its 15 years of existence.

A hypothesis, as long as it is not actually refuted, can only be judged on results; that is to say, on the previous disparate phenomena that it explains and the predictions it makes that can be tested by experiment. I have described the research programme suggested by this general hypothesis which is designed chiefly to generate even more specific predictions and hypotheses; for this we need to know much more about the mode of action of psychotomimetic drugs. The particular specific hypothesis formulated may well be false. It is certainly readily falsifiable. If it is, this possibility can be cleared out of the way and a new specific hypothesis constructed from our basic premises, but this can only be done if we have more facts. I should like to conclude by listing the known facts in this field which the general hypothesis can account for.

(1) The clinical correlation between schizophrenia and stress14 and the malignancy of the condition (through the vicious circle that would be set up if the stress mechanism were itself to elaborate highly stress-producing compounds). (2) The clinical links between schizophrenia and depressive illness and Parkinsonism (which may then be related to disorders in the metabolism of noradrenaline. 5-hydroxytryptamine and dopamine, respectively). The fact that the known hallucinogens are O- or N-methyl derivatives of the central transmitters (noradrenaline. 5-hydroxytryptamine and dopamine) or are derivatives of neurohumours (melatonin). Any theory that cannot account for this fact loses credence. (4) The therapeutic effect of the phenothiazines, which inhibit central adrenergic mechanisms18 as well as inhibiting N-methyl transferase. (5) The reported effect of methionine-monoamine oxidase inhibitor mixtures on schizophrenia16.

The hypothesis is merely a working hypothesis: however, as Kety¹⁷ has said, it is the best that we have at the moment. One should certainly keep an open mind about other hypotheses, but at present there is none in the field that can account for the phenomena.

The current sad muddle that constitutes so much of schizophrenia research might be alleviated if a coordinated interdisciplinary and interdepartmental programme were to be set up along the lines described here. In the United States brisk work along these lines is already under way and work is developing in the Soviet Union. In Britain, however, there is only a handful of people engaged in research into the biochemical basis of schizophrenia, which seems odd, because schizophrenics still occupy more than one quarter of the hospital beds in the country.

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Site of Attachment of Retinal in Rhodopsin

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On illumination, rhodopsin passes through several unstable intermediates to a final mixture of retinal and opsin. One of these intermediates, metarhodopsin II, can be reduced by sodium borohydride to bind irreversibly the prosthetic group retinal to opsin in secondary amino linkage.

THE rhodopsins are the visual pigments most abundantly found in vertebrate retinas. They contain the 11-cis isomer of retinal (vitamin A aldehyde, formerly called retinene), bound as a prosthetic group to a lipoprotein moiety (opsin). The chief action of light is to convert this isomer to the all-trans configuration, a process followed in the dark by the formation of several transient intermediates and the eventual liberation of free trans retinal (see Fig. 1). In the course of this reaction sequence, usually referred to as the bleaching of rhodopsin, nervous excitation of visual cells is initiated.

The work of Collins² and Morton and Pitt³ suggests that a Schiff base linkage binds retinal to opsin

$$\mathrm{C_{19}H_{27}CH}{=}\mathrm{O} + \mathrm{H_2N\text{-}opsin} \rightarrow \mathrm{C_{19}H_{27}CH}{=}\mathrm{N\text{-}opsin} + \mathrm{H_2O}$$

A previous paper has shown that such a linkage is in fact exposed when cattle rhodopsin is bleached by light and can be reduced by sodium borohydride

$$C_{19}H_{27}CH$$
=N-opsin $\xrightarrow{NaBH_4}$ $C_{19}H_{27}CH_2NH$ -opsin $\xrightarrow{4}$

The carbon-nitrogen double bond apparently becomes accessible as the intermediate metarhodopsin II forms; it is probably exposed by conformational changes in opsin which are known to accompany the transformation of metarhodopsin I to metarhodopsin II (Fig. 1). The product of the reduction is an N-retinyl-opsin, a rhodopsin derivative in which a stable secondary amine linkage binds the fluorescent prosthetic group to the site which it occupies in metarhodopsin II. Furthermore, this site is probably the same one which the retinyl group occupies in native rhodopsin⁴.

The irreversible attachment of the retinyl group to opsin provides a handle which can be used to probe the active site of cattle rhodopsin and to identify the group to which retinal is bound. One would like, eventually, to explain why the binding of 11-cis retinal cattle rhodopsin causes a shift in its absorption maximum from 382 mμ to 498 mμ, from the ultraviolet to the blue green portion of the spectrum. Hubbard has suggested that this shift involves the binding of 11-cis retinal to opsin as a protonated Schiff base (C₁₉H₂₁CH=NH-opsin), with groups on opsin causing a displacement of the positive charge into the polyene system of the retinyl group, thus promoting resonance and so shifting the spectrum towards the red*.

To help evaluate this and other hypotheses which have been suggested, one needs to know what groups occur

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near the retinal in rhodopsin. This article identifies the residue on opsin which binds retinal, and provides an analysis of the amino-acids which are covalently linked in its immediate neighbourhood. The picture which emerges will be completed only when one also knows which groups the tertiary structure of rhodopsin brings close to the prosthetic group, for these probably also contribute to the development of colour.

Identification of the Residue which binds Retinal in N-retinyl-opsin

N-retinyl-opsin can be prepared in quantity by irradiating rhodopsin solutions in the presence of sodium borohydride. These solutions are obtained by extracting membrane preparations of rod outer segments with 2 per cent aqueous digitonin. They contain rhodopsin on a large micelle with digitonin.

N-retinyl-opsin was prepared by irradiation for 5 min, at room temperature, of 70 ml. of a 5×10^{-5} molar solution of rhodopsin which had been brought to pH 8 with 1 normal sodium hydroxide, and made 10^{-2} molar in sodium boro-

$$\begin{array}{c} \text{Rhodopsin} \quad (498 \text{ m}\mu) \\ \downarrow \quad h^{\nu} \\ \text{Pre-lumirhodopsin} \quad (543 \text{ m}\mu) \\ \downarrow \\ \downarrow \\ \text{Lumirhodopsin} \quad (497 \text{ m}\mu) \\ \downarrow \\ \text{Metarhodopsin} \quad I \quad (478 \text{ m}\mu) \\ \downarrow \\ \uparrow \\ \text{Metarhodopsin} \quad II \quad (380 \text{ m}\mu) \\ \\ C_{19}H_{27}C = N \text{-opsin} \\ \downarrow \\ H_{10} \\ \end{pmatrix} \\ \text{All-trans retinal} \quad (387 \text{ m}\mu) + \text{opsin} \\ C_{19}H_{27}C = O + H_{2}N \text{-opsin} \\ \downarrow \\ H \\ N \text{-retinyl-opsin} \quad (333 \text{ m}\mu) \\ C_{19}H_{27}CH_{2}NH \text{-opsin} \end{array}$$

Fig. 1. Intermediates in the bleaching of cattle rhodopsin. Absorption maxima are shown in parentheses. Between —15° C and 5° C metarhodopsin I and metarhodopsin II exist in an equilibrium in which formation of metarhodopsin II is favoured by increases in temperature, acldity, and glycerol or neutral salt concentration. The normal bleaching sequence can be interrupted at the stage of metarhodopsin II by sodium borohydride, which reduces the Schiff base linkage between retinal and opsin before its hydrolysis can occur.

hydride. A Sylvania 'Sun Gun' which had been filtered to pass only wavelengths between 400 and 700 m μ was used as the light source. The N-retinyl-opsin solution was dialysed against water overnight at 4° C to remove excess borate ions, and was stored at 4° C. The fluorescence of the retinyl group, which has its excitation maximum at 330 m μ and emission maximum at about 480 m μ , could be observed in solutions and on chromatograms with an ultraviolet hand lamp. The molar extinction of the retinyl group is approximately 50,000 at 333 m μ (ref. 8).

N-retinyl-opsin was broken down to its constituent amino-acids by alkaline rather than acid hydrolysis because the retinyl group is destroyed by acids. N-retinylopsin solutions (usually 10-20 ml. 5×10^{-5} molar in the retinyl group) were made 2 normal in sodium hydroxide, evacuated and flushed three times with nitrogen in a 'Teflon' test tube, sealed under nitrogen and kept at 100° C The hydrolysate was applied to a column of for 6 h. silica gel HR (Merck-Brinkman) 15 cm long and 2.5 cm in diameter which had been prepared in chloroformmethanol-ammonium hydroxide in the volume ratio 70-27-3, and was eluted with the same solvent. fluorescent fractions were obtained, of which the second had the absorption spectrum of the retinyl group and accounted for half of the retinyl group present in N-retinylopsin before alkaline hydrolysis.

Fig. 2 shows the chromatographic mobility of this retinyl compound compared with six N-retinyl aminoacids. Each of the N-retinyl amino-acids, the synthesis of which is described in Fig. 3, was prepared in a volume

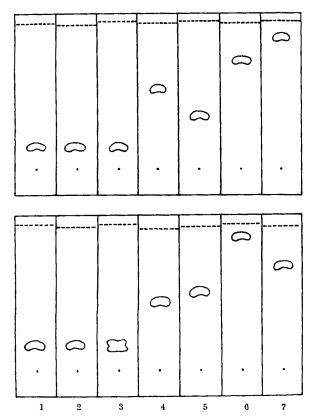


Fig. 2. Thin layer chromatography of the N-retinyl compound from N-retinyl-opsin and known N-retinyl amino-acids. A 250µ layer of silica gel MR (Merck) was applied to a 20×20 cm glass plate, scored into 3 cm zones, and 10-20 µl. portions of the retinyl compounds were applied 2·5 cm from the bottom of the plate. In the top chromatogram n-butanol-acetic acid-water in the volume ratio 80: 18: 2 was used as the eluting solvent. Chloroform-methanol-ammonium hydroxide (70: 27: 3) was used in the bottom chromatogram. Zone 1, N-retinyl-lysine; zone 2, the retinyl compound from N-retinyl-opsin; zone 3, N-retinyl-ornithine; zone 4, N-retinyl-aspartate; zone 5, N-retinyl-histidine; zone 6, N-retinyl-valine; zone 7, N-retinyl-tryptophan.

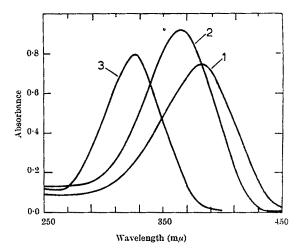


Fig. 3. Synthesis of an N-retinyl amino-acid. Curve 1 is the spectrum of all-trans retinal in 60 per cent aqueous methanol (1·7 × 10·5 molar; λ_{\max} 383 m μ). A 200-fold molar excess of alanine was added and the pH was adjusted to 9·5 by adding 1 normal sodium hydroxide. After 20 min the retinal had been converted to the Schiff base N-retinylidene—

alanine (curve 2; λ_{\max} 367 m μ ; $C_{10}H_{c2}C=N-CHC00^-$). Addition of 10 mg of powdered sodium borohydride reduced this Schiff base to the corresponding secondary amine. N-reliayl-alanine (curve 3; λ_{\max})

CH₃ 328 mμ; C₁₉H₂₇CH₂NHCHCOO).

of 20 ml. Fifteen ml. were then evaporated almost to dryness and passed through the same hydrolysis and column procedure as N-retinyl-opsin. All survived in 40-60 per cent yield, with their spectra and chromatographic mobilities remaining unchanged. The mobility of the retinyl compound obtained from N-retinyl-opsin was compared also with fifteen other N-retinyl amino-acids prepared from common amino-acids.

The retinyl compound from N-retinyl-opsin could not be distinguished from N-retinyl-lysine or N-retinyl-ornithine (the next lower homologue of N-retinyl-lysine) in thin layer chromatography9 (compare also Akhtar et al.¹⁰). Because N-retinyl-ornithine is a product of the alkaline decomposition of N-retinyl-arginine, the retinyl compound from N-retinyl-opsin could have been derived from either an N-retinyl-arginine or an N-retinyl-lysine residue which was present in N-retinyl-opsin before alkaline hydrolysis. To bind the retinyl group, however, arginine would have to be an N-terminal amino-acid of opsin, for only the a-amino group of arginine reacts with retinal to form a Schiff base. Attempts to find an N-terminal residue in either rhodopsin or opsin have so far been unsuccessful¹¹. The use of alkaline hydrolysis introduced a further ambiguity: in 2 normal sodium hydroxide at 110° (' the retinyl group undergoes an intramolecular rearrangement between the a- and s-amino groups of lysine, but does not pass to the amino groups of any other amino-acids which may be present. This rearrangement could be demonstrated by hydrolysing α -N-acetyl- ϵ -retinyl-lysine in 2 normal sodium hydroxide. The product was a mixture of α -N-retinyl-lysine and ϵ -N-retinyl-lysine. Reacetylation produced both α-N-acetyl-ε-N-retinyl-lysine and α-Nretinyl-ε-N-acetyl-lysine which (unlike α-N- and ε-Nretinyl-lysine) could be readily distinguished in thin layer chromatography8.

For this reason, alkaline hydrolysis was of limited usefulness in breaking down N-retinyl-opsin to a single N-retinyl amino-acid. Nor could acid hydrolysis be used, because it destroys the retinyl group and the ninhydrin colour of N-retinyl-lysine. Also, no combination of enzymes hay yet been found that releases a single N-retinyl amino-acid from N-retinyl-opsin⁸. Enzyme hydrolysis could, however, be used to free small peptides containing the retinyl group from N-retinyl-opsin^{8,12}. These peptides.

like N-retinyl-opsin, yielded a compound with the chromatographic mobility of N-retinyl-lysine on alkaline hydrolysis. The smallest peptide of this nature also had a free N-terminal residue which was not lysine, indicating that the retinyl group must be attached to an internal amino group. This is presumably the s-amino group of a lysyl side chain.

N-retinyl-opsin Peptides containing the Retinyl Group

Pronase, a mixture of proteases from Streptomyces griseus, was used to liberate small N-retinyl peptides from N-retinyl-opsin. A number of other enzymes, trypsin, chymotrypsin, pepsin and papain, effected some proteolysis in solutions of N-retinyl-opsin but left the retinyl group bound to a large undigested protein moiety. Proteolysis was most rapid when pronase was added directly to solutions of micellar N-retinyl-opsin (that is, still containing sufficient phospholipid and digitonin to render soluble the N-retinyl-protein present). When the digitonin and phospholipid were removed by ethanol extraction, the N-retinyl-protein precipitated and its complete digestion in aqueous suspension required several days.

In the digestion shown in Fig. 4, 10 ml. of a solution of N-retinyl-opsin (5 × 105 molar in retinyl group), containing a small crystal of thymol, was incubated with pronase under nitrogen at pH 7-3 and 30° C. Fresh pronase (Calbiochem, grade B) equal to about 2 per cent of the weight of the N-retinyl-opsin was added every 4 h and the pH was maintained at 7.3 by occasional additions of 0-1 normal sodium hydroxide. The fluorescent N-retinylpeptides freed by pronase digestion were soluble in ethanol and accounted for 60-70 per cent of the retinyl group present before digestion (Fig. 4). Their liberation was virtually complete after 14 h of digestion, and their relative concentrations and mobilities in thin layer chromatography did not change on further digestion. The retinyl material which did not become ethanol-soluble during the course of digestion remained bound to an N-retinyl-protein residue soluble in 75 per cent formic acid.

Columns of silica gel HR (Merck) were used to resolve these peptides. For the chromatogram shown in Fig. 5 an ethanol extract of a 14 h old pronase digest of N-retinylopsin (containing approximately 3×10^{-6} moles of retinyl group) was evaporated almost to dryness, dissolved in 5 ml. of chloroform-methanol-ammonium hydroxide in

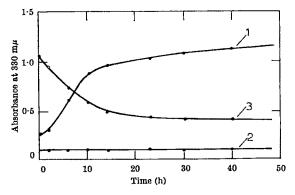


Fig. 4. Digestion of N-retinyl-opsin by pronase. Ten ml. of a solution of N-retinyl-opsin was incubated under nitrogen with pronase at pH 7·3 and 30° C. Samples (1 ml.) were removed at various times, lyophilized, and extracted with two 1 ml. portions of 99 per cent ethanol. The absorption of the pooled ethanol extracts at 330 mx is shown in curve 1. This provides a measure of the retinyl compounds extracted. The material in each aliquot left after ethanol extraction was then extracted with 2 ml. of water and the absorption of these extracts at 330 mx is shown in curve 2. Apparently no retinyl derivatives are water-soluble. Curve 3 is the absorption at 330 mx of the remaining residues dissolved in 75 per cent formic acid. This indicates the amount of rethyl material still bound to an indigestible protein fraction soluble only in 75 per cent formic acid; it accounts for one third of the retinyl group originally present in N-retinyl-opsin.

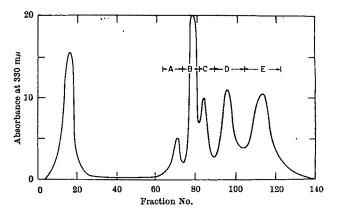


Fig. 5. Column chromatography of N-retinyl-peptides. The absorption of the first peak at 330 m μ does not reflect the presence of the retinyl group but is associated with the tail end of a large absorption band at 280 m μ ; this material moves with the solvent front and contains decomposed lipids and retinyl compounds.

the volume ratio 70: 27: 3, and put on a column of silica gel HR, 30 cm in length and 2.5 cm in diameter, which had been prepared in the same solvent. Only glass and 'Teflon' parts were used in constructing the column. A solvent reservoir containing the same solvent was connected, the system was put under nitrogen pressure of 5 lb./in.², and 2 ml. fractions were collected. The elution of discrete fluorescent retinyl-containing bands could be observed using an ultraviolet hand lamp. Of the retinyl compounds applied to these columns, 50-60 per cent were recovered in the fractions collected. The remainder was very strongly adsorbed to the columns, and could not be removed with the solvents used.

The spectra of the cluted peptides were virtually identical with the spectrum of N-retinyl-alanine shown in Fig. 3, curve 3. Peaks B, C, D and E, containing approximately half the N-retinyl material which cluted from the column of Fig. 5, were further purified by chromatography on columns of silica gel HR, 60 cm long and 0.9 cm in diameter, using the procedure just indicated. Peak A did not contain enough material to make further chromatography worth while, and N-retinyl-peptides cluted from the column after peak E (not shown in Fig. 5) have not yet been purified.

Table 1. Amino-acid analyses of n-retinyl material eluted from the column shown in fig. 5, after further purification, and hydrolysis in 6 normal hydrochloric acid

Residues present (N-		Pooled	fractions	
retinyl-lysine group)	В	C	D	E
Threonine Proline Alanine Isoleucine Phenylalanine	1·2 (1) 2·3 (2)	1·1 (1) 1·4 (1) 2·6 (3) 1·4 (1) 2·7 (3)	1·0 (1) 1·0 (1) 2·2 (2) 1·1 (1) 1·8 (2)	1·2 (1) 0·9 (1) 1·9 (2) 1·1 (1) 2·8 (3)

N-retinyl-lysine does not appear in these analyses because it is destroyed by acid.

Each of the purified N-retinyl peptide fractions was hydrolysed in 6 normal hydrochloric acid for 24 h. Longer hydrolysis did not produce further changes. These purified fractions each contained 1 to 5×10^{-8} moles of retinyl compounds. Amino-acid analyses of the rechromatographed peaks B, C, D and E are shown in Table 1, expressed as moles of amino-acid/mole of N-retinyl-lysine. N-retinyl-lysine does not appear in these analyses because both its ninhydrin colour and the retinyl group are destroyed by acid. All these peptide fractions, however, yielded a compound with the chromatographic mobility of N-retinyl-lysine on alkaline hydrolysis.

The N-retinyl amino-acid present in these peptides was ε-N-retinyl-lysine. This follows from the fact that the smallest peptide had phenylalanine as its N-terminal residue; this was identified with the N-terminal reagent 1-dimethyl-amino-naphthalene-5-sulphonylchloride⁸. With the N-terminal residue of this peptide free, the retinyl group must have been bound to an internal amino group, the ε-amino group of lysine. This smallest peptide was obtained pure in fraction B, and the compositions of the larger peptide fractions, C, D and E, were probably those indicated in parentheses in Table 1. The possibility that some of these fractions were heterogeneous, however, cannot be excluded. The largest peptide fraction had the average composition of the decapeptide: phe3, ala3, ileu, pro, thr, ε-N-retinyl-lys.

The amino-acid residues of N-retinyl-opsin listed in Table I occur adjacent to the lysine residue, the s-amino group of which binds the retinyl group. Presumably this same amino group binds retinal in metarhodopsin II and in native rhodopsin. The peptides containing these residues have overlapping compositions, as one would expect if borohydride reduction anchored the retinyl group to a single site in N-retinyl-opsin, and pronase cleaved a number of nearby peptide bonds. The smallest peptide has the composition phe2, ala, E-N-retinyl-lys, to which the larger peptides add thr, pro and ileu, plus additional alanine and phenylalanine.

The preponderance of non-polar side chains in these peptides indicates that they compose a hydrophobic region of the N-retinyl-opsin lipoprotein, perhaps not in direct contact with the aqueous medium. This might be the reason why they are difficult to free by enzyme hydrolysis, thus only the pronase mixture of non-specific proteases from Streptomyces griseus will hydrolyse these peptides. This hydrophobic environment might also tend to keep the ε-NH₂ group of lysine non-protonated at neutral pH, thus promoting its combination in Schiff base linkage with the aldehyde group of 11-cis retinal to form rhodopsin¹³. (In free solution the ε-amino group of lysine is

protonated at neutral pH, and can combine with retinal only at pH greater than 9 (ref. 14).)

It seems reasonable to assume that the retinyl group itself exists in a hydrophobic environment both in N-retinyl-opsin and in native rhodopsin. It is difficult to say what influence this environment might have on the colour of cattle rhodopsin. The significance of the residues in the peptide to which the retinyl group is covalently bound could be better assessed if one had for comparison similar data on other visual pigments. In this regard it would be most interesting to examine the peptide which binds retinal in chicken iodopsin which, although it has the same prosthetic group as cattle rhodopsin, absorbs much further in the red region of a spectrum (λ_{max} 562 m μ).

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Amino-acid Sequence of Glyceraldehyde 3-Phosphate Dehydrogenase from Lobster Muscle

bу

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The protein sub-unit of glyceraldehyde 3-phosphate dehydrogenase consists of a single chain of 333 amino-acid residues, corresponding to a molecular weight of 36,000. Amino-acid sequence studies show that the active enzyme-NAD complex with a molecular weight of 146,000 is composed of four protein chains of identical sequence.

GLYCERALDEHYDE 3-phosphate dehydrogenase (GPDH) plays an important part in carbohydrate metabolism.

In the presence of NAD and phosphate it catalyses the reversible oxidative phosphorylation of glyceraldehyde 3-phosphate to 1,3-diphosphoglycerie acid and as such it participates in the glycolytic conversion of glucose to pyruvic acid in most living organisms. Since its original isolation from yeast by Warburg and Christian¹ the crystalline enzyme has been prepared²⁻⁵ from a wide range of species and has been the subject of much chemical and kinetic investigation6,7.

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No. of residues/36,000 g of protein

A chemical study of the enzyme from pig muscle led Harris and Perham⁸ to conclude that GPDH from any given species is composed of four similar, and probably identical, protein chains, each containing approximately 330 amino-acid residues (corresponding to a molecular weight of 36,000), including one catalytically active cysteine^{9,10}. Among NAD-linked dehydrogenases, muscle GPDH is unique in having high affinity for NAD, and the active tetramer has the capacity to bind up to four moles of the coenzyme¹¹. It has been shown that the binding sites are not equivalent and that a marked conformational change^{12,13} involving some form of co-operative interaction between the protein chains¹⁴ occurs when the first molecule of NAD is bound to the apo-enzyme. The enzyme-NAD complex would thus have a molecular weight of between 144,000 and 147,000 according to its content of bound NAD, and values recently obtained 15 by sedimentationequilibrium methods are in agreement with those predicted from the chemical data8.

Studies of haemoglobin¹⁶ and of monomeric enzymes such as lysozyme¹⁷, chymotrypsin¹⁸ and ribonuclease¹⁹ have shown how a knowledge of the three-dimensional structure of an enzyme can be used, in conjunction with chemical and kinetic information, to investigate mechanisms of enzyme catalysis. Similarly, in our view, a detailed knowledge of the three-dimensional structure of the protein chains in GPDH, and of the specific arrangement by which these chains associate and interact to form the quaternary structure of the active tetramer, will be necessary before the mechanism of enzyme catalysis can be fully understood. This detailed structure can be obtained by interpretation of X-ray diffraction analysis of crystals in conjunction with a knowledge of the aminoacid sequence of the enzyme protein. Of all the GPDHs which have been crystallized, the enzyme from lobster muscle⁵ has proved to be the most suitable for analysis by X-ray diffraction methods. The crystal structure of lobster GPDH is being studied by Dr H. C. Watson and his colleagues in this laboratory²⁰. We have therefore determined the amino-acid sequence of the protein sub-unit in the enzyme and a preliminary account of this work is given in this communication. A separate study⁸ of the amino-acid sequence of GPDH from pig muscle (J. I. Harris and R. N. Perham, unpublished results) will be published elsewhere.

Determination of Amino-acid Sequence

GPDH was prepared from lobster tail muscle as previously described. The amino-acid composition, based on a molecular weight of 36,000 (ref. 8) and given in Table 1, indicates that the protein sub-unit consists of about 330 residues. This composition is in good agreement with the earlier data of Allison and Kaplan⁵, except that it indicates the presence of an additional residue of cysteine and of arginine.

Studies of the amino-acid sequence were carried out chiefly with the N-trifluoroacetyl-S[14C]-carboxymethylenzyme. This derivative was prepared by carboxymethylation of the five cysteine residues with [2-14C] iodoacetic acid in 8 molar urea at pH 8.2 (ref. 8), followed by trifluoroacetylation²¹ of the twenty-eight lysine residues in the continued presence of a large excess of S-ethyltrifluorothioacetate in 8 molar urea at pH 10.0 (unpublished results of Harris and Perham). Digestion with trypsin (1 per cent) was carried out in a pH-stat for 10 h at pH 8.5 and 25° C. The average number of peptide bonds which were hydrolysed by trypsin in these conditions was approximately equivalent to the total number of arginine residues (nine) per mole of protein.

The resulting mixture of peptide fragments gave rise to five separate components by gel-filtration on 'Sephadex G-50' in 0.1 molar ammonium bicarbonate (pH 8.0). The smaller components yielded pure peptide fractions by ionophoresis on paper, and the larger components were

purified by ion-exchange chromatography on DEAEcellulose in 8 molar urea containing tris-hydrochloric acid buffer at pH 7.5. In this manner, ten unique peptides (nine with C-terminal arginine, and one, the C-terminal peptide in the protein chain, with C-terminal alanine) were identified among the products of trypsin digestion. These peptides contained 3, 3, 4, 10, 14, 14, 32, 34, 43, and from 175–180 amino-acid residues, respectively, giving a total of about 335 residues.

Table 1. Amino-acid composition of the protein monomer in glycer-aldehyde 3-phosphate dehydrogenase (gpdh) from lobster muscle

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Amino-acid	1	2	3
Lysine	28	27	28
Histidine	4-5	5	5
Arginine	8	9	Q
Cysteine	4	5*	5
Aspartic acid	35	31	32
Threonine	20	20†	20
Serine	23	26†	25
Glutamic acid	25	25	24
Proline	11	13	12
Glycine	32	30	30
Alanine	34	31	32
Valine	37	37‡	38
Methionine	10	10*	10
Isoleucine	19	18‡	18
Leucine	18	19	18
Tyrosine	9	9	9
Phenylalanine	15	15	15
Tryptophan	4	(3)§	3

- Data of Allison and Kaplan⁶ recalculated for a molecular weight of 36,000.
 Analysis of performic acid oxidized protein.
 Calculated from the amino-acid sequence (Fig. 1).

 - * Estimated as cysteic acid and methionine sulphone, respectively, † Estimated by extrapolation to zero time of hydrolysis. ‡ Estimated by extrapolation to infinite time of hydrolysis. § Not determined.

Purified peptides which contained N-trifluoroacetyllysine were separately redigested with trypsin after removing the blocking group with M piperidine at 0° C (ref. 21). The resulting mixtures, which now contained lysine peptides in addition to the peptides containing C-terminal arginine, were purified by suitable combinations of gelfiltration, paper ionophoresis at various pHs and paper chromatography. The amino-acid sequences of the purified peptides were determined by standard methods with major reliance on stepwise analysis by the dansyl-Edman procedure²². Overlaps of lysine residues were obtained from the sequences of peptides produced with chymotrypsin, pepsin, papain or cyanogen bromide; the lysine diagonal method²³ was occasionally used to facilitate the purification of these peptides. Tryptic peptides which contain arginine occupy C-terminal positions in the original lysine-blocked fragments, and the relative order of these fragments along the protein chain was established from the sequences of peptides containing arginine, which were obtained from pepsin or chymotrypsin digests of the S-[14C] carboxymethyl-protein. The N-terminal trypsin peptide in the protein chain was found to be N-acetyl.Ser.Lys. The acetyl group was identified as acetylhydrazide after hydrazinolysis of the N-substituted peptide, and by mass spectrometry. Peptides which contained tryptophan were detected on paper by Ehrlich's reagent and the number of tryptophan residues in each of the purified peptides was established by the dansyl-Edman method (residues 192 and 309 (Fig. 1)) or by spectrophotometric analysis (residue 83). By these methods three residues of tryptophan were found in peptide fragments in contrast to the value of four obtained by spectrophotometric analysis of the intact enzyme protein. The reason for this discrepancy has not been established, but it is possible that the value obtained by direct analysis of the protein is high because of the presence of bound NAD. Amide

groups have been provisionally assigned from the electrophoretic mobilities²⁴ of peptides which contained aspartic and glutamic acids.

These results enable us to propose the following provisional amino-acid sequence for the protein sub-unit of lobster GPDH (Fig. 1). It consists of a single chain of 333 amino-acid residues (including N-terminal N,acetylserine), corresponding to a molecular weight of 36,003. The active tetramer would thus have a molecular weight of 144,012, or 146,650 if it contains four moles of bound NAD (compare pig GPDH, refs. 8 and 15).

Details of the experimental results on which the sequence is based cannot be given in this short communication. We therefore present the following evidence in support of its

(1) The amino-acid composition derived from the proposed sequence is in excellent agreement with the composition calculated from the experimentally determined amino-acid analysis of the enzyme protein (Table 1). (2) All the tryptic peptides which occurred in major yield were purified and are unambiguously accommodated in the sequence. The sequence of 333 residues thus represents a complete recovery of the analysed parts. (3) All the lysine and arginine residues have been unequivocally overlapped, many with peptides derived from more than one type of digest. (4) Stepwise analysis by the Edman method, coupled with the identification of successively released amino-acid residues as their dansyl derivatives, was carried out through all the peptide bonds in the sequence (in many instances on more than one type of

fragment, and on separate occasions), with the exception of the bonds between residues 1-2, 2-3, 83-84, 98-99, 135-136, 158-159, 175-176, 190-191 and 255-256. Sequences obtained by the dansyl-Edman method were in agreement in all cases with the amino-acid compositions of the analysed peptides and in most cases the sequences obtained in this way were confirmed by analysis of other fragments obtained by digestion with suitable proteolytic enzymes. (5) Finally, the validity of the sequence of the lobster enzyme receives additional support from a comparison with the sequence of the pig muscle enzyme (unpublished results of Harris and Perham). In many instances sequence homologies between the two proteins provide a valuable check on the accuracy of the two independently determined sequences. Further confirmatory evidence will continue to be sought in the course of work which is being undertaken to establish the identity of other reactive groups in the enzyme.

Discussion of Methods

The use of methods based on the reversible blocking of lysine residues has greatly facilitated the sequence analysis of lobster GPDH. With proteins of smaller size (for example, ribonuclease)²⁵ the conventional first step has been to isolate and to work out the sequence of all the peptides from a total trypsin digest of the complete protein. The order in which the various trypsin peptides occur in the protein chain is then established from the sequences of a selection of different peptide fragments containing lysine and arginine; these are obtained by

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Acetyl. Ser. Lys. Ile. Gly. Ile. Asp. Gly. Phe. Gly. Arg. Ile. Gly. Arg. Leu. Val. Leu.
Arg. Ala. Ala. Leu. Ser. Cys. Gly. Ala. Gln. Val. Val. Ala. Val. Asn. Asp. Pro. Phe. Ile. 20
Ala. Leu. Glu. Tyr. Met. Val. Tyr. Met. Phe. Lys. Tyr. Asp. Ser. Thr. His. Gly. Val. Phe.
Lys. Gly. Glu. Val. Lys. Met. Glu. Asp. Gly. Ala. Leu. Val. Val. Asp. Gly. Lys. Lys. Ile. 60
Thr. Val. Phe. Asn. Glu. Met. Lys. Pro. Glu. Asn. Ile. Pro. Trp. Ser. Lys. Ala. Gly. Ala. 80
Glu. Tyr. Ile. Val. Glu. Ser. Thr. Gly. Val. Phe. Thr. Thr. Ile. Glu. Lys. Ala. Ser. Ala. 90
His. Phe. Lys. Gly. Gly. Ala. Lys. Lys. Val. Val. Ile. Ser. Ala. Pro. Ser. Ala. Asp. Ala. 110
Pro. Met. Phe. Val. Cys. Gly. Val. Asn. Leu. Glu. Lys. Tyr. Ser. Lys. Asp. Met. Thr. Val. 130
Val. Ser. Asn. Ala. Ser. Cys. Thr. Thr. Asn. Cys. Leu. Ala. Pro. Val. Ala. Lys. Val. Leu. 148 150
His. Glu. Asn. Phe. Glu. Ile. Val. Glu. Gly. Leu. Met. Thr. Thr. Val. His. Ala. Val. Thr. 170
Ala. Thr. Gln. Lys. Thr. Val. Asp. Gly. Pro. Ser. Ala. Lys. Asp. Trp. Arg. Gly. Gly. Arg 180 182
Gly. Ala. Ala. Gln. Asn. Ile. Ile. Pro. Ser. Ser. Thr. Gly. Ala. Ala. Lys. Ala. Val. Gly. 200
Lys. Val. Ile. Pro. Glu. Leu. Asp. Gly. Lys. Leu. Thr. Gly. Met. Ala. Phe. Arg. Val. Pro. 220
Thr. Pro. Asp. Val. Ser. Val. Val. Asp. Leu. Thr. Val. Arg. Leu. Gly. Lys. Glu. Cys. Ser. 240
Tyr. Asp. Asp. Ile. Lys. Ala. Met. Lys. Thr. Ala. Ser. Glu. Gly. Pro. Leu. Gln. Gly. 260
Phe. Leu. Gly. Tyr. Thr. Glu. Asp. Asp. Val. Val. Ser. Ser. Asp. Phe. Ile. Gly. Asp. Asn. 270
Arg. Ser. Ser. Ile. Phe. Asp. Ala. Lys. Ala. Gly. Ile. Gln. Leu. Ser. Lys. Thr. Phe. Val. 290
Lys. Val. Val. Ser. Trp. Tyr. Asp. Asn. Glu. Phe. Gly. Tyr. Ser. Gln. Arg. Val. Ile. Asp. 310
Leu. Leu. Lys. His. Met. Gln. Lys. Val. Asp. Ser. Ala. COOH 330 \phantom{0000} 333
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Fig. 1. Amino-acid sequence of glyceraldehyde 3-phosphate dehydrogenase from lobster muscle. The sequences of the tryptic peptide containing Cys-148 (residues 139 to 158); the N-terminal dipeptide (X.Ser).Lys, and the C-terminal tetrapeptide Val(Asp,Ser).Ala were determined previously (Allison and Harris**; W. S. Allison, unpublished results).

alternative methods of fragmentation such as pepsin, chymotrypsin or cyanogen bromide. Trypsin digests of larger proteins contain on average a correspondingly larger number of peptides. A digest of lobster GPDH, for example, contains a total of more than forty peptide fragments (including products of partial digestion of slowly hydrolysed bonds, and of cleavages at bonds other than those involving the twenty-eight lysine and nine arginine residues), and it has proved difficult to obtain all the necessary peptides in pure form from complex mixtures such as these^{8,28}.

In order to reduce the number of peptides to be fractionated from such a mixture, we attempted to limit the action of trypsin to the nine arginyl bonds in lobster GPDH by reacting the \(\epsilon\)-amino groups of the lysine residues with S-ethyltrifluorothioacetate^{21,23} (or with maleic anhydride²⁶, unpublished results). This procedure proved to be highly advantageous in that trypsin digests of the resulting lysine-blocked proteins, like their counterparts from the pig enzyme (unpublished results of Harris and Perham), were completely soluble, and readily amenable to fractionation by standard methods. The ten major peptide components which were obtained in this way (see earlier section) could then be studied separately.

Peptides which contained blocked lysines were unblocked to regenerate lysine residues, and the unblocked peptides were then separately redigested with trypsin. digests were relatively easy to fractionate because in each case they contained only those tryptic peptides that occur between two consecutive arginine residues in the protein chain. The isolation of the tryptic peptides from lobster GPDH was thus accomplished more simply by a series of separate fractionation steps from lysine-blocked fragments than by direct fractionation of the complex mixture of more than forty peptides which is produced by digestion of the unblocked protein with trypsin. Similarly, peptides which overlap lysine residues are also obtained more easily (for example, by the lysine diagonal method 23,28) from suitable digests of lysine-blocked fragments than from the considerably more complex mixtures of peptides that occur in similar digests of the whole protein. In this way the sequences of N-trifluoroacetyl- (and of maleyl26) peptides can be determined in a series of separate operations. The complete sequence of the protein chain is then established by overlapping arginine residues with peptides obtained from suitable digests of the whole protein as described earlier.

The overall strategy which has been developed for determining the amino-acid sequence of lobster GPDH is clearly applicable to other proteins of similar, or even larger, size. Many other important enzymes such as yeast and liver alcohol dehydrogenases, lactic dehydrogenase and aldolase, to mention but a few, are known to consist of sub-units comparable in size to those of GPDHs, and we suggest that the method of reversible lysineblocking could be used to similar advantage in the sequence analysis of these enzymes. Ideally, it is desirable that the protein to be studied should contain a sufficient number of lysine and arginine residues, and the method will work best if there are from two to three times as many lysines as there are arginines; moreover, it is also advantageous if arginine residues are fairly evenly distributed along the protein chain. This is exemplified by the case of lobster GPDH where the uneven distribution of arginine residues gave rise to one large lysine-blocked fragment (residues 18 to 193, Fig. 1) containing 176 amino-acid residues, including sixteen lysines. This fragment was obtained in low yield partly because of its susceptibility to chymotrypsin-like cleavages during trypsin digestion, and also because of severe losses during chromatography on DEAE-cellulose. Consequently, in order to determine the sequences of some of its constituent lysine-containing peptides, it proved in some circumstances to be more convenient to obtain additional amounts of these peptides from a tryptic digest of the unblocked protein.

Relationships between Structure and Activity

The amino-acid sequence of lobster GPDH presented here provides strong evidence for the chemical identity of the four protein chains comprising the active enzyme molecule. At first sight the active enzyme could therefore be envisaged as consisting of four structurally equivalent monomer chains, each chain (α) containing a reactive cysteine which in conjunction with NAD and phosphate would be capable of promoting the catalytic reaction within the quaternary structure of the tetramer.

X-ray diffraction studies²⁰ of crystals of the enzyme-NAD complex suggest, on the other hand, that the tetrameric molecule could consist of structurally identical pairs of sub-units. This concept is supported by recent spectrophotometric evidence which indicates that the molecule exhibits chemical as well as structural asymmetry (S. A. Bernhard, personal communication). In this respect, the GPDH-NAD complex is similar in structure to haemoglobin¹⁶ in which two chemically different sub-units are related in pairs to form the tetrameric molecule.

One way of reconciling these results with the identity of chemical sequence would be to postulate that crystals of the apoenzyme possess a symmetrical tetrameric (α_4) structure which changes to the $\alpha_2\alpha_2$ ' type of structure as the result of conformational changes which occur when NAD binds to the protein sub-units. Conformational changes involving co-operative interactions between the monomer chains are known to occur when NAD interacts with the apoenzyme in solution^{12–14}. Attempts to compare the crystal structure of the NAD-enzyme with that of the apoenzyme have not so far been possible because the apoenzyme, unlike that of lactic dehydrogenase²⁷, does not seem to crystallize in the absence of the coenzyme.

In a previous study of lobster GPDH, Allison and Harris²⁸ showed that Cys-148 reacts selectively with iodoacetic acid in the native enzyme. Moreover, the amino-acid sequence around this reactive cysteine (residues 143–155, including a second but unreactive cysteine in position 152) was shown to be identical with the sequences around the corresponding reactive cysteine in GPDHs from several other species^{8,10,28}. It has now emerged that the other three cysteines in lobster GPDH (positions 22, 129, 249) are not homologous with cysteine residues in other GPDHs. For example, in pig GPDH the corresponding positions are occupied by serine, methionine and valine, respectively (ref. 8 and unpublished results of Harris and Perham); the positions of the other two cysteines in the pig enzyme, on the other hand, are occupied by Val-243 and Ser-280 in the lobster enzyme. These variable cysteines are therefore unlikely to be specifically involved either in the catalytic activity or in maintaining the tertiary structure of active muscle GPDH.

Cys-148 participates in the catalytic reaction by attaching the substrate in thioester linkage to the enzyme (for example, refs. 9 and 10). In the absence of substrate (but in the presence, or absence, of NAD) it can also form an intrachain disulphide bond with Cys-152 (ref. 8). This shows that the two sulphydryl groups can occur in close proximity in the three-dimensional structure, and that the apparently unreactive sulphydryl group of Cys-152 could also prove to be an essential feature of the active molecule.

Another glimpse of the three-dimensional structure of the apoenzyme is provided by the observation that the acetyl group which is bound initially to Cys-148 (in the pig and rabbit muscle enzymes) during the enzyme-catalysed hydrolysis of p-nitrophenylacetate^{9,10} and acetylphosphate²⁹, respectively, is able to transfer to a specific lysine residue^{29,20} (shown to be Lys-182 in the pig enzyme)³¹ at a higher pH. The same reaction has now been shown to occur with lobster GPDH (unpublished re-

sults of B. E. Davidson) and a comparison of the sequences around the two reactive amino-acids in the pig32 and lobster enzymes reveals that forty-two of the forty-eight residues in this segment of the chain (positions 143-190) are identical in the two species. Moreover, the changes that have occurred are all of a conservative nature and could all have arisen as the result of a single base change in the Escherichia coli code33.

The remarkable conservation of the amino-acid sequence around the reactive cysteine and lysine residues in enzymes from such distantly related species as pig and lobster suggests that the three-dimensional conformation of this part of the protein chain has also been conserved, possibly because it contributes significantly to the structure of the "catalytic centre" of the enzyme. In this context the transfer of an acetyl group from the sulphydryl group of a cysteine in position 148 to the ϵ -amino group of a lysine in position 182 shows that these two groups can approach each other very closely within the three-dimensional structure of the tetrameric apoenzyme. Little is as yet known about the detailed arrangement of the protein chains within the tetramer and it therefore remains to be established whether the acetyl transfer reaction occurs within or between monomers and, more specifically, to what extent (if any) the interaction of amino-acid side chains from more than one monomer may be involved in the catalytic reaction itself.

The amino-acid sequence of the protein monomer in lobster GPDH provides a framework for the precise interpretation of the results of further chemical, kinetic and X-ray crystallographic studies on the active tetrameric molecule in solution, and in the crystalline state. GPDHs from all sources are likely to possess a similar three-dimensional structure and hence a common reaction mechanism. It is to be hoped that the combined results of chemical and X-ray diffraction studies now in progress will lead to the identification of the amino-acid side chains that bind the substrate, NAD and phosphate to the protein sub-unit, as a step towards elucidating the mechanism of enzyme catalysis.

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Specific Binding of Sodium and Potassium Ions in Erythrocyte Membranes

by

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In erythrocytes, the binding of sodium and potassium ions to sites in the membrane is specific and the number of sites is extremely small. Addition of ATP to the medium increases the binding of sodium ions by 50 per cent or more and it is suggested that ion binding is connected with the ion pump.

THE sodium-potassium ion pump which has been described in erythrocytes and in many other types of cell is one of the best known examples of a device for transduction of chemical energy into osmotic energy. It has been shown that ATP is the immediate source of energy for the ion pump in erythrocytes. It is also known that the ATPase system ("transport ATPase"), concerned with active ion transport, requires the simultaneous presence of Na+ and K+. The existence of two separate reaction sites is thus indicated^{1,2}. Whittam³ and Ĝlynn⁴ have provided proof that these two separate sites are

on opposite sides of the erythrocyte membrane. This spatial separation makes the system anisotropic, an a priori requirement for the coupling of a chemical reaction to diffusional flows across a membrane.

Recently^{5,6}, a model of the Na⁺-K⁺ pump in the erythro-

cyte membrane was proposed and analysed thermodynamically. This model is based on many others3,7,8. and is graphically represented in Fig. 1.

The principal features of this model are the existence of the specific binding sites for Na+ and K+ and their cyclic interconversion by chemical reactions, one of which in-

volves ATP as a reactant. In such a model, an increase in specific binding of Na+ should be at the expense of the specific binding of K+. Specific binding of Na+ and K+ has not been observed in erythrocyte membranes or other biological membranes, but its existence has been inferred from kinetic studies^{1,2}. Cohen⁹ has demonstrated the existence of specific binding sites for rubidium in the Chlorella membrane, and has studied their properties extensively. One remarkable feature was that the specific binding ability was retained in cells which had been treated with boiling ethanol. This clarifies somewhat the nature of the binding sites and has also very important practical application in our experiments. studies10,11 and also our own results, as will be shown later, indicate that the amount of "carrier" or number of binding sites is extremely small, of the order of 10-9 moles/ml. cells. It is very difficult to measure specific binding in such small amounts. By using hot ethanol on membrane fragments we have been able to concentrate the number of binding sites, thus enabling measurement of the specific binding of Na+ and K+.

The membrane preparation we have used resembles that used for measuring Na+-K+-activated ATPase by Dunham and Glynn¹² and Post et al. 13. After haemolysis, the erythrocyte membrane fraction was thoroughly washed in the cold with large volumes of 30 mmolar tris-hydrochloric acid buffer at pH 8.1, until no haemoglobin appeared in the supernatant. The membrane fraction was finally washed with 30 mmolar tris-hydrochloric acid at pH 7.2 containing 20 mmolar magnesium chloride. The resulting preparation was kept in a deepfreeze, and was tested for ATPase which had been activated with Na+ and K+ before the binding experiments were undertaken. A few experiments were also carried out on a rat brain membrane preparation using Skou's method¹⁴, modified somewhat by Edelman (personal communication). Such membrane preparations were treated either with 80 per cent boiling ethanol, with 5 per cent cold TCA, or by freezing in liquid air. The pellet resulting from any one of these treatments was resuspended in cold 20 mmolar magnesium chloride and 30 mmolar tris-hydrochloric acid at pH 7.2. Three different methods were used to measure the binding of Na+ and/or K+.

In the first method, 2 ml. of the suspension was filtered through a 'Millipore' filter (THWP). This filter, which was 25μ thick, was chosen because it contained only 7 mg of water when wet. The filtrand was rinsed several times with magnesium chloride and *tris* solution and was then equilibrated for at least 10 min with the same solution, to which radioactive Na⁺ had been added. Tests were made to show that longer periods of equilibration did not bring about more binding. The amount of bound ions was calculated as the difference between total counts and the

"solution correction". This last quantity represents the radioactivity of the solution which was trapped in the filter and the filtrand. The "solution correction" is the most important parameter in this experiment. It was checked that no radioactivity had been bound by the 'Millipore' filter and that, when the membrane preparation was treated with a concentrated solution of very low specific activity, all the measured radioactivity could be accounted for by trapped solution. The chief error is caused by some drying which occurs while draining the filtrand by the vacuum pump. It was found that this error could be minimized by standardizing the procedure.

In the second procedure, a known volume of membrane suspension was centrifuged in a weighed test tube. The supernatant was sucked off, and the test tube and pellet were weighed. Buffer solution containing a known amount of radioactive sodium was added and incubated with the membrane particles for 30 min. The supernatant was removed by centrifugation, its radioactivity was measured and its value was corrected for the water content of the original pellet. The difference between the original and corrected final radioactivities represented the ions bound by the membrane fragments.

According to the third method, the suspension of membrane fractions was centrifuged and washed three times in cold magnesium chloride and tris buffer containing 0·1 mmolar sodium chloride and 0·1 molar potassium chloride. In the final washing solution, there was no change in the concentration of Na⁺ and K⁺, which were thus assumed to be in equilibrium with ions in the membrane fragments. The pellet was then weighed before and after drying at 104° C and was transferred to a 'Vycor' test-tube (Low Na glass) and converted to ash in 2 ml. 'Analar' nitric acid. The ash was analysed for sodium and potassium. The results were corrected for the amount of supernatant which was trapped and were considered to be the amount of sodium and potassium bound.

A very important feature of our measurements is that the binding of Na⁺ and K⁺ was measured in the presence of relatively high concentration of Mg²⁺ and tris ions. The contention is that all the non-specific sites become occupied by these ions, especially by the divalent Mg⁺⁺. When the concentration of Na⁺ and K⁺ is 10⁻⁴ molar, the Mg⁺⁺ concentration is 200 times, and that of the tris ions 300 times, higher; hence we assume that the Na⁺ and K⁺ binding found here is specific.

The figure indicated by the first two methods described is $2-4\times10^{-6}$ moles Na/l. of cells: these methods rely on equilibrium being reached with radioactive Na⁺. With the third method, a much higher value— $2-4\times10^{-6}$ moles of Na/l. of cells—is obtained. It is possible that in this case a large fraction of bound Na⁺ is not readily exchangeable

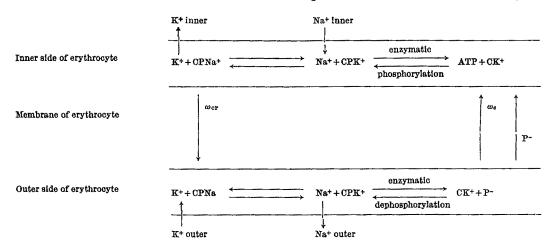


Fig. 1. Model for ion-exchange pump. C, Carrier; CP, phosphorylated carrier; ω , mobility of carrier.

with Na $^+$ in the solution. The results for K^+ are of the same order of magnitude but are more variable than the Na $^+$ results.

Glynn¹⁰ and Solomon et al.¹¹ have calculated from inhibition experiments that the carrier concentration in erythrocytes is about $2 \times 10^{-7} - 2 \times 10^{-8}$ moles/l. of cells. Our results indicate that the amount of specifically bound Na+ is much larger. It can be argued that this large amount is not all required for pump activity but might be related to passive cation permeability. In order to test the effect of ATP on binding, membrane fragments were incubated in a medium of the same composition as that which was used for measuring Na+-K+-ATPase activity. The incubation mixture was left at 37° C for 3 min and the reaction was then stopped, either with boiling ethanol or with cold 5 per cent TCA. The mixture was centrifuged and the residue was washed three times as described for the binding experiments.

Tables 1 and 2 show that ATP increased the binding of Na+ by 50 per cent or more when measured by equilibration of membrane fragments with radioactive Na+. There were no important differences between membranes treated with hot ethanol and those treated with cold TCA. Detailed studies were undertaken using the method of direct analysis of specific binding. Results are shown in Table 3. In all cases a big increase in Na+ binding is seen when membrane fragments were incubated with ATP and Na+ and K+. The omission of K+ from the incubation medium, or addition of ouabain either decreased the effectiveness of the ATP, or else decreased the specific binding below the control values. Similar

Table 1. EFFECT OF ATP ON BINDING OF Na+ BY ERYTHROCYTE MEMBRANE FRAGMENTS

Experiment No.	Treatment	Means by which reaction was terminated	Na bound c.p.m./mg dry weight $\pm S.E$.
1	-ATP	Boiling	42 ± 2.1
2	+ ATP ATP +- ATP	Ethanol	79 ± 1·3 282 ± 25 457 ± 38
3	-ATP		162±14
4	+ ATP - ATP + ATP	5 per cent cold TCA	253 ± 19 202 ± 58 314 ± 15

Membrane fragments (see text) were incubated in a solution containing 30 mmolar tris-hydrochloric acid, pH 7·4, 3 mmolar magnesium chloride, 100 mmolar sodium chloride, 100 mmolar potassium chloride and 3 mmolar ATP for 3 min at 37° C. The reaction was terminated either with bolling ethanol (final concentration 80 per cent) or with cold TCA (final concentration 5 per cent). After centrifugation the pellet was resuspended in solution B (20 mmolar MgCl, and 30 mmolar tris-hydrochloric acid at pH 7·2). After filtration on 'Millipore' filters the filtrand was washed with solution B, and was then equilibrated with solution B, and radioactive Na⁺ was added at a low concentration (10⁻⁵ molar) of Na⁺. After draining, the filter was counted.

Table 2. EFFECT OF ATP ON BINDING OF NA. BY ATP ON ERYTHROCYTE MEMBRANE FRAGMENTS

Treatment		Na ⁺ concentration in the equilibration solution (moles/l.)	Na^+ bound (mole/ml, cells $\pm S.E.$)
- ATP	Native	4×10 ⁻⁴	$11.1 \pm 3.2 \times 10^{-10}$
+ ATP		4×10 ⁻⁴	$19.2 \pm 1.82 \times 10^{-10}$
- ATP	Boiling	1×10-4	5.5 ± 0.82 × 10-10
+ ATP	ethanol		13.6 + 5.2 × 10-10

Membrane fragments were incubated in the same solutions as in Table 1, after which they were either treated with bolling ethanol, or else were centrifuged down. They were then washed three times in solution B (see Table 1). After centrifugation the pellet was resuspended in solution B containing radioactive Na⁺, and incubated for 30 min. After centrifugation, the radioactivity of the supernatant was measured.

Table 4. EFFECT OF ATP ON SPECIFIC BINDING OF NA* BY RAT BRAIN

	THOROSOMES	
Treatment	Na bound (c.p.m./mg membrane)	(u)
-ATP +ATP -ATP +ATP -ATP	$ \begin{array}{c} 189 \pm \frac{4 \cdot 3}{5 \cdot 3} \\ 236 \pm \frac{5 \cdot 3}{5 \cdot 3} \\ 350 \pm 10 \\ 412 \pm \frac{9}{10} \end{array} $	
- ATP + ATP - ATP + ATP	147 ± 10 3 249 ± 40 3 250 ± 63 2 295 ± 50 4	(4)
– ATP + ATP + ATP + ouabain	336 ± 0 433 ± 29 235 ± 32	(")

Brain microsomes were prepared by the method described by ${\rm 8kou^{14}}.$ Incubation with ATP as described in Table I.

- (a) After incubation with ATP, the microsomes were treated with ethanol, and subsequently as in Table 1 for different experiments.
- (b) After incubation with ATP, the microsomes were treated with 5 $\,\rm pct$ cent cold TCA, and subsequently as in Table 2.

results were obtained for K+, though the effect was usually less.

Preliminary results with rat brain are shown in Table 4. It is clear that results were similar to those obtained from erythrocytes.

It is possible to claim that one step of a chemo-osmotic machine was obtained in vitro, a chemical reaction bringing about an increase in the amount of bound ions. This is an important step in the transduction of chemical energy into osmotic energy. In our experiments, only one step occurred in the cycle of a chemo-osmotic machine, that is, increase in ion binding. The next step to be found should be a chemical reaction which causes the desorption of the bound ions by a decrease in the specific binding capacity.

It is tempting to suggest that there is a close analogy between the effect of ATP on ion binding and its effect on active transport in erythrocytes in vivo as well as on the transport ATPase system in the erythrocyte membrane. In all three cases, addition of ouabain or omission of K abolishes the ATP effect. Our results could thus indicate that the ion pump in vivo might work in the same way as in our experiments. The conclusion that ion binding. which we measured, is connected with the ion pump is reinforced by a correspondence which we have noted between the activity of the Na+-K+-activated ATPase and the degree of binding brought about by ATP. Most of the work reported so far was done on erythrocytes which had been stored for several weeks in the cold in a blood bank. It was found that incubation of the cells with I per cent glucose at 37° C for I h before haemolysis increased by more than three times both the Na⁺-K⁻activated ATPase and the ability of ATP to increase Naand K+ binding (Table 5).

The results obtained here are not consistent with the model of the ion pump presented at the outset, in which there are assumed cyclical alternate changes in Na⁺ and K⁺ binding. The model implies that inhibition by ouabain or omission of K⁺ should increase either the amount of Na⁺-bound substance or the amount of K⁺-bound substance. In our experiments, the addition of ATP appeared to increase both the Na⁺- and K⁺-bound substances. Such a result is consistent with Skou's model^{1,2}, which depicts simultaneous increase of binding of Na⁺ and K⁺ by ATP, but not with the other, generally accepted, model.

Table 3. EFFECT OF ATP ON THE SPECIFIC BINDING OF SODIUM AND POTASSIUM IONS TO ERYTHROCYTE MEMBRANES

Treatments	Experin	periment A Experiment B		ment B	Experiment C		Experiment D		Experiment 1:	
	Na	ĸ	Na	ĸ	Na	K	Na	ĸ	Na	K
-ATP	100 ± 12	100 ± 11	100 ± 19	100 ± 16	100 ± 8		100 ± 8	100 ± 10	100 ± 12	100 ± 12
+ATP, -K +ATP	$88 \pm 9.7 \\ 138 \pm 12$	101 ± 22 138 ± 22	$72 \pm 6 \\ 270 \pm 23$	72 ± 12 $128 + 24$	170 ± 22 287 ± 19	_	220 ± 9	150 ± 14	170 ± 17 570 ± 27	105 ± 11 170 ± 28
+ATP, +ouabain	63 ± 0.4	102 ± 23	66± 6	79 ± 12	170 ± 23		103 ± 6	110 ± 12	210 ± 22	120 ± 18

Membrane fragments were incubated, as described in Table 1, in a medium containing 30 mmolar tris-hydrochloric acid at pH 7·4, 3 mmolar magnesium chloride and 100 mmolar sodium chloride. 15 mmolar potassium chloride, 3 mmolar ATP and 0·01 mmolar ouabain were added as appropriate. After 3 min at 37° C, boiling ethanol was added and, after centrifugation, the pellet was resnapended and centrifuged three times with cold solution, containing 20 mmolar magnesium chloride, 30 mmolar tris-hydrochloric acid at pH 7·2, 0·1 mmolar sodium chloride and 0·1 mmolar potassium chloride. The pellet was ashed and resuspended in distilled water. S.E. of mean are given. A, B, C, D, E are experiments on different batches of erythrocytes.

Table 5. Effect of atp on specific binding of Na+ and K+ by erythrocyte membrane fragments previously incubated with glucose

		(moles/ml.	$cells \pm S.E.$)	
Treatment		ucose	+ G	lucose
	Na	ĸ	Na	K
ATP +- ATP +- ATP + ouabain	$1.56 \pm 0.24 \times 10^{-8}$ 1.92 ± 0.61 1.66 ± 0.28	$0.61 \pm 0.14 \times 10^{-4}$ 0.80 ± 0.36 0.70 ± 0.14	$1.42 \pm 0.22 \times 10^{-8}$ 3.5 ± 0.61 0.95 ± 0.44	$0.56 \pm 0.16 \times 10^{-8}$ 0.9 ± 0.06 0.38 ± 0.24

Before haemolysis the cells were incubated with 1 per cent glucose at 37° C. Subsequent procedure as in Table 3.

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Ions bound

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Ferric Oxyhydroxide Core of Ferritin

Ьу

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Department of Physiology, University of Edinburgh Medical School Ferritin is an iron-protein complex which plays an important part in the storage of iron. Here an atomic structure is proposed for the iron containing core and its synthesis is discussed.

THE characteristic red brown colour of the iron storage protein, ferritin, is caused by the presence of a large amount of ferric iron in the molecule (about 20 per cent of the dry weight). This iron is deposited as (FeOOH)₈ (FeOOPO₃H₂) (ref. 1) within a confined space, about 75 Å across, at the centre of a roughly spherical shell of protein about 25 Å thick²⁻⁴. Native ferritin consists of a mixture of molecules which are unfilled or partially or com-pletely filled apoferritin shells. A high proportion of the molecules contain well filled cores (molecular weight of core about 400,000).

The protein shell has been shown to consist of a symmetrical arrangement of protein sub-units3. electron microscope the cores can be seen without staining. They seem to be rather variable in shape and sometimes to be cleaved into two or more parts5. It is not clear from these studies whether the cleavage seen in the electron microscope has resulted from drying of the sample during examination, or whether the cores are sub-divided into smaller particles in the natural material. The cores give an electron diffraction pattern⁵ indicating that they are composed of crystalline material of average particle size 40 to 50 Å, but again it is uncertain from electron diffraction studies alone whether they are crystalline in the natural state or whether the degree of crystallinity is dependent on the state of hydration. Low angle X-ray scattering from ferritin4 solutions shows that to a resolution of 20 Å the core approximates to a single uniform density object of average diameter 75 Å.

We have now examined ferritin cores under a variety of conditions, wet and dry, by X-ray and electron diffraction, and have obtained essentially the same diffraction pattern both in intensity and line breadth in each case. As previously reported, the electron diffraction pattern of ferritin cannot be identified with that of any of the common oxides or oxyhydroxides of iron. We have considered possible arrangements of oxygen and iron atoms and describe here one which accounts reasonably well for the observed pattern.

The spacings and approximate intensities of bands common to the X-ray and electron diffraction patterns of ferritin are listed in Table 1. A number of small spacing bands were obtained by electron diffraction. Some of these were not clearly visible above the background with X-rays or were beyond the wavelength limit of the FeKa radiation used. Essentially the same pattern has been obtained with all the samples listed in Table 2. The source in each case was horse spleen ferritin, or ferritin reconstituted from horse apoferritin by methods described below. For X-ray diffraction wet samples were mounted in thin-walled glass capillary tubes, while dry powders were pressed into a cylindrical shape with a small amount of gum tragacanth as adhesive.

The results show that iron cores of native ferritin are similar with respect to structure and crystallinity in a variety of conditions (wet or dry, attached to the protein or separated from it). Attention is drawn protein or separated from it). especially to the following observations.

(1) The procedure for separating the cores from the protein (treatment with 1 normal sodium hydroxide for several minutes at room temperature) has previously been shown to remove most of the phosphate from the cores (23 per cent of the original phosphate remaining in the cores, 77 per cent appearing in the supernatant). Cores separated by treatment of ferritin with 1 normal sodium hydroxide for periods up to 2 h gave the same pattern as intact ferritin. The phosphate does not therefore seem to be an essential structural component of the core crystallites, or to contribute significantly to the diffraction pattern. This is confirmed by the result that ferritins, reconstituted from apoferritin by three different procedures, in the absence of added phosphate gave patterns similar to those of the native ferritin (although the crystallites were smaller in some cases). The cores released by alkali treatment also maintain much of their original shape as seen in the electron microscope, although the crystallites of each core become apparently more tightly clumped on removal of protein and the cores aggregate to form a gross precipitate.

Table 1					
hkil	d_{o} Å	d: Å	Iobs*	Iobs†	$I_{\mathrm{calc}} \ddagger$
$\{\begin{array}{c} 10\bar{1}0\\ 10\bar{1}1\\ 0004 \end{array}\}$	2.5 approx.	2·55 2·47 2·35	Strong	10.4	2·5 8·5 1·5
1012 1013 1014	2·23 1·99 1·72	2·24 1·98 1·73	Strong Medium Weak	4·7 2·5 3·9	6·8 3·4 2·9
1015 1120 1016	1·50 1·47 1·33	1·51 1·47 1·34	Very weak Strong Very weak	5·7 14·2	6.0 14.7 3.5
1124 } 2022 } 0008 }	1·23 1·18	1·25 1·23 1·18	Weak Weak	Broad (Medium)	0·8 0·5
20 <u>2</u> 3 5 20 <u>2</u> 4 20 <u>2</u> 5	1·11 1·05	1·18 1·12 1·06	Very weak Medium	Broad (Weak)	0·2 2·0
11 <u>7</u> 8 10710 3030	0·91 0·88 0·84	0·92 0·88 0·85	Weak Very weak Medium	Beyond FeI	Ca limit

- * Electron diffraction, visual estimations.
- * X-ray, scaled to Icale, estimated errors ± 20-50 per cent.
- † X-ray.

Table 2. SAMPLES GIVING SIMILAR DIFFRACTION PATTERNS

Sample	Conditions	Radiation
Native ferritin I*	Vacuum dried	Electrons
Native ferritin II†	Vacuum dried	Electrons
Native ferritin	Air-dried acetone precipitate	X-rays
Native ferritin	Large, wet, single crystal continuously rotated	X-rays
Native ferritin	Large, wet, single crystal, stationary	X-rays
Isolated cores	Washed alkali precipitate, wet	X-rays
Isolated cores	Washed alkali precipitate, dry	X-rays and electrons
Reconstituted ferritin A	Vacuum dried	Electrons
Reconstituted ferritin B	Vacuum dried	Electrons
Reconstituted ferritin C	Vacuum dried	Electrons

^{*} Contained some residual cadmium after crystallization from cadmium sulphate solution.

(2) Powder patterns, typical of ferritin core patterns, could also be obtained from large wet single crystals of ferritin. The most interesting of these are photographs of a stationary single crystal in which sharp spots, caused by the protein crystal lattice, and very weak diffuse rings, caused by the iron cores, can be seen simultaneously. The latter give no indication of preferred orientation with respect to the crystal lattice. This observation supports conclusions, previously derived from comparisons of single crystal photographs of ferritin and apoferritin, that the atomic arrangement within the cores is not specifically related to the structure of the protein. This suggests either that the protein does not have specific binding sites or that, if it does have such sites, the core material is randomly bound to them.

Reconstitution experiments are of some interest in relation to the biosynthesis of ferritin. Details of this work will be published elsewhere, but some points are relevant to this discussion. In vivo evidence suggests that apoferritin is formed first in biosynthesis and indeed the injection of ferrous salts into animals stimulates de novo synthesis of apoferritin. The iron (as

Fe²⁺) enters the intact apoferritin shells, becomes oxidized to Fe³⁺ and is precipitated inside the molecule as ferric oxyhydroxide (FeOOH). The ferric oxyhydroxide crystallites are either bound to the protein or are too large to move out through the spaces between sub-units in the protein shell (which allow the passage of Fe²⁺). Several reconstitution procedures were attempted, of which three are reported here. In each case the starting materials were apoferritin and ferrous ammonium sulphate or ferrous sulphate, the procedure varying as indicated.

(A) Oxidation by oxygen in bicarbonate buffer. pH 6.8 to 8.0 (ref. 9). In the absence of apoferritin this gives

a-ferric oxyhydroxide.

(B) Oxidation by potassium iodate in sodium thiosulphate, pH 5.4 to 7.4. This procedure was first used by Orme-Johnson, Gilchrist and Collins (private communication). In the absence of apoferritin it gives γ -ferric oxyhydroxide.

(C) Oxidation by oxygen in imidazole buffer, pH 6.7 to 7.0. In the absence of apoferritin this gave a third, as

yet unidentified, product.

Reconstituted ferritins (that is molecules which had the usual red brown colour, which had sedimentation coefficients considerably larger than that of apoferritin, and which could be seen in the electron microscope to contain an electron-dense core) were obtained by each of these procedures. Each of them gave a ferritin-like diffraction pattern, although in procedure (A) the crystallites formed inside the protein were considerably smaller than those in native ferritin. Because, in the absence of apoferritin, different products were obtained in each case, the protein clearly has an effect on the mode of crystallization of the ferric oxyhydroxide inside the protein shell. Apoferritin also appeared to affect the rate of oxidation as well as the product obtained. The role of the phosphate, if any, has yet to be determined. Some of it is probably attached to the protein, and may influence the core-protein interaction. Apoferritin is reported to contain some phosphorus¹⁰ (well under half that present in ferritin), but it is not known where this is located. In attempting to interpret the diffraction photographs we have, for the time being, ignored the phosphate and investigated arrangements of oxygen and iron atoms only which might account for this pattern.

The X-ray diffraction patterns of ferritin cores are typical of a rather poorly crystalline material. The lines are broad and weak relative to the background. Densitometer traces were obtained, however, and attempts were made to measure both line breadths and integrated intensities. Crystallite sizes, estimated from the half-width of two lines (spacings, 2.23 and 1.47 Å), are in reasonable agreement within the range of experimental error, for both air-dried precipitated cores and whole ferritin. Qualitative comparisons of the 1.47 band from wet samples indicate similar crystallite sizes under these conditions. The crystallite size, 75 ± 30 Å, obtained from the X-ray patterns is somewhat larger than that estimated from electron diffraction patterns, 40-50 Å. difference may not be significant, but crystallite sizes measured directly from electron micrographs¹¹ appeared to be even smaller, possibly as low as 10-20 Å. Because of the breadth and low intensities of the lines and difficulties in assessing the background, the X-ray intensities could not be measured accurately. Some lines (for example, those with spacings of 1.47 and 1.51 Å) could not be completely resolved from one another on the densitometer trace. On the other hand, the line at about 2.5 Å, which seems, to the eye, to be a single rather broad reflexion, on densitometry could be seen to be composed of at least two overlapping peaks, a strong one at about 2.47 Å and a weaker one at about 2.55 Å. In the electron diffraction patterns a broad, strong line was reported at a spacing of 2.55 Å. This line is measured against a steeply rising background. When re-examined at short exposure times, so that background scatter and

[†] Cadmium free.

peak intensities are reduced, the peak moves to about 2.51 ± 0.03 Å. It therefore seems probable that this line is also composed of two (or more) unresolved lines, as suggested by the X-ray pattern.

As already stated, the diffraction pattern of ferritin cores is not identical to that of any known minerals. It has some similarity, however, to that of δ-ferric oxyhydroxide. The latter has a hexagonal cell with a = 2.94 Å and c=4.51 Å^{12,13}. In the ferritin pattern, strong lines are observed, which would correspond to the (1010) and (1120) reflexions of this cell, but other lines, such as that at spacing d = 1.99 Å, do not fit this simple cell. We can index the ferritin pattern, however, on a hexagonal cell, which has a=2.94 Å and c=9.40 Å (rather more than twice the c axis in δ-ferric oxyhydroxide). The spacings calculated for this cell are compared with the observed ones in Table 1.

The oxides, oxyhydroxides and hydroxide of iron consist essentially of close-packed layers of oxygen atoms, stacked in various ways, with iron atoms in the interstitial holes. These holes are of two kinds, octahedral and tetrahedral, in which the iron is surrounded respectively by six or four oxygen atoms. Octahedral co-ordination of iron is the more common. Fig. 1 shows a structure for the ferric oxyhydroxide of the ferritin core which would fit the hexagonal unit cell described. It has an alternating sequence of four close-packed oxygen layers of the type ABAC ABAC . . . (in hexagonal close-packing there is a sequence of oxygen layers ABAB..., while in cubic close-packing the sequence is ABCABC...). Possible sites for the iron atoms between the oxygen layers are as shown. If the structure is ferric oxyhydroxide, there must be two oxygen atoms for every Fest. In the unit cell in Fig. 1 there are four oxygen sites (we have not distinguished between O2- and OH- oxygen atoms) and a total of twelve iron sites, eight tetrahedral and four octahedral. Various distributions of the two iron atoms among these sites are possible and calculations of expected intensities were made for several of these. agreement was obtained with iron atoms equally distributed among all the available sites in a random manner, so that each site was occupied, on the average, by one sixth of an iron atom. Calculated intensities for this arrangement are shown in Table 1 in comparison with those observed. Lorentz, polarization, multiplicity corrections and a temperature factor, $\exp(-B\sin^2\theta/\lambda^2)$, with B=7.5 Å² have been applied to the calculated intensities. Other arrangements involving variations in the oxygen stacking were also considered (for example, mixtures of cells with ABAB . . . and ABCABC . . . stacking), but none of those tried gave as good agreement between observed and calculated intensities as that

The structure proposed for ferritin has a similar stacking arrangement of oxygen layers to that suggested for "green rust II" (a ferrous ferric oxide hydrate? Fe₃O₄, 4/3H₂O), although the cell dimensions of the latter are larger (a=3.17 Å, c=10.94 Å) and intensity differences suggest that the iron distributions are not identical. In δ-ferric oxyhydroxide the oxygen stacking is of the hexagonal ABAB . . . type with a layer separation of 2.25 Å, and the iron atoms are probably divided among all the various tetrahedral and octahedral sites in the ratio 20-80 per cent. In ferritin cores a higher occupancy of tetrahedral sites may account for the increased separation of oxygen layers, 2.35 Å. There seems, however, to be greater coherence between oxygen layers in ferritin cores than in 8-ferric oxyhydroxide, because in the X-ray patterns of the latter no reflexions with l index greater than 2 are observed as compared with l equal to at least 5 in ferritin. This may reflect a greater degree of hydrogen bonding between layers in ferritin cores, which possibly result from the manner in which the cores are formed inside the protein. Apoferritin, however, may not play a unique part in the formation of

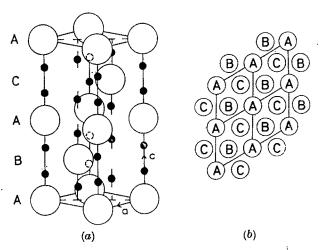


Fig. 1. Proposed unit cell for the FeOOH core of ferritin showing atomic sites.

a, Sites in the unit cell:

Oxygen, 0, 0, 0; 0, 0, $\frac{1}{2}$; $\frac{2}{3}$, $\frac{1}{3}$, $\frac{1}{4}$; $\frac{1}{3}$, $\frac{2}{3}$, $\frac{3}{4}$; Iron, octahedral, $\frac{1}{3}$, $\frac{2}{3}$, $\frac{1}{8}$; $\frac{1}{3}$, $\frac{2}{3}$, $\frac{3}{8}$; $\frac{2}{3}$, $\frac{1}{3}$, $\frac{5}{8}$; $\frac{21}{33}$, $\frac{7}{8}$; tetrahedral, 0, 0, $\frac{1}{8} + u$; 0, 0, $\frac{3}{8} - u$; 0, 0, $\frac{5}{8} + u$; 0, 0, $\frac{7}{8} - u$; $\frac{2}{3},\ \frac{1}{3},\ \frac{1}{8}-u;\ \frac{2}{3},\frac{1}{3},\ \frac{3}{8}+u;\ \frac{1}{3},\ \frac{2}{3},\ \frac{5}{8}-u;\ \frac{1}{3},\ \frac{2}{3},\ \frac{7}{8}+u;$

b, Stacking of the ABC oxygen layers for four unit cells viewed along the hexagonal axis.

this structure. Two other diffraction patterns, resembling those of ferritin cores, have been reported; one from a mineral deposited in immature radular teeth of Cryptochiton stelleri14, and the other15 from an iron oxide gel formed by the addition of ammonia to a solution of ferric nitrate (with an estimated crystallite size of 30 Å). These two materials may therefore have structures closely similar to that of ferritin cores.

Further work on ferritin cores is in progress, including a study of fractions of different iron contents.

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Note added in proof. Since this manuscript was submitted, our attention has been called to a recent paper on the constitution of colloidal "hydrous ferric oxide". This material, a hydrolysate of ferric nitrate, also gives a diffraction pattern resembling that of ferritin cores. The authors propose a structure which differs from the one we suggest for ferritin, although the two structures have closely related unit cells. (Towe, K. M., and Bradley, W. F., J. Colloid and Interface Sci., 24, 384; 1967.)

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New Type of Restriction to the Expression of a Structural Gene in Bacteria

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Penicillinase synthesis in a strain of Staphylococcus aureus provides evidence for a new type of restriction to the expression of structural

THE expression of structural genes in bacteria may be limited in a number of ways. In some cases, the limitation is expressed by the product of a second-or regulatorygene¹⁻⁸, while in others the rate of expression is set by a region known as the promoter^{7,8}. A third type of limit to

gene expression is found in mutants of the "polar" type⁹⁻¹¹.

In addition to at least the first two of these means of limitation, penicillinase synthesis in Staphylococcus aureus seems to be restricted by the availability of sites at which enzyme synthesis can occur. This type of limitation is only noticeable in cultures which carry two penicillinase structural genes and is shown by the fact that such strains synthesize only as much penicillinase as the haploid strain, and not twice as much, as would be expected on the basis of gene complement. The experiments described here therefore suggest that some cellular differentiation with respect to protein synthesis can exist, at least in these

strains of Staphylococcus aureus.

The expression of two penicillinase genes in a single cell can be studied by using a plasmid diploid clone which contains both the p_A^+ and p_C^+ genes¹² (the genes responsible for the synthesis of A- and C-type penicillinase¹³). This arrangement permits accurate measurement of the expression of each structural gene by immunological techniques^{12,12}. Furthermore, because diploids of this type usually segregate to give both the anticipated haploid segregants, the expression of the same structural genes may be easily studied in the same cytoplasm unaccompanied by a second plasmid¹⁴. The synthesis of A- and C-type penicillinase by the diploid $147(\alpha \cdot i^+p_A^+ \dots \gamma \cdot \text{ero}^R/\beta \cdot i^-p_C^+ \cdot \text{ero}^S)$: for terminology—see Novick and Richmond¹⁴) has been studied, in the presence and absence of inducer, and compared with the synthesis of A-type enzyme by the segregant strain $147(\alpha.i+p_1^*...\gamma.ero^R)$ and of C-type by $147(\beta \cdot i^-p_{\sigma}^+ \cdot \text{ero}^s)$. The results are shown in Table 1. Whereas the p_{λ}^{+} gene is expressed to a level of about 300 U/mg dry weight of bacteria and the p_{σ}^{+} to about 260 U/mg, when fully induced in the cytoplasm of strain 147, the total amount of enzyme synthesized in the diploid $147(p_{\lambda}^{+}/p_{\sigma}^{+})$ is about 290 \overline{v}/mg , of which about half is the immunological A-type variant and half C-type. Clearly, the total penicillinase synthetic ability of the cell is not therefore increased by the presence of two penicillinase genes, and the available penicillinase synthetic ability is shared approximately equally between the two structural genes concerned. In the haploid segregants, however, the full synthetic ability of the cell, with respect to penicillinase synthesis, can be taken over by either structural gene in the absence of the other. As this demonstration of the limitation in penicillinase expression in the diploid is dependent to a great extent on the accuracy of the assay methods used, a series of eight independently isolated diploid clones was tested for their level of penicillinase expression in the presence of inducer and compared with separately isolated segregants of each genotype (Table 2). These results reinforce those shown in Table 1.

Using the known specific enzyme activity of pure penicillinase^{13,15}, calculation shows that a rate of synthesis of 300 penicillinase units/mg dry weight of bacteria is equivalent to about 7.5 µg/mg dry weight of bacteria or to

about 15 µg/mg of cell protein, assuming that about half the cell dry weight is protein. It is difficult to believe that an increase from 1.5 to 3.0 per cent in penicillinase molecules among the cell proteins, which is required for the free expression of two genes rather than one, could not be accommodated by a small reduction in the synthesis of all other proteins, if all the protein synthetic potential in the cell were available for penicillinase production. The limit to penicillinase expression therefore seems to reflect a localized restriction in some component essential for penicillinase but not for total protein syn-

Although these experiments suggest that there is a limit to the amount of penicillinase which can be produced by a staphylococcal cell, they do little to indicate where the limitation occurs. By using certain mutant strains which synthesize very small amounts of penicillinase protein—the so-called "micro-mutants" (ref. 16)—it is possible to investigate more closely the point of limitation in penicillinase formation. Although it is not yet certain that the penicillinase molecule synthesized by the "micro-mutants" is

Table 1. PHENOTYPIC BEHAVIOUR OF THE DIPLOID $147(a.i^+p_A^+...\gamma.ero^R/$ β . $i - p_G^+$. eros) and of the two segregants 147(α . $i + p_A^+$. . . γ . eror) AND $147(\beta \cdot i^-p_C^+ \cdot \text{eros})$

Strain	Genotype	Type of enzyme pro- duced A		cific enzy (enzyme : dry wt. i iduced : C-type	units/m oacteris	g i)
Diploid	$147(a \cdot i^+p_A^+ \dots \gamma \cdot \text{ero}^R)$					
	$\beta \cdot i - p_C^+$ eros)	A and C	_	· -	143	148
			~			91
	7 A M C 14			11	_	91
Segregant	$147(\alpha \cdot i^+ p_A^+ \dots \gamma \cdot \text{ero}^{\text{R}})$) A	8		312	
	$147(\beta \cdot i^{-}p_{C}^{+}, eros)$	C		217		269

Table 2. EXPRESSION OF VARIOUS CLONES OF THE DIPLOID 147(a.i.pt ... γ . ero^R/ β . $i^-p_G^+$. ero^S) and of the segregants $147(\alpha$. $i^+p_A^+$. . . γ . ero^R) (SEGREGANT 1) AND $147(\beta . i-p_C^+ . eroS)$ (SEGREGANT 2)

Specine enzyme activity			
(enzym	e units/mg d	ry wt.)	
	Induced		
Total	A-type	C-type	
284	157	127*	
297	136	151*	
306	161	145*	
812	158	154*	
284	150	134*	
272	137	135*	
261	145	116*	
202		161*	
		140	
	312		
	284		
	293		
	327		
	269		
	300		
	-	284	
		261	
	****	292	
	*******	244	
		263	
	-	247	
		249	
		271	
	261 202 288 ———————————————————————————————	261 145 292 181 292 181	

Table 3. PHENOTYPIC BEHAVIOUR OF THE DIPLOID 147(a. i+ . MICRO-INDUCIBLE . $p_A^+\dots\gamma$. elob/ β . $i^-p_G^+$. eros) and of the two segregants 147(a . i^+ . MICRO-INDUCIBLE . p_A^+ . . . γ . ero^R) AND 147(β . $i^-p_U^+$. ero^S)

Strain	Genotype	Type of enzyme	- (6	eific enzy enzyme i dry wt. b	inits/m acteria	g. .)
		pro- duced		duced C-type		uced C-type
Diploid	$147(a \cdot i^+ \cdot micro-indu-$					
	cible . p_A^+/β . $i - p_C^+$. eros)	A and (0.5	8	2	135
				8	1	37
Segregant	s $147(a \cdot i^{+} \cdot micro-indu-$					
	cible $p_A^+ \dots \gamma$ eroR)	A	0.1	-	3.7	_
	$147(\beta$, $i^ p_{\mathcal{O}}^+$, ero ^S)	C		261		283

identical with the wild type, it is certain that the very low level of enzyme activity synthesized by these strains does genuinely reflect the formation of small amounts of protein and not normal amounts of a protein of low specific enzyme activity. So, if a diploid culture is constructed with an $(i^-p_c^+)$ plasmid as one component of a diploid and an $(i^+$ micro-inducible p_{λ}^+) plasmid as the other, it should be possible to decide whether the low rate of expression of the p_A^+ gene (caused by the micro mutation and expressed in a diploid6) allows a correspondingly increased expression of the p_{σ}^{+} gene. In other words, can expression of the p_{θ}^{+} gene in the diploid fill the gap by the very low level of expression of the p_A^+ gene?

The diploid $147(\alpha \cdot i^+)$, micro-inducible $p_A^+ \cdot p_A^+ \cdot$ $\beta \cdot i^- p_{\sigma}^+$ ero⁸) was constructed and its behaviour in the presence and absence of inducer compared with that of the segregants $147(\alpha, i^+)$ micro-inducible p_A^+ . p_A^+ expected from previous experiments16, there was no repair of the micro-inducible phenotype in the diploid: the total amount of A-type enzyme synthesized in the presence of inducer was no more than 2 enzyme units/mg dry weight of bacteria-which is about the same rate of synthesis found in the induced haploid (i+ . micro-inducible p_{A}^{+} segregant. In a fully induced diploid, however, the level of C-type enzyme synthesis was about 135 units/mg-which is about half that found when the same genome was present in the haploid state in strain 147($i^-p_0^+$). Furthermore, this level of expression is similar to that found in the diploid $147(\alpha \cdot i^+p_{\pi}^+\dots\gamma \cdot \operatorname{ero}^n/\beta \cdot i^-p_{\pi}^+\dots\operatorname{ero}^s)$ —see Tables 1 and 2. It seems therefore that the presence of the $(\alpha.i^+$ micro-inducible p_{λ}^+ plasmid restricts the expression of the $(\beta . i^-p_0^+)$ plasmid to about half that found in the haploid segregant $147(\beta . i^-p_0^+)$ despite the fact that little A-type enzyme is synthesized by the diploid. These experiments therefore show that the limitation in penicillinase production in diploid cultures is not caused by competition between penicillinase protein molecules themselves for a limiting site; and accordingly, these results seem to rule out the possibility of a simple competition for a liberation site at the cell surface. The results do suggest, however, that a competition at some level of protein synthesis earlier than the formation of the intact molecule itself does occur—and that this could well be competition between the messenger RNA products of the two penicillinase genes for a restricted number of protein synthesizing sites in the cell.

The possibility that there may be restrictions in the number of sites which are used to express certain structural genes in Staphylococcus aureus raises the question whether this compartmentation reflects the architecture of the staphylococcal cell in any way. Staphylococcal penicillinase is a true extracellular protein¹⁷ (as defined by Pollock¹⁸) which—in the case of the strains used here—is excreted to a limit of about 40 per cent under steady state conditions in media in which the phosphate concentration is high19. Examination of the location of the residual "cellbound" enzyme (60 per cent) shows that about 5 pe: cent may be removed from the cell by washing in 0.6 molar sodium chloride at pH 8.0 but that the great majority of the remaining enzyme is firmly bound to the cytoplasmic membrane. Not more than 2 per cent of the total

penicillinase activity of the cell seems to lie free inside the cytoplasmic membrane—and this small amount could well arise by contamination of the fraction during preparation-particularly because the fractionation requires disruption of intact cells (my unpublished results). It seems probable therefore that staphylococcal penicillinase is synthesized on or in the cytoplasmic membrane, and that expression of the penicillinase structural genes occurs at a limited number of protein synthetic sites in this structure and not generally among all the synthetic sites of the cell.

Although a limitation in the expression of two structural genes in a diploid occurs with penicillinase synthesis in these strains, it is not a universal phenomenon as far as bacteria are concerned, or even for all strains of Staphylococcus aureus. Jacob and Monod¹, Cohn-Bourgeois20 and Revel²¹ have all studied the level of expression of β-galactosidase in diploid Escherichia coli cultures and in all cases there is a reasonable correlation between enzyme activity in the culture and the complement of β -galactosidase structural genes. Even in Staphylococcus aureus strain PS 80 (ref. 22) the synthesis of penicillinase in diploid cultures may reflect the number of penicillinase structural genes in diploids in certain circumstances (unpublished work of E. H. Asheshov and K. G. H. Dyke). In both the β-galactosidase and penicillinase diploids, however, where there is no restriction on enzyme expression, one genetic component of the diploid is chromosomal and the other extrachromosomal. In the penicillinase diploids described here, where restriction does occur, both the penicillinase structural genes are carried in the extra-chromosomal state on plasmids, and this may be related to the limitation in gene expression.

If the synthesis of certain proteins is restricted to certain regions in the bacterial cell, the question immediately arises as to how this is achieved in molecular terms. One possibility is that message synthesis occurs only in a restricted region in the cell and that widespread diffusion of the message to all parts of the cell is impossible. In view of the apparent restriction of penicillinase synthesis to a limited number of sites in some staphylococcal strains, it seems possible that these sites may provide a stimulus to allow message transcription from the gene to occur, but only when the gene and the relevant protein synthesizing sites are in the correct spatial juxtaposition. If this hypothesis is correct, it implies that the positional relationship between various portions of the bacterial genome and various sites of protein synthesis in the cell could well be of crucial significance to the functional differentiation of bacterial cells.

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LETTERS TO THE EDITOR

PLANETARY SCIENCE

Ionospheric Effects caused by Electrons precipitated from the Outer Radiation Belt

DURING the period October 1962 to March 1963, there was a disturbance in the ionosphere at the Antarctic station Sanae (70° 18' S., 2° 22' W.) whenever a high flux of precipitated electrons was observed in the magnetically conjugate area by the satellite Alouette1. Together with Gledhill, we' have shown that similar disturbances are produced at other stations lying near the same magnetic shell (L=4) in both the northern and southern hemispheres. We established that flux values above a certain level are observed only when the ionosphere is disturbed, and that the percentage of total time for which this level is exceeded at each station is almost exactly the same as the percentage time for which the ionosphere is disturbed. Our results showed that the frequency of occurrence of ionospheric disturbances on L=4 increases markedly in the vicinity of the South Atlantic geomagnetic anomaly. This observation was attributed to the fact that the mirror points of particles trapped in the outer radiation belt are much lower there than anywhere else, being responsible for the high intensities of charged particles observed there3,4.

There seems no reason why the effects² should be confined to the shell L=4. Several workers³⁻⁶ have observed high counting rates of charged particle fluxes at shells as low as L=2. It has also been observed⁷ that electron bombardment makes a contribution to the ionization of the F region in the South Atlantic both by day and night, and it has been suggested⁶ that the "winter anomaly" in D region absorption could be explained by mid-latitude electron precipitation. It therefore seems probable that electron bombardment could be an important cause of ionospheric disturbances at all sites which lie below the outer radiation belt. Unfortunately, satellite observations are not available to us at present for these locations, so this theory cannot be directly tested. Here we use the facts already established² to extend the treatment to lower L shells.

The behaviour of the ionosphere was examined at each of a large random sample of 2 h periods at several northern and southern hemisphere stations lying on the shells $1.88 \leqslant L \leqslant 4.47$ for the period October 1962 to March 1963. The percentage of total time for which the ionosphere was disturbed at each station was determined using the previous method¹. We have, however, made one change. We included the parameter f_0F_2 so that the ionosphere is classified as disturbed if any one of the three parameters f_{\min} , $h'F_2$ or f_0F_2 lies outside the calculated undisturbed range.

One of the shortcomings of this method is that the disturbances do sometimes occur on magnetically quiet days, and as a result of this the selection of the undisturbed range is not definite. We are at present improving this method and so the values given here should be regarded as relative. The results are shown in Tables 1 and 2. The results for the six stations examined previously² are also shown. In the case of Marion Island, data for the period October 1957 to March 1958 were used, because these are the only data in our possession for this station.

It is clear that the frequency of occurrence of ionospheric disturbances does increase over the South Atlantic geomagnetic anomaly, as is the case for L=4. In the light of previous results¹, this strongly suggests that the correlation between electron flux and ionospheric disturbances found at L=4 will persist at all sites which lie below the outer radiation belt. A detailed study of latitudinal longitudinal, seasonal and diurnal variations is being carried out at present.

It has been shown that the frequency of ionospheric disturbances increases markedly over the South Atlantic We should therefore expect the geomagnetic anomaly. frequent occurrence of this phenomenon to be reflected in the normal behaviour of the ionosphere in this region. This implies that a longitudinal variation should be found in the behaviour of the ionosphere at locations below the outer radiation belt. (The parameters f_{\min} and $h'F_{\perp}$ were unsuitable for direct comparison of values at various stations, because f_{\min} is dependent on the instrument used and $h'F_2$ could give misleading results because it is not simply related to the real minimum height of the F_z layer.) To investigate this theory we have used monthly world-wide radio prediction charts issued by the Central Radio Propagation Laboratories. From these maps it is possible to read values of the maximum usable frequency for propagation at vertical incidence (MUF(0)) at any desired location. Because MUF(0) is actually f_xF_2 , the extraordinary wave critical frequency of the F2 layer MUF(0) is equal to $f_0F_2+f_H/2$, where f_H is the gyro frequency at a given location.

MUF(0) charts available to us at present cover the period May 1963 to July 1967. We therefore chose to examine data for May 1963, this being the month nearest to the period studied. In Fig. 1, MUF(0) at local midnight is plotted against longitude for various values of L. In order to compare F_2 region behaviour in the anomaly with that elsewhere, we also plot MUF(0) against longitude for the northern hemisphere. In this case, we use data for November 1963 which is the corresponding seasonal period. We restrict our attention to the night-time period which is best suited for a study of electron induced effects, because, as far as is known, there are no other causes of ionization operative then.

Also marked on each curve is the longitude of minimum geomagnetic intensity, B_{\min} , indicating the longitudinal centre of the geomagnetic anomaly.

The southern hemisphere curves show unmistakably that MUF(0) decreases in the region of B_{\min} , passes through a minimum and then rises to a maximum to the west of the lowest B. The minima also move westward with B_{\min}

Table 1. FREQUENCY OF IONOSPHERIC DISTURBANCE IN THE SOITHERN

нем	ISPHERE		Ionospheri
$^{L}_{ m at~300~km}$	Lat.	Long.	disturbance
2·50 4·47 4·20 1·88	65·2° S. 75·5° S. 70·3° S. 34·1° S.	64·3 W. 26·6 W. 2·4° W. 18·3° E.	53 58 65 56 45
Stations out 2·98 2·08 4·20	side the anon 42.9° S. 35.3° S. 52.5° S.	147·2° E. 149·0 L. 169·1° E.	17 28 31 30
	L at 300 km Stations i: 2-50 4-47 4-20 1-88 2-81 Stations out 2-98 2-08	at 300 km Stations in the anomal 2:50 65:2° S. 4:47 75:5° S. 4:20 70:3° S. 1:88 34:1° S. 2:81 46:8° S. Stations outside the anon 2:98 42:9° S. 2:08 35:3° S. 4:20 52:5° S.	L Lat, Long. at 300 km Stations in the anomaly 2·50 65·2° S. 64·3 W. 4·47 75·5° S. 26·6′ W. 4·20 70·3° S. 2·4° W. 1·88 34·1° S. 18·3° E. 2·81 46·8° S. 37·9′ E. Stations outside the anomaly 2·98 42·9° S. 147·2° E. 2·08 35·3° S. 149·0 D. 4·20 52·5° S. 169·1′ L.

^{*} Stations examined previously2.

Table 2. FREQUENCY OF IONOSPHERIC DISTURBANCE IN THE NORTHERN

	пел	JAMENE		Ionospheri
Station	$_{ m at~300~km}^{L}$	Lat.	Long.	disturbane
Winnipeg* Washington Ottawa* St. Johns* Slough Dourbes Juliusruh-Rugen Miedzeszyn	4·38 2·73 3·78 3·63 2·53 2·31 2·72 2·37	49·9· N. 38·7° N. 45·4° N. 47·5° N. 50·1° N. 54·6° N. 52·2° N.	97·4° W. 77·1° W. 75·7° W. 52·8° W. 0·6° W. 4·6° E. 13·4° E. 21·2° E.	31 32 26 23 31 29 21 37

^{*} Stations examined previously.

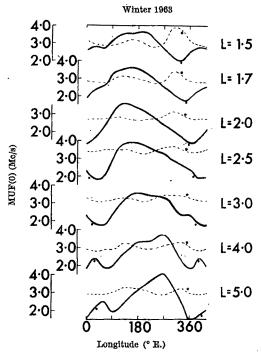


Fig. 1. Longitudinal variation of MUF(0) in the northern (---) and southern (---) hemispheres along various L shells at local midnight.

towards lower L shells. As is to be expected, the longitudinal variation of MUF(0) is very much less pronounced in the northern hemisphere, as the anomalous decrease in geomagnetic intensity is not very marked there. There are also two peaks in the northern hemisphere. One is approximately conjugate to the southern hemisphere minimum. This peak becomes more pronounced at lower L shells and at L=1.2 the northern hemisphere variation is larger than that in the southern hemisphere. It practically disappears at L=3 where the precipitated electron flux intensity was observed to decrease during the period May to July 1963 (ref. 5). Another peak is also discernible at about 150° E. which corresponds with the small increase in precipitation observed there3.

The southern hemisphere variations observed in MUF(0)are greatest in magnitude at L=5; there is a decrease towards L=3, an increase at L=2 and then a decrease again toward lower L values. This is consistent with the variation of precipitated electron flux intensity across L space for May to July 1963 (ref. 5). Preliminary analyses of the same type for other times of day, year and solar cycle indicate that the same behaviour persists during the six summer months, but not during winter. A detailed investigation of these phenomena is being carried out at present.

An explanation of the causes of this phenomenon will not be attempted here. Preliminary results, however, suggest that corpuscular bombardment of the atmosphere may give rise to two competing effects: an increase in electron density because of direct ionization, and a decrease in electron density because of a temperature increase which may increase both the scale height and the effective recombination rate.

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Cavernous Weathering in the Taylor Dry Valley, Victoria Land, Antarctica

TAYLOR VALLEY (77° 33' S., 163° 25' E.) is part of a deglaciated valley complex called the McMurdo Oasis, which is located about 80 km to the west of Ross Island across McMurdo Sound. Since the IGY many New Zealand and American geological parties have worked in this area1-3, which is easily accessible by helicopter from Scott Base and McMurdo Station at the southern end of Ross Island. Now that the topographical and geological reconnaissance stage has been completed and adequate maps cover the 2,500 km² of the McMurdo Oasis, more attention is being devoted to the study of various anomalies and problems.

One interesting geomorphological phenomenon, found in the Taylor Valley, is the cavernously weathered granite and gneiss erratic boulders (or taffonis). Processes of physical weathering which occur in this cold desert area are more involved, and probably more rapid, than has previously been imagined. Rock disaggregation in the McMurdo Oasis is not simply explained by the processes of freeze and thaw or wind, and evidence is mounting concerning the part which growth of soluble salt crystals plays in the development of cavernous weathering.

To establish the basis for more detailed studies, the important concentration of cavernously weathered erratics was mapped during autumn 1966 (Fig. 1). Many of the

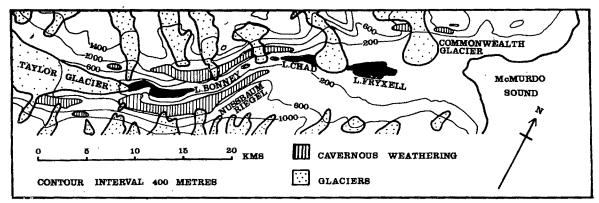


Fig. 1. Taylor Valley, Victoria Land, Antarctica, showing distribution of principal areas of cavernously weathered erratic boulders.

boulders observed were more than 2 m in height and were from 4 to 5 m in diameter, with pits from 1 to 2 m in diameter which rose gradually inwards. As weathering proceeds, however, the boulders are progressively lowered to a deeply pitted stage of coalescing disintegration, with the upper part being completely removed. As was observed in the Victoria Valley, no preferred orientation of honeycombing was noticed4.

Weathering processes in this locality have to be assessed against the nature of the environment and its climate. Taylor Valley extends for 40 km from the Taylor Glacier to McMurdo Sound (Fig. 1). Taylor Glacier which drains from the polar plateau is a former outlet glacier but now penetrates only a short distance into the valley. Tongues from several alpine glaciers descend into the valley from either side, and during the summer period streams of melt water drain into highly saline lakes, such as Bonney, Chad and Fryxell. Nussbaum Riegel (1,000 m) divides the valley into a lower, and more open, eastern section, and an upper, and more narrow, western section.

Taffonis chiefly occur in the upper Taylor Valley (Fig. 1) and, although they are found in the lower Taylor Valley, they do not appear with the same intensity, except for two small areas on either side of the Commonwealth Glacier. This disparity in location of taffonis is probably caused either by lack of erratic boulder deposition in the lower Taylor Valley, or by their removal or destruction by subsequent ice advances from the direction of McMurdo Sound. In the upper Taylor Valley, taffoni concentration is predominantly on a broad moraine covered bench, on the south side of the valley, 200 to 600 m above sea level, which runs from Nussbaum Riegel towards the Taylor Glacier, and on narrow benches, on the north side of the valley and at similar heights, which are broken by the tongues of the steep alpine glaciers as they descend towards the valley floor (Fig. 1). In some places, taffonis are located close to the snouts of these alpine glaciers, which indicates that the regimes of these glaciers were sluggish, even during the late Pleistocene.

Multiple glaciation has occurred in the Taylor Valley, and the moraines on which the taffonis lie were probably deposited during Péwé's Taylor Glaciation⁵ and Calkin's Bull Drift Episode of his Victoria Glaciation, to which he

has put a minimum age of 30,000 yr6.

In relating weathering forms to climate there is still a great lack of information on the heat balance of this area. It is known, however, that the mean annual temperature is in the vicinity of -17° C, although summer temperatures are usually in the range -4° to 0° C, and at times the air temperature can reach 10° C and ground temperatures over 20° C. It has been shown that in the active layer of the soil in this area, some twenty to sixty freezethaw cycles occur each year?. Precipitation, or snow accumulation, totals about 15-20 cm a year near the lower end of the Taylor Valley and tends to decrease as one moves inland. A strong positive radiation balance for the area of about 29,000 cal cm-2/years, however, soon disperses any snowfall, and indeed evaporation tends to continually enlarge the ice free area, with the low albedo of the bare rock surfaces causing an increase in summer temperatures and a decrease in relative humidity.

Winds blow predictably up or down valley and show a seasonal alteration. During summer, easterlies pre-dominate and are cooler and more humid than the drier katabatic westerlies which predominate in winter and are heated adiabatically in their rapid descent from the polar plateau of the continental interior. During summer, the influence of these warm, dry westerly winds is largely confined to the upper part of the Taylor Valley. The sublimating character of these westerly winds also tends to keep the valley snow free, even during the winter months. Although reliable comparative data are lacking, the upper part of the Taylor Valley, which is where the best examples of cavernous weathering exist, is a drier and a less cloudy and humid area than the lower part of

the valley where consequently rates of evaporation and sublimation seem to be less.

The existence therefore of favourable environmental conditions plus a plentiful supply of salts has led to the conviction that salt weathering is the dominant erosive process in the McMurdo Oasis. Other physical processes such as the influence of the wind can certainly no longer account for the development of cavernous weathering10, and frost fracturing is effective chiefly at the permafrost level and in areas open to insolation, whereas the development of salt crystallization favours the shady and sheltered situations of pits and hollows. In Corsica, where taffoni form resembles those in the McMurdo Oasis, it is noted that taffonis are commonest near the coast, where there is a plentiful supply of salt with only one weak frost a year, but at higher altitudes where there is a diminished salt supply but greater frost action, taffoni formation soon disappears11. Laboratory experiments have shown12 that crystallization of salts, such as sodium chloride and gypsum, can exert disintegrating forces in excess of rock strength when rapid cooling or evaporation produces a small degree of supersaturation.

The origin of the widespread occurrence of salts throughout the McMurdo Oasis has been under discussion^{7,13}, but it now seems probable from a trace element study of lakes in the McMurdo Oasis14 that the salts were principally extracted from continental rocks by glaciers and were fed into the lakes by streams of melt water during the summer. Origin apart, rock breakdown of the coarsely crystallino erratics begins to develop when the crystallization point of one of the salts is reached. The process is quite distinct from chemical weathering and does not seem to lead to widespread mineral alteration 10,15,16. Future investigations should, however, provide more relevant data with regard to the nature of the rock meal found in the interior of taffonis, and also make available more macro- and micro-climatic information, particularly on the mechanics of microgelivation and supersaturation.

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Large Scale Earth Resistivity Experiment in New Zealand

In 1965 a high voltage d.c. line, 580 km long, began operation, bringing power from the Benmore hydroelectric power station in the South Island of New Zealand to the Haywards terminal in the North Island (Fig. 1). Initially, the return path of the current was through the ground from which an approximately dipole pattern of potential might be expected on the Earth's surface. This potential would not in general be separable from other ground potentials of natural or artificial origin. Load changes in the Benmore-Haywards system are, however, carried out in steps of 20 MW, or 80 amp in line current. The consequent abrupt changes in ground potential can be observed for considerable distances from the electrodes provided there is no great telluric activity at the time.

These measurements have been used to investigate ground resistivities, the method being similar in principle to normal resistivity surveying but on a much larger scale. The New Zealand Electricity Department agreed to a programme of 20 MW load changes after midnight, when other earth current activity is at a minimum. Potentials were measured between orthogonal pairs of iron spike electrodes about 800 m apart. Observations were successfully obtained at forty-eight stations, chiefly in the South Island, and the results are shown in Fig. 1.

There is clearly an overall pattern which, however, departs considerably from a simple dipole. The most noticeable feature is the marked difference in the patterns around the two electrodes. The northern electrode is in good contact with the sea and vectors in its vicinity are small and varied in azimuth. In contrast, the Benmore electrode is sited in the middle of the South Island, where the chief rock types are schist, greywacke and argillite. The large radial gradients indicate a more resistive and

uniform medium here. On this pattern is imposed considerable variation in azimuth and amplitude, much of which is likely to be caused by near surface inhomogeneities. For example, accessible sites were frequently down in valleys rather than on exposed ridges, and the shape and conductivity of the valley sediments could well affect the current vector.

Observations around the southern electrode are sufficiently regular to be discussed in terms of a unipolar model. If a current of I amps flows into a uniform half space of resistivity ρ , from a point source on the surface, the surface potential gradient is

$$E = \frac{\rho I}{2\pi r^2} \text{ volt/m}$$

at a distance r metres from the source. Twenty-four stations have been selected as reasonably close to Benmore to be largely unaffected by the northern electrode. The observed values of the gradient are shown in Fig. 2, plotted against distance from the Benmore electrode. The dashed lines are the theoretical curves for a homogeneous medium with resistivities of 2,500 and 5,000 Ω m.

There is some systematic variation here. The gradients for stations more than 100 km distance from Benmore are

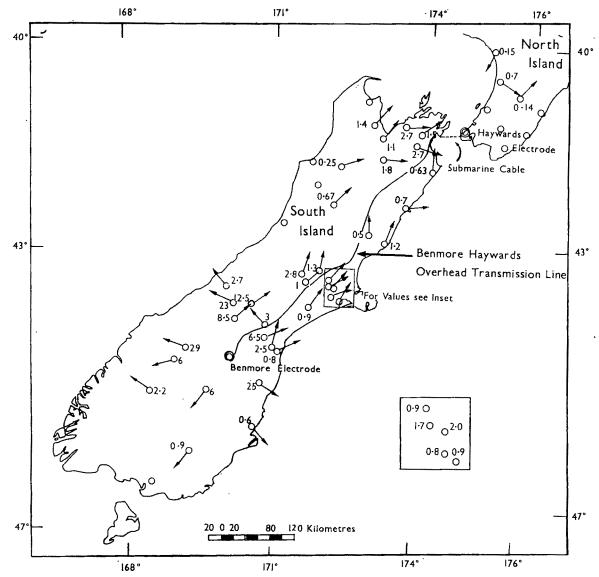


Fig. 1. Map showing positions of the two electrodes and the field stations. The arrows indicate the direction of maximum potential gradient at the station which is situated at the base of the arrow. The figures are the values of the potential gradient in mV/km. Circles without arrows indicate stations where no recognizable change in signal occurred on switching. Directions assume current entering ground at Benmore electrode.

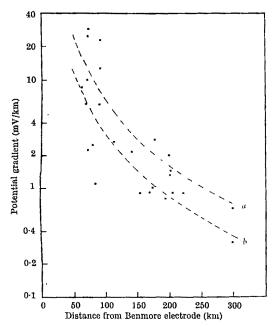


Fig. 2. A plot of the resultant potential E against distance from the Benmore electrode. The change in current flowing is 80 amp in all cases. The theoretical curves for $\varrho=5,000~\Omega m~(a)$ and 2,500 $\Omega m~(b)$ are shown.

less scattered than for stations closer to the electrode, again suggesting lateral inhomogeneities nearer the surface. The apparent resistivities, computed from the formula given previously using the observed gradients, range from 1,500 to 15,000 Ωm with a mean of 5,000 Ωm . These are rather high values but are not inconsistent with soundings in similar depths elsewhere. Lundholm¹ found resistivities of 14,000 Ωm beneath Sweden, and Krajev found resistivities of 2,000 Ωm beneath Karelia², with dipole separations of up to 600 km and 75 km, respectively. Cantwell, Nelson, Webb and Orange³ found values usually less than 1,000 Ωm for separations of up to 200 km in north-west United States.

The depth of penetration of the current is affected by the presence of the sea but must be several tens of kilometres at least under the South Island. The resistivity contrast between the Benmore region and the sea is of the order of 10,000 to 1, and suggests that to a first approximation the sea may be an equipotential. Deeply penetrating currents from the southern electrode would return then to the northern electrode through the sea bed and the sea. The observed surface vectors do not, however, fully support this and show some tendency to a dipole flow pattern. Further work is in progress.

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Age of the Paresis Complex, South-West Africa

MESOZOIC igneous activity in South-West Africa is shown as lava plateaux and dyke swarms of basaltic composition and as a group of central volcanic complexes representing a highly variable range of rocks¹. The Paresis complex (20° 15′ S., 15° 42′ E.) is one of the latter and is made up of a consanguineous suite of syenites, bostonites, rhyolites and associated foyaitic rocks, as well as minor basalts and gabbros. From structural and geochemical evidence Siedner²³ concluded that the basaltic rocks of Paresis represent locally preserved remnants of the regional Stormberg volcanics coeval with, but genetically unrelated to, the central felsic suite. The age of Paresis is thus significant because it dates both the complex and the regional volcanism. The only other published chronological datum on Mesozoic igneous centres in South-West Africa is a potassium-argon age of 190±8×10° yr on biotite from the Klein Spitzkop granite⁴.

For this study five quartz-bearing members of the central igneous suite, previously described and analysed3, were selected. Rubidium and strontium concentrations were determined by stable isotope dilution on a 6 in. mass spectrometer, and the isotopic composition of strontium was measured on a 12 in. mass spectrometer. In calculating the data it was assumed that the rubidium-85/rubidium-87 ratio of natural rubidium is 2.591 (ref. 5) and the strontium-86/strontium-88 ratio of natural strontium is 0·1194 (ref. 6). The strontium-87/strontium-86 ratios were normalized to strontium-86/strontium-88 = 0.1194. The errors are estimated to be 2 per cent in the rubidium-87/strontium-86 ratios and 0.05 per cent in the strontium-87/strontium-86 ratios. A straight line (Fig. 1) was fitted to the data (Table 1) by the method of York?. The weights assigned were the reciprocals of the squares of the estimated errors. The slope of the fitted line is 0.001872 ± 0.000060 from which the age of $135 \pm 4 \times 10^8$ yr was calculated, assuming $\lambda = 1.39 \times 10^{-11}$ yr⁻¹ (or 127 ± 4×10^6 yr if $\lambda = 1.47 \times 10^{-11}$ yr⁻¹). The intercept at rubidium-87/strontium-86 = 0 is 0.7086 ± 0.0014 . All the errors quoted are 99.5 per cent confidence limits. A potassiumargon age of 136×106 yr on a basalt from Paresis8 supports the present results.

Table 1. RUBIDIUM AND STRONTIUM CONCENTRATIONS AND ISOTOPE RATIOS OF ROCKS FROM THE PARESIS COMPLEX

210		
56·1 16·4 12·1	10·5 64·4 93·7	0·7105 0·7285 0·8282 0·8843 0·9765
	56·1 16·4	16·4 64·4 12·1 93·7

The Mesozoic volcanics of South-West Africa are, on stratigraphic grounds, usually correlated with the Stormberg Series of the Southern African Karroo System. Recent age measurements on rocks from Rhodesia^{9,10}. South Africa^{10,11} and Swaziland¹² confirm the Triassic to early Jurassic age of the Stormberg volcanism in those areas. Data reported here and elsewhere⁸ indicate that the climactic phase of Mesozoic volcanism in South-West

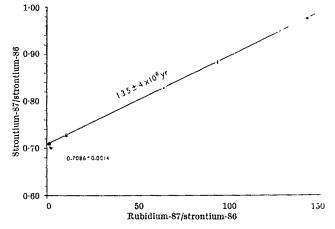


Fig. 1. Total rock isochron for a differentiated suite from the Paresis igneous complex, South-West Africa.

Africa, however, occurred between 110 and 135×10^6 yr ago. The apparent interval of at least 40×10^6 yr between the formation of the Stormberg volcanics in these two broad regions raises serious doubts about their stratigraphic equivalence. Age determinations on these rocks are unfortunately still too few and discontinuous to justify a high degree of confidence about the chronological pattern of the Stormberg volcanism in southern Africa and the nature of its westward migration. Further ageinvestigations on Mesozoic basalts from South-West Africa, Lesotho and Rhodesia are in progress.

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Life on the Surface of Venus?

It is said (for example, refs. 1-3) that our knowledge of the surface of the cloud covered planet Venus is extremely fragmentary and ambiguous; that there are alternative non-thermal explanations of the microwave emission; that, even if the surface is hot, the polar regions may be cold enough to support life, or sufficiently high mountains may exist, and so on. It seems appropriate to relate some of this speculation to continuing work on the

physical environment of Venus.

The principal objection to life on the surface of Venus is the high inferred temperatures. Ground-based measurements of the disk-integrated microwave brightness temperature as a function of wavelength4-6 and of phase angle7, as well as radio interferometry^{8,8} and the Mariner 2 microwave experiment^{10,6}, all have a simple and natural interpretation if the surface of Venus is a hot dielectric sphere with mean thermometric temperatures of about 700° K. A comparison of the radar and visual diameters of the planet combined with the most likely temperature structure of the atmosphere, within the range of probable errors, again suggests 700° K (ref. 11); this argument is independent of passive microwave results. The agreement among these different modes of measurement is striking. Those alternative, non-thermal, explanations of the microwave emission which have been made in sufficient detail to be tested disagree seriously with one or more of the microwave observa-This includes free-free transition models of ionospheric emission^{10,12}, synchrotron radiation from presumed Van Allen

radiation belts9,13, glow discharges as a result of charge separation¹⁴ and electrical discharge between adjacent liquid droplets in the clouds¹⁴. Some other non-thermal model may explain the microwave observations, but no such model yet exists. Although it has been difficult to account for the high surface temperature, recent developments in the construction of non-grey greenhouse and other atmospheric models have made possible successful generation of the requisite surface temperatures.

There has been speculation³ that the appearance of Earth as photographed by Lunar Orbiter 1 is sufficiently similar to that of Venus to suggest that the surface environments of the two planets are also similar. Fig. 1 compares photographs of crescent Earth and crescent Venus. At optical frequencies the overcast of Earth is about 50 per cent, and the overcast of Venus almost 100 per cent. No unambiguous breaks in the clouds of Venus have ever been reported. While the visible clouds of Venus certainly play a part in maintaining the high surface temperatures15, infrared absorption by the atmosphere must contribute much more to the total opacity in any successful greenhouse model. Thus not only are the cloud covers very different for Earth and Venus, but cloud cover is not a very useful criterion for comparing surface temperatures.

The conclusion that the surface is very hot is still inferential, and direct measurements by atmospheric entry probes are certainly needed (see addendum at end of communication). The complete evidence, however, strongly suggests average surface thermometric temperatures of about 700° K. In such hot surface models, the coldest spots will be at the poles; temperatures there can be estimated from interferometric observations and from symmetry arguments as $470^{\circ} \pm 95^{\circ}$ K (refs. 7 and 8). Even the polar temperatures are therefore probably above the normal boiling point of water. There are some radar data suggesting mountains on Venus, although it is actually areas of enhanced local roughness which have been found. The maximum possible lapse rate of temperature in the lower Venus atmosphere is the adiabatic, about 8° K/km. A mountain top in a typical locale with a temperature of, say, 350° K would have to be some 44 km high. Such mountains are impossible on Venus-the hydrostatic pressures at their bases exceed the tensile and yield strengths of all geochemically abundant materials. This problem even applies to hypothetical mountains at the poles of Venus.

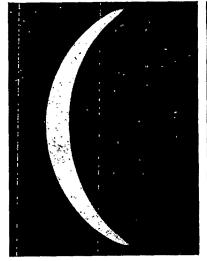




Fig. 1. Left: photograph of crescent Venus in integrated visible light (courtesy, Mt. Wilson and Palomar Observatories). Right: photograph of crescent Earth in integrated visible light by the ATS-1 satellite (courtesy, Goddard Space Flight Center, NASA). The surface resolution of Earth is here several times better than for Venus, but the contrast discrimination is worse. It is clear that, if the resolutions and shades of grey were degraded to the same level, surface detail would be visible on Earth and not on Venus.

The critical point of water is 647° K and 218 atm. Thus liquid water is impossible at average Venus surface temperatures. At the deduced polar temperature near K, the saturation vapour pressure over liquid water is 83 atm. In most models of the atmosphere of Venus the total pressure is less than this 16. Furthermore, the opacity at millimetre wavelengths caused by even I atm of water vapour would be grossly inconsistent with the microwave spectrum (compare refs. 4-6). Large quantities of water vapour are also inconsistent with the observed radar reflectivities. At cold spots the water vapour partial pressure is at most 1 per cent of the saturation value, and probably less.

Enzymes are rapidly inactivated, proteins denatured, and most biological organic molecules pyrolized at temperatures much less than 700° K, and so it seems safe to conclude that the mean surface temperatures of Venus exclude terrestrial forms of life. Alternatives such as biological refrigerators pumping heat across the surface boundary layer temperature gradient can perhaps be imagined, and some thermodynamic studies along these lines may be useful, but the overall chances of life on the surface of Venus remain bleak. The possibility of life in the clouds has already been discussed?

Addendum.The recent success of the Venus 4 spacecraft of the Soviet Union supports the conclusions reached here, which were written before the entry of the Venus 4 capsule into the atmosphere of Venus. Surface temperatures of 550° K near the nightside equator are roughly consistent with the interferometric and phase observations7,8; but because these temperatures are somewhat smaller than the microwave results, the strictures against life at the very poles may be slightly relaxed.

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PHYSICS

Relationships between the Masses of Subatomic Particles

In previous communications^{1,2}, I have shown that all subnuclear particles are probably composed of x tamaids combined with y bachs according to definite quantum conditions controlled by a quantum number z. In the

case of positively charged subatomic particles I developed a relationship as follows

Particles external to the core = (x-6)+y=(3+13z) (1)

One of the most remarkable features of the tamaid-bach stationary states is that the experimental data support the conclusion that there can be a facile interchange of tamaids and bachs as between the core and the external stationary states. I consider it very probable that the cores of subnuclear particles of positive charge contain a constant number of six entities regardless of their physical nature, the stationary states containing either three units or successive shells of thirteen unit particles in conformity with equation (1).

The structures indicated in Table 1 follow for the common mesons, baryons, etc., t^0 being the tamaid and b the bach.

Table 1. CHARGED SUBNUCLEAR PARTICLES

Symbol	z	Mass (MeV)	Core	First shell	Second shell	Third shell	Fourth shell	Fifth shell
$\mu^+ \ \pi^+ \ \mathrm{K}^+$	0 2 1	105·659 139·57 493·78	$ \begin{array}{r} 4t^0 + 2b \\ 5t^0 + 1b \\ 6t^0 \end{array} $	3b 3b 3b	13b 13t°	13b		
Q+ D Σ+	3 3 4	758-30 938-256 1,189-53	6t° 6t°	3b 3b 3b	13t° 13t° 13t°	$10t^{0} + 3b$ $13t^{0}$ $13t^{0}$	$13b$ $4t^{0} + 9b$ $13t^{0}$	1t° + 12b

The structures for the muons and the pi meson are particularly interesting because they imply the existence of bachs in the core.

It is known that modern atomic theory does not accept the presence of the electron within the atomic nucleus because of its large de Broglie wavelength, and it is also known that the neutron can nevertheless decay to give an electron. It is, however, improbable that, in the core of a subnuclear particle, a single bach combines with a single tamaid to form an entity comparable with the neutron because, according to the quantum equation (1), all the bachs seem to exert their specific influence even when they are apparently in the core.

Whether the shells given in Table 1 are characteristic of true orbitals or whether the various particles merely agglomerate in quantized layers is a matter for future speculation.

Some of the masses of certain subnuclear particles have been determined by various workers with very considerable precision3, and these have been used in Table 2 to give the best statistical value for the rest-masses of the bach and tamaid in MeV units, the normal method of least-squares analysis being employed. It is very unlikely that the bound mass of the tamaid remains absolutely constant, and this must affect the accuracy with which the absolute value of the mass of the bach can be determined.

Table 2 Particle Structure Mass (experimental) Mass (calculated) $4t^{\circ} + 5b$ $4t^{\circ} + 5b$ $5t^{\circ} + 30b$ $5t^{\circ} + 30b$ $5t^{\circ} + 16b$ $19t^{\circ} + 3b$ $19t^{\circ} + 3b$ $19t^{\circ} + 16b$ $36t^{\circ} + 12b$ $36t^{\circ} + 16b$ 105·429 105·429 139·570 139·570 134·982 493·989 493·989 498·250 938·050 $\begin{array}{c} 105 \cdot 659 \pm 0.002 \\ 105 \cdot 659 \pm 0.002 \\ 139 \cdot 577 \pm 0.014 \\ 139 \cdot 577 \pm 0.014 \end{array}$ 134-974 ± 0·014 134-974 ± 0·018 493-78 ± 0·17 493-78 ± 0·17 497-82 ± 0·25 938-256 ± 0·005 939-550 ± 0·005

From the series of simultaneous equations presented in Table 1, the best values for the bound rest-masses of the tamaid and bach are found to be $t^0 = 25.9477$ ± 0.009 MeV and $b=0.3277\pm 0.010$ MeV, respectively. The masses of the various subnuclear particles have been calculated using these figures and the results are given in the ultimate column of Table 2. The agreement shown with the experimental values suggests that the mass defect effect produced by adding unit tamaids to the structure of subnuclear particles must be remarkably constant.

I thank O. F. Newman for undertaking the statistical

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Some Preparative Details of the Nd3+/SeOCl2 Laser

Heller and Lempicki^{1,2} have reported a liquid laser, using a solution of neodymium ions in selenium oxychloride and tin tetrachloride, which operates at room temperature. My investigations have shown that the successful operation of this system is critically dependent on the purity of the components and the method of preparation. The rapid quenching of Nd³⁺ fluorescence by impurities, containing low atomic weight elements, makes it necessary to purify carefully the solvents and exclude moisture during preparation. Impurities can also be detrimental to the optical quality of the solution. A method of preparation is described here which has been found to give a working solution. Some preliminary results using a simple cell are briefly reported.

All handling and distillations were carried out in a dry 'Teflon' sleeving was used on all nitrogen atmosphere. glass to glass joints. Selenium oxychloride (B.D.H.) was fractionally distilled at reduced pressure (60° C at 14 mm of mercury). Marked decomposition occurred if the distillation was done at or near atmospheric pressure. Tin tetrachloride (B.D.H.) was allowed to stand over mercury for 24 h before fractional distillation. The mixed solvent was made by adding selenium oxychloride to

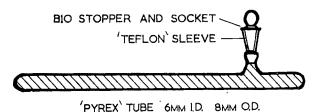
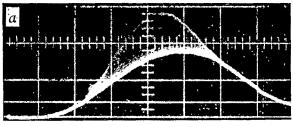


Fig. 1. 'Pyrex' cell with flame-sealed ends.



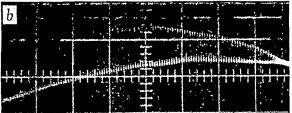


Fig. 2. a, Cell output at 600 Joule input energy, divisions = 75 μ sec; b, expanded portion of the spiking at the same energy input, divisions = 20μ sec. Detector rise-time approximately 0.5 μ sec.

11.5 ml. tin tetrachloride to give 50 ml. of solution. The prepared solvent was refluxed with neodymium oxide (Johnson, Matthey and Co. Ltd., 99.9 per cent) until complete solution occurred (2-3 min) and the solution was filtered through a ground-glass sinter. The rate of solution was dependent on the particle size of the oxide and further grinding may be necessary. The final solution had a pale reddish violet colour.

A 0.5 normal Nd3+ solution was tested in a simple cell, similar to that described recently by Lempicki and Samelson³ (Fig. 1). This cell was pumped in a 12.5 cm long elliptical cavity, and the output, through a filter centred at 10,500 Å, band pass 300 Å, is shown in Fig. 2. Feedback occurs through total internal reflexions within the tube, and the stimulated emission is not collimated. The regular nature of the spiking is shown on the expanded time base in Fig. 2b. No value has been obtained for the laser threshold in this cell because the minimum energy input with the available equipment is 300 joules. A high gain for the material was, however, indicated by decreasing the pumped length of the tube to 5 mm; at an input of 600 Joules distinct spiking was still observed.

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Taylor Vortices with Eccentric **Rotating Cylinders**

THE stability of circumferential laminar flow between rotating concentric cylinders has been extensively investigated, the classical work being that of Taylor¹. The effect of eccentric positioning of the cylinders, however, has received relatively little attention, although this further complication is of technological interest in connexion with operating journal bearings at very high speed in the so-called superlaminar regime. I have carried out experiments with an eccentrically positioned rotor inside a stator, the axes being parallel, and I have already described how the Taylor vortex pattern still appears, but with the critical speed raised appreciably above Taylor's value for concentric cylinders.

The vortex pattern, made visible by suspending small aluminium particles in the test fluid, is substantially similar in appearance for both concentric and eccentric positioning of the cylinders, but the spacing diminishes at high eccentricity ratios (that is, displacement of centres divided by radial clearance) and the pattern then becomes confused because of reverse flow and vortex waviness.

In a recent communication², I described a simple and sensitive method for detecting the onset of Taylor vortices, using a small constant-temperature heated probe which traversed slowly and axially in the clearance space. The percentage increase in critical speed over the concentric value was almost the same at a given eccentricity ratio, for clearance ratios (that is, radial clearance divided by rotor radius) of 0.478 and 0.274 (ref. 2). I have now extended these measurements to six clearance ratios from 0.138 to 0.478, using oils and air as test fluids, and I have again found that the clearance ratio has little effect on the critical speed ratio.

These results, obtained using a heated probe, all showed critical speeds above those determined by visual observation, and it seemed desirable to check the determination by direct measurement of rotor torque, for this is the quantity of most practical significance. I have made torque measurements using a torsion-mounted motor for a clearance ratio of 0.207, and in Fig. 1 it can be seen that there is the expected increase of torque as centrifugal effects begin to modify the laminar flow regime. For concentric conditions the change of slope of the torque against speed graph is clearly marked, but, because the cylinders are made eccentric, the critical change becomes progressively less sharply defined, especially for eccentricity ratios over 0.5. The critical speed is clearly seen to increase with eccentricity ratio and, as shown in Fig. 2, the critical conditions determined by heated probe

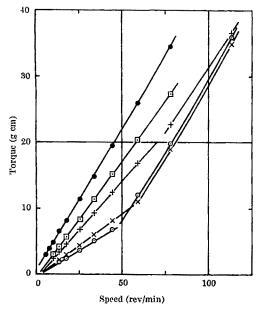


Fig. 1. Variation of torque with eccentricity ratio, ϵ . Rotor dimensions; 2-48 in. diameter, 14 in. long and 0-26 in. radial clearance; oil 10-5 cst. The various eccentricities, ϵ , are \bigcirc , 0; \times , 0-47; +, 0-59; \square , 0-71; \bullet , 0-83.

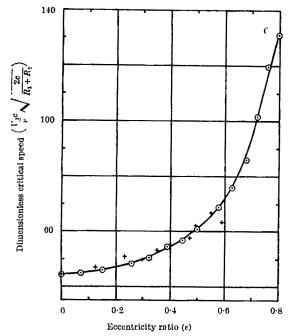


Fig. 2. Effect of eccentricity ratio on critical speed. The dimensionless critical speed is $\frac{V_1c}{\nu} \sqrt{\frac{2c}{R_1+R_1}}$ where V_1 is the surface velocity, ν is the kinematic viscosity, and R_1 and R_1 are the inner and outer radius, respectively. \odot , Is a heated probe indication, and + are torque measurements.

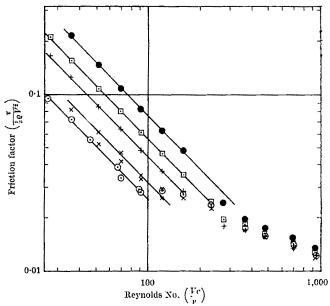


Fig. 3. Effect of eccentricity ratio on the friction factor. The friction factor is $\frac{\tau}{\frac{1}{2}QV^2}$ where τ is the shear stress at the rotor and g the density. The various eccentricities, ϵ , are: \bigcirc , 0; \times , 0.47; +, 0.59; \square , 0.71; \bigcirc , 0.88,

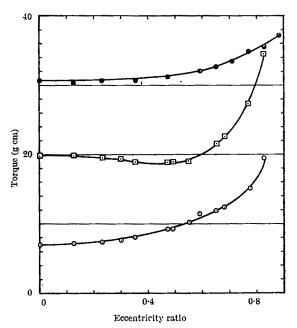


Fig. 4. Effect of eccentricity ratio on torque. •, 152 r.p.m., 3.5 cst; (superlaminar); •, 78 r.p.m., 10.5 cst (intermediate); •, 44 r.p.m., 10.5 cst (laminar).

traverse and by torque measurement are in adequate accord.

If the measured torques are expressed conventionally as the ratio of shear stress to dynamic pressure, the resultant friction factor against Reynolds number diagram is as shown in Fig. 3. There is some tendency for the critical condition to occur at a constant level of friction factor, and the superlaminar friction factors are less dependent on eccentricity ratio than the laminar friction factors. This last point is also brought out in Fig. 4, where the actual torque readings are seen to increase more rapidly with eccentricity ratio for a speed in the laminar range (and in agreement with calculations based on viscous flow theory) than for a speed in the superlaminar range. The intermediate curve shows the inter-

esting situation where an initially superlaminar flow at low eccentricity ratios becomes laminar at the same speed for higher eccentricity ratios with a consequent decrease in friction at intermediate eccentricity ratios.

These results show that the critical condition is difficult to determine accurately by measuring gross torque at other than small eccentricity ratios. I therefore investigated the use of a small constant-temperature heated probe mounted flush with the surface of the stator to detect the change in local heat transfer coefficient associated with the onset of Taylor vortices.

Under concentric conditions and at eccentricity ratios up to about 0·3, the rate of heat transfer was observed to change very rapidly at the critical condition, the direction of the change depending on the axial position of the probe relative to the vortex system. As the eccentricity ratio was progressively increased, the change became less definite, which indicated a change of flow regime, and consistent results could not be obtained.

I therefore regard the detection method using a traversing heated probe² as relatively the most satisfactory, but further studies at high eccentricity ratios are desirable.

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CHEMISTRY

Structure of the NH Stretching Band and (NH).. N Hydrogen Bond Frequencies in Purine

THE AH stretching band of a strongly hydrogen bonded AH... B system is usually very broad and may present a complicated structure consisting of numerous sub-bands. In order to explain these sub-bands, the frequency modulation theory—that is, that the sub-band frequencies can be represented by the expression $v = vAH \pm nv(AH) ... B$, where $n = 0, 1, 2 \dots$ and $\nu(AH) \dots B$ is any hydrogen bond frequency—has been advanced in the literature 1-3. In the case of imidazole, which contains strong NH..N hydrogen bonds, we have suggested that the hydrogen bond-frequencies do not seem to be a decisive factor in determining the sub-band structure of the vNH absorption4. In this communication, we wish to show that the same is true for purine, which contains a similar hydrogen bonded system, by comparing the behaviour of the vNH sub-bands and the far infrared hydrogen bond frequencies of different isotopic species at different temperatures.

The crystal structure of purine has been determined⁵. The molecules are linked in infinite chains by short hydrogen bonds, the N. N distance being 2.85 Å. The unit cell contains four molecules, and the symmetry of the orthorhombic crystals belongs to the space group $Pna2_1$. There are twenty-one lattice vibrations which can be represented by $5A_1 + 6A_2 + 5B_1 + 5B_2$. Thus only fifteen lattice modes are infrared active.

In the observed spectrum of crystalline purine there are ten absorption bands in the 300–33 cm⁻¹ region (Fig. 1, Table 1). Two of them, at 268 and 230 cm⁻¹, correspond to the internal modes while the remaining bands are likely to be caused by the external vibrations, which necessarily include the hydrogen bond motions. No detailed assignment of the latter can be given at the present; however, the strongest band at 110 cm⁻¹ is believed to be caused by

Table 1. FAR INFRARED FREQUENCIES OF PURINE AND PURINE-8-d.

Purine		Purir	ne-8-d,
25° C	−180° C	25° C	–180° C
268 s	271	268	270
230 w	230	224	225
168 m	178	168	178
130 sh		130	
110 s	117	110	117
	112		
91 w	94	91	95
73 w	73	71	72
57 m	62	57	62
51 m	55	51	55
41 m	47	41	47

Table 2. Sub-bands of the νNH absorption bands of solid purine and purine-8-d_1

Pu	rine	Puri	ne-8-d,
25° C	−180° C	25° C	−180° C
3,070 3,010 2,942 2,865 2,780 2,725 2,680 2,610	3,072 3,002 2,935 2,886 2,782 2,728 2,678 2,608	2,987 2,939 2,760 2,712 2,634	2,990 2,940 2,758 2,711 2,631
2,557 2,538	2,562 2,540	2,580	2,575

a predominantly hydrogen bond stretching mode, by analogy with the imidazole crystal.

There is an important temperature effect on the lattice vibrations of purine. As shown in Table 1, the frequencies shift to higher wave numbers when the crystal is cooled to liquid nitrogen temperature. The isotopic effect—that is, when the CH group between the imidazolic nitrogens is deuterated (purine-8-d₁)—on the other hand, does not shift the lattice frequencies within the experimental error $(\pm 1 \text{ cm}^{-1})$.

In the 4,000–2,000 cm⁻¹ region of crystalline purine only the NH and CH stretching frequencies are expected. The vNH absorption band of purine (Fig. 2) has its centre of gravity near 2,700 cm⁻¹ and a half-width of about 600 cm⁻¹. There are ten relatively well defined and strong sub-bands which disappear in the spectrum of ND substituted purine (purine-7-d₁) and are thus caused by hydrogen bonded NH groups. Their frequencies are given in Table 2. The CH stretching fundamentals, between 3,100 and 3,000 cm⁻¹, are much narrower and can be easily distinguished from sub-bands by deuteration.

The temperature and isotope effects on the lattice frequencies and on the sub-maxima of the vNH band can be compared in order to check the relationship required by the frequency modulation theory. The sub-maxima on the high frequency side of the vNH band centre would be expected to shift to higher and those on the low frequency side to lower wave numbers on cooling the crystal; furthermore, the difference bands should show a marked intensity decrease. This result is not observed. In fact, the temperature shifts of the sub-bands are irregular (Table 2) and the intensity of the low frequency side sub-bands increases at low temperature. The vNH band sub-maxima of the isotopically substituted purine-8-d₁, on

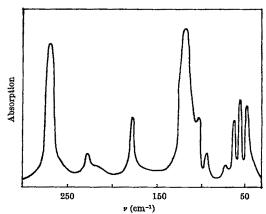
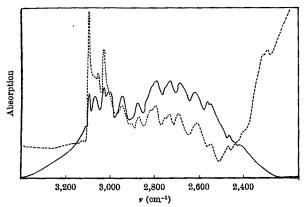


Fig. 1. Far infrared spectra of purine at liquid nitrogen temperature; nujol mull.



2. Infrared spectra of purine (—) and of partially deuterated purine-7-d₁ (....); NH/ND=10/90. Mulls in 'Fluorolube'.

the other hand, should not vary appreciably in either position or intensity, because the lattice frequencies do not shift and the NH..N bond remains unaltered. In fact, the centre of gravity of the vNH band of purine-8-d1 has the same frequency as that of purine; its structure, however, is different. There are only six well defined subbands and their frequencies differ considerably from those of purine (Table 2). Even more profound changes are observed in the structure of the vND bands of purine-7-d1 and purine-7,8-d₂, which show only two or three sub-bands while their lattice frequencies vary very little (our unpublished results).

We therefore conclude that the experimentally observed lattice frequencies, which include hydrogen bond vibrations, do not seem to determine the sub-band structure of the vNH absorption of purine and we must look for other reasons. It has been shown recently for solid alcohols that the splitting of the vOH band is caused by nearest neighbour coupling of adjacent OH groups and that this is the predominant reason for band broadening. In the case of purine, splitting of the vNH band into three infrared active components (A_1, B_1, B_2) may be expected for the crystal and two (nearest neighbour coupling) for the isolated chain approximation. We have investigated the infrared spectrum of a mixed crystal containing 90 per cent purine-7-d, and 10 per cent purine. stretching vibration can then be considered as decoupled and the structure of the vNH band is expected to be simpler. It turns out, however, that this is not the case; essentially the same sub-bands as for purine are observed in the spectrum (Fig. 2). This indicates that the chief submaxima belong to an isolated NH..N system.

The assumption that the sub-bands are caused by the overtones and combinations of internal vibrations in Fermi resonance with the NH stretching mode, as found for imidazole4, seems to be the most satisfactory. An assignment of the sub-bands is not given, but their behaviour with different isotopic species is consistent with the fundamentals which may give rise to binary combinations in the 3,200-2,200 cm⁻¹ region.

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Solvation Forces in Soap Films

THE stability of soap films and colloidal dispersions involve the same forces, thus leading to the same type of curves for potential energy plotted against distance. Such curves frequently show two minima¹ (Fig. 1). Stabilization in the secondary minimum at relatively large inter-particle separation distances (102-103 Å) is controlled by electrostatic double layer repulsion forces and this region of the curve has been investigated in some detail² for aqueous soap films in air. Stabilization in the primary minimum at small separation distances (10-20 Å) has been considered to be governed by a short range repulsive force as a result of interaction between solvation layers a few molecules thick which are associated with the two flanking surfactant monolayers3. Any closer approach of the two interfaces entails desorption which manifests itself as a rapidly increasing repulsive force as successive solvation layers are removed4. Because of the lack of specific data, this short range repulsive force is conventionally represented in the potential energy diagram by a very steeply rising curve at a certain critical separation distance¹, implying that primary minimum, or Perrin, films must be subjected to very large pressure differences before they can be further thinned. One method for investigating this region of the potential energy curve is to measure film thickness as a function of relative water vapour pressure.

Equilibrium film thicknesses can be conveniently determined by an optical reflexion method⁵ using the appropriate three layer optical model^{6,7} to interpret the photometric data. The equilibrium thickness of vertical black films, formed from aqueous solutions of decyltrimethylammonium decyl sulphate (5×10-4 molar) containing varying amounts of sodium bromide (0.05-2 molar), has been determined optically, in the first instance for films in contact with bulk solution at their lower meniscus.

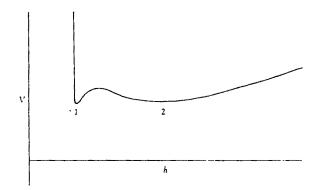


Fig. 1. Schematic representation of the total potential energy (V) per unit area of a vertical soap film as a function of thickness (h) at an arbitrarily low ionic strength. 1 is the primary, 2 the secondary, minimum,

The films were formed on a rectangular $(2 \text{ cm} \times 5 \text{ cm})$ glass frame mounted within a glass vessel which was totally submerged in a water thermostat at 25° ± 0.01° C. The electrolyte concentrations were chosen to give freedraining "mobile" films. The variation in total film thickness (h) with electrolyte concentration is shown in Fig. 2. An isolated film, supported on the same glass frame, rapidly attained isopiestic equilibrium with a bulk solution placed in the same vessel. Thus starting with a film, formed from a bulk solution containing 0.05 molar sodium bromide, the thickness could eventually be reduced to 46 Å when a saturated solution of sodium bromide $(p/p_0=0.577)$ was introduced into the system. The changes in thickness with varying water vapour pressure were shown to be reversible for single films which had lifetimes of the order of several days.

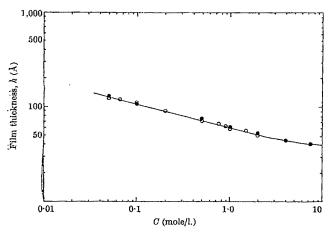


Fig. 2. Equilibrium thickness (h) of black foam films as a function of sodium bromide concentration (C) of bulk solution. \bigcirc , Equilibration through the bulk phase; \bigcirc , equilibration through the vapour phase.

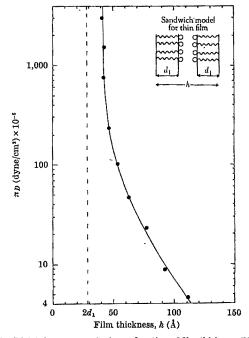


Fig. 3. Disjoining pressure (π_D) as a function of film thickness (h). The thickness of two surface layers, $2d_1 = 28$ Å.

Thicknesses are in good agreement with the previous equilibrium measurements (Fig. 2). The introduction of a series of saturated salt solutions of progressively lower partial pressures down to $p/p_0 = 0.11$ has enabled film thicknesses at these low water activities to be measured. Even though the water vapour pressure was considerably lower than the value for saturated sodium bromide, film lifetimes at the lowest partial pressure which were studied were of the order of 3 h. Achievement of these low thicknesses by the hydrostatic compression method² would require pressures in the range 10²-10³ atmospheres.

In describing the thermodynamics of thin film systems, Derjaguin has introduced the concept of a disjoining pressure^{8,9} which results from the net effect of the various surface forces operating in the thin layer. At equilibrium, the internally generated film pressures must balance the externally applied osmotic pressure. Thus the removal of water from a thin film to the infinitely thick pure liquid phase as standard state can be related to the disjoining pressure (π_D) by

$$\pi_D = -\frac{RT}{v} \ln (p_h/p_0) \text{ dyne em}^{-2}$$

where R is the gas constant, T the absolute temperature. v the partial molar volume of water, p_h the vapour pressure of the film and p_0 the vapour pressure of pure water. Fig. 3 shows that at high pressures the total film thickness (h) as a function of π_D tends asymptotically to a limiting value of 40 Å corresponding to an aqueous core thickness of 12 Å (ref. 7). These results clearly indicate the existence of an equivalent strong repulsive force which prevents film collapse at this limiting equilibrium thickness. magnitude of this force is of the correct order to permit its identification with that required for desolvation4.

These experiments have thus demonstrated the equilibrium nature of these very thin Perrin films and the importance of short range solvation forces in determining their thickness.

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The Elovich Equation in Chemisorption Kinetics

THE equation first proposed by Roginsky and Zeldovich¹ but now generally known as the Elovich equation has been extensively applied to chemisorption data2,3. The equation is

$$\frac{\mathrm{d}q}{\mathrm{d}t} = a\mathrm{e}^{-aq} \tag{1}$$

where q = amount of gas chemisorbed at time t and aand a are constants.

The most common method of determining a and α involves the integrated form of equation (1)

$$q = \frac{2 \cdot 3}{\alpha} \log (t + t_0) - \frac{2 \cdot 3}{\alpha} \log t_0 \qquad (2)$$

where

$$t_0 = \frac{1}{\alpha a} \tag{3}$$

A value of t_0 is chosen to linearize a plot of q against log $(t+t_0)$. The gradient of this plot gives a value for α , and a can then be calculated from equation (3).

It should not be forgotten that using equation (2) in this way is merely a graphical method of curve analysis. The acid test of any method of calculating a and α values is the ability of these values to reproduce the experimental data when applied to equations (1) or (2). This test has seldom been applied to published Elovich parameters. I intend to show that the accepted method of using equation (2) often leads to a and a values which do not reproduce experimental data, and to describe a modified analysis.

The calculated a value should equal the experimental rate at t=0 (equation (1)) but in most published analyses this is not the case². This has been attributed²⁻⁴ to the presence of a fast initial uptake which does not follow

equation (1). Nevertheless, t_0 values are selected to linearize q against log $(t+t_0)$ plots over the entire course of chemisorption. In my opinion, it is quite wrong to impose linearity on a section of the integrated plot where equation (1) is believed not to apply; this will result in an incorrect choice of t_0 and thus to incorrect a and α values. A better approach would be to determine the extent of the rapid initial stage and then to choose a t_0 value giving linearity only over the later stages which do obey Elovich kinetics. This approach has already been successfully adopted by Ritchie and myself5, using the following method.

Despite the imprecisions in computing rates from q against t curves, the linearity of \log_{10} (dq/dt) against q plots (equation (1)) may be taken as at least a qualitative test of the adherence of data to Elovich kinetics. This test involves no choice of a disposable parameter, and if such a plot is not linear at any stage it will be wrong to choose a t_0 which linearizes the integrated plot over that stage. Various photoadsorptions on titanium dioxide were found to follow parabolic kinetics

$$(q+q_0)^2 = kt + q_0^2 (4$$

where k and q_0 are constants, over their initial stages. Plots of $\log_{10} (dq/dt)$ against q were linear only over subsequent stages. Elovich parameters were calculated by taking zero time as the time at which Elovich kinetics began to apply and then choosing t_0 to linearize the integrated plots only from that time onwards. calculated a values then agreed with the experimental rates at the times of change-over from parabolic to Elovich kinetics, that is, at t=0 on the new time scale.

I have now applied such an analysis to selected chemisorption data from the literature. The chemisorption of oxygen on carbon films6 and on metal chromites7, of hydrogen on chromic oxide8 and of iodine on graphite8 followed parabolic kinetics (equation (4)) over their initial stages, and Elovich kinetics over their later stages. In other cases, for example, oxygen on nickel¹⁰, nitrogen on Fe-Al₂O₃-K₂O (ref. 11) and oxygen on carbon films (unpublished results of J. C. Orr and myself) no kinetic expression could be assigned to the initial non-Elovichian chemisorption. In still other cases12, the complete linearity

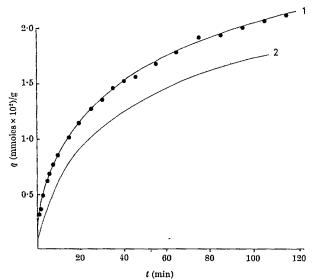


Fig. 1. Points represent experimental data? for chemisorption of iodine on graphite at 400° C.

Curve 1. t=0 to t=7 $(q+q_0)^2=kt+q_0^2$, k=750 (mmoles $\times 10^2$)?/min, q_0 negligible, t=7 to t=20, Elovich kinetics, a=1.76 (mmoles $\times 10^3$ /g)⁻¹, $q_0=12$ min. $t_0 = 12$ min. t = 20 onwards, Elovich kinetics, $\alpha = 1.57$ (mmoles $\times 10^2/g)^{-1}$,

Curve 2. Elevich over entire chemisorption, $\alpha = 1.76$ (mmoles $\times 10^2/g)^{-1}$,

of \log_{10} (dq/dt) against q plots showed that the chemisorptions were Elovichian over their entire course.

In all cases there was satisfactory agreement between avalues and the appropriate experimental rates. The α values from integrated plots agreed with the less precise values determined from \log_{10} (dq/dt) against q plots (gradient -0.434α).

Abrupt changes of slope are a feature^{2,3} of many integrated Elovich plots. The q values at which "breaks" occur depend on t_0 and thus are different when the modified analysis is applied. The a values of second and subsequent Elovich sections agreed reasonably with the experimental rates at the beginning of the sections when they were calculated from

$$a = \frac{1}{(t_0 + t^*)\alpha} \tag{5}$$

where α was obtained from the gradient of the section and t^* is the time for which previous Elovich section or sections had been in force. No mechanistic significance is yet attached to this empirical observation.

Only in a few cases¹² were Elovich kinetics followed over the entire chemisorption. There the accepted method of analysis gave parameters which reproduced the data. In all other cases⁵⁻¹², a and α values obtained by the present modified analysis described the experimental data accurately, in contrast to those obtained by linearization of integrated plots over the entire chemisorption. This situation is exemplified in Fig. 1.

Because values of a and a have featured in chemisorption models^{2,4} and in activation energy calculations² it is important to obtain reliable values of these parameters. It seems that many values quoted in the literature may be suspect.

I am grateful to the authors of refs. 6, 9, 10 and 12 for sending me copies of their original experimental data.

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MOLECULAR STRUCTURE

Molecular Structure of p-Bromocarbobenzoxy-Glycyl-I-Prolyl-I-Leucyl-Glycine

WE have studied by X-ray analysis a series of oligopeptides, such as carbobenzoxy-Gly-Pro(OH), carbobenzoxy-Gly-Pro-Leu(OH), carbobenzoxy-Gly-Pro-Leu-Gly(OH) and carbobenzoxy-Gly-Pro-Leu-Gly-Pro(OH), which were prepared in order to examine the relationship between the structure of collagen and the substrate specificity for the enzyme reaction of collagenase, and have already reported some of the results1-3. Recently, the threedimensional analysis of the tetrapeptide, p-bromocarbobenzoxy-Gly-Pro-Leu-Gly(OH), has been almost completed and a unique conformation was found in its structure.

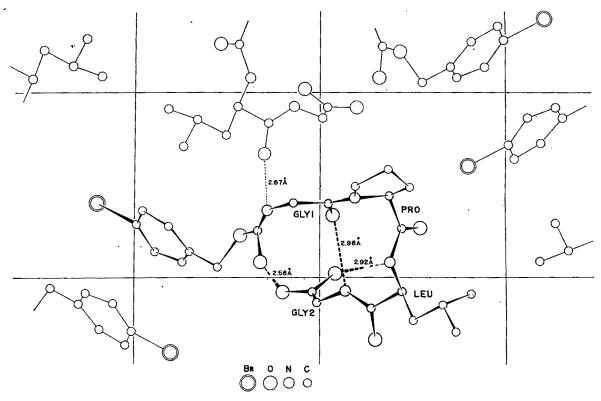


Fig. 1. Part of the crystal structure of the peptide, looking down the b axis.

The peptide chain, shown in Fig. 1, is folded back at the proline and leucine residues so as to form the intramolecular hydrogen bond (2.96 Å) between NH of Gly(2) and O of Gly(1). The conformation of the peptide backbone of this molecule is very similar to those of cyclohexaglycyl⁴ and the cyclic hexapeptide in ferrichrome-A (ref. 5). The same type of hydrogen bond has been found in these cyclic hexapeptides.

The striking feature of this peptide is that, unlike other peptides^{6,7}, it has the $C\alpha$ —H bond cis to the C'=O bond of the proline residue⁶. This may have something to do with the inactivity of this peptide to the enzyme collagenase. Both the intramolecular hydrogen bond and the conformation of the proline residue seem to be important for the stabilization of this folded structure.

The hydrogen bond, 2.92 Å, between N of the Leu residue and the carboxyl group of Gly(2) of the next upper molecule along the b axis may also play an important part in the structure. The side view of the molecule is shown schematically in Fig. 2. The terminal Gly(2) residue goes down, and one of its oxygen atoms makes a very strong hydrogen bond of 2.56 Å with the carbonyl oxygen atom of the carbobenzoxy group of the lower next molecule. Thus, by way of the strong hydrogen bond, an endless chain of the peptides is made up parallel to the b axis, and the chain as a whole looks like a somewhat deformed helix.

The dimensions of the peptide linkages are very similar to those given by Corey and Pauling⁹. The angles of rotation in the peptide backbone are listed in Table 1. The dihedral angles between neighbouring peptide linkages are 84° , 73° and 103° . The *p*-bromocarbobenzoxy group

Table 1. INTERNAL ROTATION ANGLES IN THE PEPTIDE BACKBONE

Residue	$\varphi(N-C_{\alpha})$	$\psi(G_{\alpha}-C'$
Gly(1) Pro Leu Gly(2)	269° 117 74 74	351° . 151 195

The definitions for these internal rotation angles are those given by Edsall et al. 10. Those in Pro-70 in lysozyme 11 are: $\phi=132\cdot0^\circ$ and $\psi=141\cdot4^\circ$.

seems to have little influence on the conformation of the peptide backbone.

The crystal data of p-bromocarbobenzoxy-glycyl-l-prolyl-l-leucyl-glycine, $C_{23}H_{31}N_4O_7Br$, are: $a=14\cdot25$, $b=6\cdot21$, $c=29\cdot67$ Å, space group $P2_12_12_1$, and Z=4. The X-ray intensity data of the reflexions with the spacing

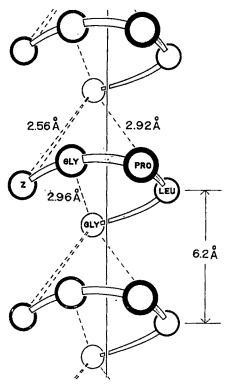


Fig. 2. Side view of the peptide, drawn schematically.

(d) larger than 1.2 Å were collected by means of a single crystal diffractometer. The R factor for 918 non-zero reflexions is 0.06 at the present stage of the structure refinement. The details will be published elsewhere.

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glucometasaccharinic acid (pathway B). All the intermediates and final products were identified by paper chromatography and paper electrophoresis.

This scheme for the alkaline degradation of glucose-6phosphate is strikingly similar to the well known anaerobic enzyme metabolism of carbohydrates3. Glucose-6-phosphate is produced in muscle either by the action of muscle hexokinase on glucose, or by the glycolysis of glycogen. through the intermediate formation of an a-glucose-1phosphate. In muscle, glucose-6-phosphate reversibly isomerizes into fructose-6-phosphate, a reaction which is catalysed by the enzyme isomerase, in a way analogous to the alkaline isomerization (pathway A) and probably also involving an enediol anion intermediate⁴. In glycolysis, however, the splitting of fructose-6-phosphate to the three-carbon compounds initially requires its phosphorylation by adenosine triphosphate to fructose-1,6-diphosphate (enzyme-phosphofructokinase), which only then splits to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Several further steps are involved until the final conversion into lactic acid. A very similar scheme occurs in the alcoholic fermentation of glucose (the Embden-Meyerhof pathway), except that the final product is ethanol and carbon dioxide. There is no known metabolic analogue, however, to the reaction chain B leading to 6-phosphoglucometasaccharinic acid.

The similarity between the alkaline and the metabolic degradation seems too close to be a coincidence. It suggests that the metabolic pathways are earlier in evolution than are the enzymes which catalyse these pathways. Astronomical evidence tells us that the atmosphere of primitive Earth must have been reducing, its main components having been hydrogen, ammonia, methane and water. Oceans and lakes in contact with such an atmosphere must have been alkaline. Such alkaline conditions are thus similar to those shown to cause the alkaline

degradation of glucose-6-phosphate.

We thank Drs D. Chipman and W. P. Jencks for stimulating discussions.

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BIOGENESIS

Chemical Evolution of Carbohydrate Metabolism

CONSIDERABLE efforts have been made to trace the evolutionary pathways of carbohydrate synthesis1. To understand more completely the origin of metabolizing systems on Earth, it is interesting also to examine the evolution of the breakdown metabolism of carbohydrates.

Recent investigations have shown that the alkaline degradation of glucose-6-phosphate (above pH 8.7) proceeds without enzyme action, by several parallel and consecutive reactions, as demonstrated in the following

 $scheme^{2}$

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OH $glucose \hbox{-} 6-phosphate \hbox{\rightleftarrows-} glucose \hbox{-} 6-phosphate \hbox{-} 1,2-enediolate$ 6-phosphoglucometasaccharinate fructose-6-phosphate glyceraldehyde-3-phosphate + dihydroxyacetone orthophosphate + methylglyoxal lactic acid

From glucose-6-phosphate-1,2-enediolate, alkaline degradation branches off in two ways, leading finally to lactic acid and orthophosphate (pathway A), and to 6-phosphoTopper, Y. J., Aldose-Ketose Transformations, in The Enzymes, second ed. (edit. by Boyer, P. D., Lardy, H., and Myrbäck, K.), 5, 429 (Academic Press, New York, 1961).

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BIOCHEMISTRY

Rapid Isolation of Active Mitochondria from Plant Tissue

Most of the methods which have been described for the isolation of mitochondria from a variety of tissues1-3 are essentially very similar. The cells are gently disrupted in a suitable medium, the homogenate is then filtered through a coarse filter and the supernatant obtained is fractionated into nuclear, mitochondrial, microsomal(+) soluble components by differential centrifugation. The mitochondrial fraction, which is obtained by centrifugation at 5,000-15,000g for 15-30 min, is then washed and resedimented. One of the drawbacks of this conventional technique is that it is both time and labour consuming, making it impractical to isolate mitochondria from several different sources (for example, tissue receiving different experimental treatments) on the same day, or to compare the effects of different buffers, in the isolation media, on the subsequent activity of the mitochondria.

The results presented here were obtained from a series of experiments which were carried out to investigate whether it might be possible, by changing the method of filtration and centrifugation, substantially to reduce the time necessary to isolate biochemically active mitochondria from Jerusalem artichoke tubers. In all the experiments which I have described, the metabolic activity of the mitochondria was assayed at 25° C in 2.0 ml. of a medium consisting of 0.25 molar sucrose, 0.05 molar tris (pH 7.2), 0.05 molar potassium hydrogen phosphate, 0.01 molar malate, 0.1 per cent bovine serum albumin and 2 mg of mitochondrial protein (measured using biuret reagent) using a vibrating platinum electrode polarograph (G.M.E. 'Oxygraph'). The ADP: O ratio and the value for the respiratory control were measured by adding aliquots of 5 µl. of a solution containing 0.5 µmoles of ADP to the reaction medium.

Experiments were then carried out with the aim of significantly accelerating the isolation procedure. method of isolating mitochondria similar to that described by Wedding and Black⁴ was adopted as a reliable basis for obtaining active mitochondria. This basic method of isolation takes 70 min to carry out. The results are shown in Table 1, column A. All the solutions and apparatus used in the isolation procedures were maintained at 1°-4° C. Using a stainless steel vegetable grater (holes 2 mm in diameter), 60 g of peeled Jerusalem artichoke tubers was grated in 50 ml. of a medium consisting of 0.5 molar sucrose and 0.05 molar tris (pH 7.8). homogenate was then stirred and filtered through four layers of muslin and the resulting solution was centrifuged at 3,000g for 10 min to remove cell debris and nuclei. The supernatant was then further centrifuged at 15,000g for 25 min and the pellet which formed was resuspended in 25 ml. of 0.4 molar sucrose and 0.05 molar tris (pH 7.2) using a loose fitting Teflon homogenizer. The mitochondria were then sedimented at 15,000g for 25 min, and finally resuspended in 1.0 ml. of 0.4 molar sucrose and 0.05 molar tris (pH 7.2) and their respiratory activity was measured. Columns B, C and D in Table I show the results which were obtained when the basic method of isolation was modified in various ways. In treatment B the tissue was homogenized as already described and the homogenate was then filtered through two layers of nylon fabric (mesh 50 strands/cm) (ref. 5). No precipitate was found after centrifugation at 3,000g for 10 min, so this step was omitted. Mitochondria were removed from the supernatant after they had been centrifuged at 15,000g for 15 min. The pellet was resuspended in 25 ml. of the washing medium and recentrifuged at 15,000g for a further 15 min. The mitochondria were then suspended in 1.0 ml. of medium and their respiratory activity was measured. Treatment C was similar to B except that the supernatant and the washed mitochondria were centri-

fuged at 40,000g for 5 min. In treatment D, the homogenate was filtered through the nylon fabric and the supernatant was centrifuged at 40,000g for 1.5 min. The mitochondrial pellet was not washed, but was suspended in 1.0 ml. of 0.4 molar sucrose and 0.05 molar tris (pH 7.2) and assayed for respiratory activity.

Table 1. RESPIRATORY ACTIVITY OF MITOCHONDRIA ISOLATED BY MEANS OF DIFFERENT PROCEDURES

		Isolation	procedures	
	A	\boldsymbol{B}	` <i>c</i>	D
Time elapsed during the isolation procedure	70 min	37 min	15 min	7·5 min
Total protein in the mitochondrial fraction	46 mg	32 mg	35 mg	31 mg
Rate of oxygen uptake "state 3" (µmoles oxygen/mg protein/min)	0.026	0.043	0.044	0.043
Rate of oxygen uptake "state 4" (µmoles oxygen/mg protein/min)	0.009	0.011	0.009	0.008
Respiratory control value ADP: O ratio	2·8 2·9	3·9 2·9	4·9 2·8	$5.3 \\ 2.9$

Isolation treatments were: A, filtration through muslin and sedimentation between 3,000–15,000g for 25 min, followed by washing and resedimentation at 15,000g for 25 min; B, filtration through nylon fabric and sedimentation between 0–15,000g for 15 min, followed by washing and resedimentation at 15,000g for 15 min; C, the procedure was the same as for B except that sedimentation in both cases was carried out at 40,000g for 5 min; D, filtration through nylon fabric and sedimentation at 40,000g for 1.5 min only.

The new method reduced the time taken for isolation of active mitochondria from 70 min to just over 7 min. This was achieved by more efficient filtration, by increasing the speed of centrifugation and by omitting the final washing procedure. The values for the respiratory control increased from 2.8 to 5.3 as the time taken to isolate the mitochondria was decreased. The increase in respiratory control was chiefly the result of a significant increase in the rate of oxygen uptake during "state 3" respiration, although a slight decrease was apparent in the rate of oxygen uptake during "state 4" respiration. The ADP: O ratio remained constant in all the preparations. The increase in the rate of oxygen uptake per unit of protein in the mitochondrial fraction could be the result of rapid removal of the mitochondria from the supernatant which may contain endogenous inhibitors like quinones and fatty acids. A further advantage of this rapid isolation technique is that when it is used in conjunction with a polarographic method of assay, it is possible for mito-chondria to be isolated and assayed for rate of oxygen uptake, ADP: O ratio and respiratory control can be obtained in less than 10 min, thus enabling many different samples to be compared in one day.

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Determination of Free Radicals in Gamma Irradiated Proteins

THE long-lived free radicals produced by gamma radiolysis of dry proteins at room temperature have been previously studied by electron spin resonance1 (ESR). The carbon radicals which can be observed by ESR have been interpreted² as being predominantly located on glycine. Recently, the use of tritiated hydrogen sulphide as interceptor of free radicals has been proposed as a new experimental technique for determining the distribution of free radicals located on earbon atoms. The method involves exposing the gamma irradiated lyophilized proteins to tritiated hydrogen sulphide in order to form carbontritium bonds in the protein. Tritium distribution among the amino-acids can be determined by subsequent hydrolysis, amino-acid analysis and tritium counting; this measures the "secondary" free radical distribution. The terms "primary" and "secondary" free radicals refer, respectively, to (a) those radicals formed on irradiation at 77° K, and (b) those which arise from them on subsequent warming or which can be observed after radiolysis at room temperature.

In the labelling reaction, SH radicals are generated as

shown in equation (1)

$$C + HSI \rightarrow C - I + HS. \tag{1}$$

Although the bond dissociation energy of HS-H (90 kcal)⁴ is considerably lower than that of most C-H bonds, the possibility cannot be ruled out a priori that SH radicals abstract hydrogen from carbon (reaction (2))

$$C-H + HS - - C + H_2S$$
 (2)

to produce a new class of carbon radicals which were not initially formed by gamma radiolysis. This new class of radicals would not be expected to be identical with the initial class and thus could lead to an erroneous tritium distribution. Furthermore, reaction (2) could lead to a chain reaction for tritium incorporation by way of reaction (1). In order to examine the possible contribution of reaction (2) to labelling with tritiated hydrogen sulphide, we used tritiated hydrogen iodide as the radical interceptor. For this reagent, the reaction analogous to (2) (abstraction of hydrogen from carbon by iodine atoms) cannot occur because of the low bond dissociation energy of the hydrogen iodide molecule [71 kcal⁴]. This has been demonstrated by experimental work in which iodine and hydrogen iodide have been used as radical scavengers⁵.

The experimental procedures with tritiated hydrogen iodide were similar to those previously reported³ for tritiated hydrogen sulphide, with the exception that tritiated hydrogen iodide was separated from the iodine formed by self-radiolysis by two distillations from traps at the temperature of dry ice. The reaction of free radicals with hydrogen iodide can be followed by observing the electron spin resonance spectrum as a function of time. Fig. 1 shows the result of a typical experiment in which ribonuclease, gamma irradiated with a dose of 4.2Mrads at room temperature, was subsequently exposed to hydrogen iodide at a pressure of 150 torr. After 12 min. a marked decrease of the doublet peak occurs as a result of carbon radicals. After 4 h, all of the carbon radicals have reacted, leaving only the sulphur signal. The latter is caused by sulphur radicals, formed during gamma radiolysis, which do not react with hydrogen iodide.

It is important to distinguish between tritium labelling induced by β -decay of the tritiated radical interceptor (" β -labelling") and that introduced as a consequence of radical formation by gamma radiolysis ("gamma label-ling"). The extent of "β-labelling" was determined in experiments without prior gamma radiolysis, in which 20 mg of ribonuclease or chymotrypsinogen was exposed to tritiated hydrogen sulphide or tritiated hydrogen iodide. obtained from New England Nuclear Corporation, Boston. Mass. [3.0 ml. of gas containing 30 me.], for 4 h. After removal of all exchangeable tritium and after acid hydrolysis, the average specific activities (µc./mg) for ribonuclease were 0.073 (tritiated hydrogen sulphide), 0.085 (tritiated hydrogen iodide) and for chymotrypsinogen 0.038 (tritiated hydrogen sulphide), 0.078 (tritiated hydrogen iodide). The combined "γ+β" labelling was measured by irradiating the proteins with 5 Mrads in a cobalt gamma source in the absence of air (10-3 mm of mercury), and then by exposing the proteins to tritiated hydrogen sulphide and tritiated hydrogen iodide for 4 h in the conditions already mentioned. The average specific activities for ribonuclease were 0.76 (tritiated hydrogen sulphide), 0.23 (tritiated hydrogen

iodide) and for chymotrypsinogen 0.76 (tritiated hydrogen sulphide), 0.32 (tritiated hydrogen iodide). The observation that the amount of incorporated tritium is of the same order of magnitude for both interceptors is consistent with the absence of a chain reaction due to SH radicals. Because "β-labelling" with tritiated hydrogen iodide introduced tritium predominantly into phenylalanine and tyrosine, the "β-labelled" distribution must be subtracted from the observed " $\gamma + \beta$ " tritium distribution to obtain the corrected "\gamma-labelled" distribution. Fig. 2 shows the gamma distribution of tritium among the amino-acids of ribonuclease and of chymotrypsinogen when tritiated hydrogen sulphide and tritiated hydrogen iodide are used as radical interceptors. The results for each protein represent the average of two to five separate labelling experiments. The bar lengths in Fig. 2 are proportional to the normalized specific activities (NSA) of individual residues. NSA is defined as

$$\frac{S_i \times 100}{\Sigma S_i}$$

where S_t is the specific activity of a given amine-acid in counts/min/ μ mole and ΣS_t is the sum of specific activities of all of the amine-acids of the protein under consideration. There are many similarities between the tritium distributions for tritiated hydrogen sulphide and tritiated hydrogen iodide. The product moment correlation coefficient for ribonuclease is 0.68 and for chymotrypsinogen is 0.92.

From these results it follows either that hydrogen abstraction by SH radicals is not a significant process in these experiments, or that SH radicals abstract from the carbon-hydrogen bonds of the various amino-acids at relative rates which accidentally parallel the "secondary" radical distribution produced by gamma radiolysis. The second hypothesis appears to be less likely. The results with tritiated hydrogen iodide support the three major con-

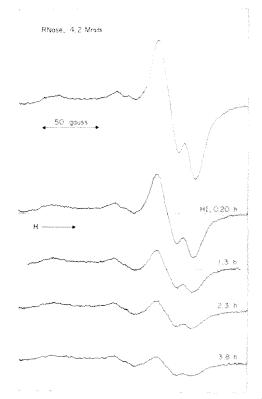


Fig. 1. Electron spin resonance spectra of irradiated ribonuclease before and after addition of hydrogen iodide. Ribonuclease was gamma irradiated (dose 4·2 Mrads) in vacuum, at room temperature (upper curve). Hydrogen iodide (150 torr) was subsequently added and spectra were recorded at different times. The curves represent the first derivatives of the absorption curves.

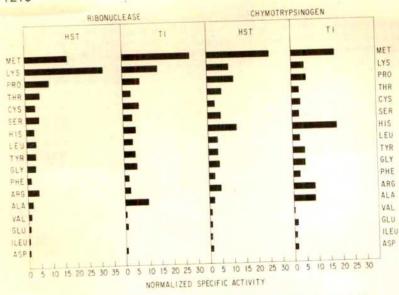


Fig. 2. Distribution of tritium among the amino-acids of ribonuclease and chymotrypsinogen using tritiated hydrogen sulphide and tritiated hydrogen iodide as radical interceptors. Bar lengths are proportional to the normalized specific activity (defined in text).

clusions previously obtained with tritiated hydrogen sulphide. First, it can be seen that tritium is widely distributed among amino-acid residues. Second, in a given protein, the specific activities vary from very high to very low. Third, the specific activity of glycine is very low, indicating that glycine does not play a special part as a free radical site in these proteins. The specific activities of certain amino-acids, however, are significantly different when the two reagents are compared. Thus the specific activity of lysine in ribonuclease labelled with tritiated hydrogen iodide is lower and that of alanine is higher than for labelling with tritiated hydrogen sulphide. It seems therefore that some distortion of distributions can occur when tritiated scavengers are employed.

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Multiple Forms of Cobra Venom Phospholipase A

MULTIPLE forms of enzymes catalysing identical reactions can exist even in one tissue1. Cobra venom is a rich source of phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4)2. This enzyme catalyses the conversion of lecithin and other phospholipids to lysophosphatides, by removal of a fatty acid residue in the second position of the phospholipid molecule³.

The haemolytic effect of phospholipase A on erythrocytes is caused by the action of lysophosphatides. A study of a cytotoxic factor from cobra venom4 which is selectively destructive to certain cells showed that at least seven cobra venom protein fractions brought about lysis of washed erythrocytes in the manner of phospholipase A.

In the present work a visual technique was developed to detect phospholipase A activity in the bands separating out after starch gel electrophoresis. The protein bands were adsorbed on paper strips which were then transferred to blood agar plates. At the end of an incubation period, clear and sharp haemolytic zones were observed, corresponding to the proteins with phospholipase A activity. Whatman 3 MM paper strips were soaked in a solution of isotonic saline containing 0.1 per cent calcium chloride because it is known that phospholipase A needs Ca++ for its activity5. strips were then dried and placed on the starch gel electrophoretic pattern for 15 min at 37° C. On removal they were again dried in air and replaced on blood agar

plates containing egg yolk emulsion as a source of external phospholipids. (The composition of blood agar plates was: 8 ml. of 6 per cent washed human erythrocytes + 0.5 ml. of 10 per cent egg yolk emulsion in 1.3 per cent agar in isotonic saline.) The plates were incubated for 15 min at 37° C. Then the paper strips were removed, but the plates were incubated for a further period and observed periodically for the appearance of clear haemolytic zones. In these conditions, the bands of haemolysis were discreet and sharp. If the strips were kept in contact with agar longer the bands became diffused and tended to coalesce. The rate of appearance of haemolytic zones may be taken as a rough quantitative measure of the amount of enzyme present.

Fig. 1a and b is a photograph and diagram of the electrophoretic pattern of cobra venom proteins obtained by the technique of Poulik⁶ (with 8 molar urea) and the corresponding haemolytic zones produced on blood agar plates. There are fourteen bands: of these, six protein fractions migrate to the cathode and eight towards the anode. Clear haemolytic zones are seen corresponding to the negatively charged protein bands. To distinguish between phospholipase A effect and the direct haemolytic factor, present in cobra venom known to cause lysis of erythrocytes without a phospholipid source, the electrophoretic patterns described in Fig. 1 were tested on blood

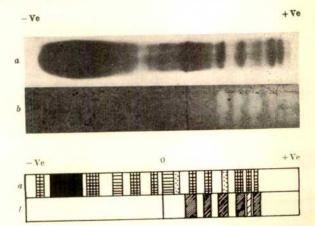


Fig. 1. Electrophoretic pattern of snake venom protein and haemolytic zones produced on blood agar plates (conditions: 200 V, 7 h, pH 8-6 and temperature 20° C). a, Cobra venom mixture (amido black); b, cobra venom mixture (haemolytic zones).

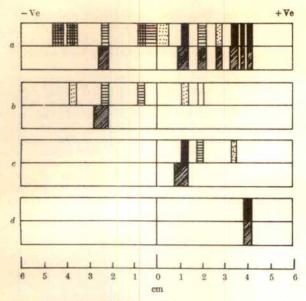


Fig. 2. Phospholipase 4 bands in purified cobra venom protein fractions (conditions as in Fig. 1). In each pair of diagrams the top half is the amido black pattern, and the lower half is the haemolytic zones, a, Perchloric acid precipitate; b, c and d, protein fractions obtained by purification of a.

agar plates prepared without egg yolk. In these conditions, no haemolysis was produced, so the involvement of the direct lytic factor is excluded. A similar study of four other varieties of snake venom indicated that cobra venom mixture had the most multiple forms of phospholipase A. There were two zones of phospholipase A type of activity in Mamushi venom, whereas Russell's viper, Bothrops alternata and Bothrops jararaca venom showed a single band. All forms of phospholipase A

detected migrated towards the anode.

The various forms of phospholipase A from cobra venom were purified by treatment with perchloric acid in controlled conditions followed by fractionation in CM cellulose and 'Sephadex'. Fig. 2a is the electrophoretic diagram for the perchloric acid precipitate fraction which shows the presence of seven haemolytic bands in the blood agar diagram (lower half of a). One of these was found to migrate towards the cathode. Haemolytic zones produced by three active preparations obtained on further purification of the perchloric acid precipitate are given in Fig. 2b, c and d. These have distinctly different mobilities. One of the fractions (Fig. 2d) is apparently homogeneous on starch gel electrophoresis for protein and haemolytic activity. Thin-layer chromatography of products formed on incubating the fractions with lecithin showed the presence of lysolecithin and a fatty acid, confirming that the lytic activity was the result of phospholipase A.

The separation of various protein bands with phospholipase A activity could not result from artefacts produced by urea which is known to cause splitting of the protein molecules, for similar patterns were obtained in its absence. Urea was included in the starch gel because it did not affect the enzyme activity and produced sharper separation of protein bands. One of the active zones migrating to the cathode appeared only after treatment of venom with perchloric acid. This could be caused by a concentration effect because treatment with perchloric acid resulted in complete precipitation of all haemolytic activity present in the starting material, but only 50 per cent of the total protein was precipitated.

Because cobra venom is a complex mixture of several proteins, differences in mobility could have arisen by interaction of an active protein with other proteins. This possibility seems unlikely because at least three active protein fractions showing different mobilities have been separated from the crude mixture after extensive purification using ion exchange column and 'Sephadex' filtration. The mobilities of two of these compare well with the original bands observed in the crude venom, suggesting that there are at least seven different molecular species of phospholipase A of varying electrophoretic mobility in cobra venom.

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Altered Duration of DNA Synthesis and Cell Cycle in Non-target Tissues of Mice treated with Oestrogen

THE effects of ovarian hormones on the cell cycle of target tissues have been extensively studied. It has been shown that high doses of oestrogens of exogenous origin can alter the duration of DNA synthesis (S phase) in target tissues when administered alone1.2 or in association with progesterone3.

The present work was undertaken to evaluate the degree of specificity of this action of oestrogen on the cell proliferation. For this purpose we studied the kinetics of the mitotic cycle of non-target tissues (namely, the intestinal crypt epithelium) in spayed mice, treated with doses of 17-3-oestradiol which, as previously shown4, reduces the duration of DNA synthesis and the length of mitotic cycle in uterine and vaginal epithelia.

Swiss albino mice, of average weight 25 g, were ovariectomized. The group of treated mice received sub-cutaneous injections of 17-β-oestradiol daily for 7 days, beginning 20 days after castration. The daily dose of hormone injected was 5 μg on days 1-3 and 7.5 μg for

the following 4 days.

On the seventh day the controls and the treated animals were subjected to the double labelling techniques to estimate the duration of DNA synthesis and the generation time of the intestinal crypt epithelium. We employed the procedure described by Maurer et al.5, using two separate injections of 3H-thymidine (1 µc./g and 10 μc./g, respectively). Autoradiographs of the labelled tissues showed two types of labelling: heavily labelled and weakly labelled nuclei. The latter corresponded to the cells which went out of the DNA synthesis phase during the interval between the two injections. number of weakly and heavily labelled nuclei was determined, and the duration of S phase was calculated from the relationship $H/W = S/t_t$ (ref. 5), where H represents heavily labelled nuclei, W weakly labelled cells, t_t the interval between the two injections (1 h in this case) and S the duration of DNA synthesis, or S phase. The labelling index (that is, the number of labelled cells/100 cells) was also measured. This made it possible to estimate the generation time (GT) by the relationship

$$\mathrm{GT} \,=\, \frac{S}{\mathrm{LI}} \,\times\, 100$$

where LI represents the labelling index.

Sections, 3–4 μ thick, were covered with Ilford K5 emulsion, by dipping the lides in the melted emulsion. The autoradiographs were exposed for 3 weeks at 4° C. After development, they were stained with haematoxylin-eosin and mounted in 'DPX' mounting medium (Michrome) for observation under oil-immersion.

As shown in Table 1, treatment with oestradiol produced a reduction in DNA synthesis time in the erypt epithelium of the duodenum, jejunum, ileum and colon.

Table 1. Effect of 17- β -oestradiol on the duration of the S phase and cell cycle in the crypt epithelium of the intestinal tract of spayed mice

()	S phase (h) Oestradiol		Generation time (h)* Oestradiol	
Organ	Control	treated	Control	treated
Duodenum Jejunum Ileum Colon	6·5 6·5 6·7 6·2	4·6 4·2 4·3 4·7	14·5 14·8 15·2 27·7	12-2 10-5 9-7 22-5

* Calculated on the basis of the labelling index.

The duration of the mitotic cycle, calculated with the labelling index, also seems to be shortened by treatment with oestrogen. This decrease in generation time is not covered by the reduction in the duration of the S phase, which indicates that at least one other phase of the cycle is also accelerated. Similar observations were made in target tissues (uterine and vaginal epithelium) of the same mice4. In that case, however, the acceleration of the mitotic cycle and the shortening of S phase were sharper than in the non-target tissues. This suggests that, as regards their action on the cell cycle, oestrogens act in the same way in both target and non-target tissues, the differences in the response being only a matter of degree.

A reduction of the mitotic cycle in a non-specific organ. the corneal epithelium, has been observed by Epifanova² in the mouse treated with oestrone. In this case, however. no alteration of the duration of the S phase is reported. If not linked to tissue characteristics, this disagreement with our results could be caused by the different concentrations of hormone used, Epifanova's being somewhat lower (2 \times 2.5 μg of oestrone). She assumed that oestrogens could act by accelerating the initiation of the DNA synthesis. Our findings suggest that higher doses of the hormone extend its effect to the S phase itself.

A similar situation seems to prevail in the case of the joint action of oestrone and progesterone. It has been observed that a higher dosage of exogenous ovarian hormones than that existing in adult virgin mice is necessary to affect the duration of DNA synthesis in the mammary gland^{3,6}. The level of endogenous hormones is, however, sufficient during pregnancy and lactation to induce such a reduction in the duration of the S phase?. During oestrus, the increase in the concentration of endogenous eestrogen parallels a reduction in the duration of the mitotic cycle in the vaginal basalis from 71 h to 25 h without alteration of the DNA synthesis time⁸. In our experiments with high doses of 17-β-oestradiol, a similar reduction of generation time (from 71 to 20 h) was observed in this tissue, but the duration of the S phase was also modified, being reduced from the 6.3 h in the controls to 2.3h in the treated mice4. This seems to be very similar to our observations in the non-target tissues.

High regional concentrations of oestrogen could be necessary to alter the duration of DNA synthesis. This may readily be attained in target tissues, because of their high affinity for the hormone. The same result can be reached in non-target organs if very high doses of oestrogen are injected, thus overcoming the low affinity of the cells for the hormone. Experiments are now in progress to test this hypothesis of a dose-response relationship in the degree of alteration of the S phase induced by oestrogen in both target and non-target

tissues. Physiological and experimentally induced variations in the oestrogen level are being studied. In such studies the double labelling technique has the advantage of making possible more instantaneous measurement of the DNA synthesis phase than the wave of labelled mitoses methods, usually used.

This work also shows that the duration of the S phase in duodenum, jejunum, ileum and colon can be reduced below the value of 6-7 h formerly considered to be nearly constant. Observations of higher durations 10,11 have already invalidated this suggestion of an approximately constant S phase duration. The present results, together with previously reported observations on a very short DNA synthesis phase 10,11, suggest that the former suggestion cannot be replaced by another a priori plausible suggestion¹⁻⁴, that the figure of 6-7 h represents an approach to the minimum time required for DNA doubling in mammalian cells.

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Bidirectional Transport of Radioactively Labelled Axoplasmic Components

It is well established that axoplasm moves somatofugally from the neurone cell body toward the axon terminals1. Somatofugal axoplasmic transport was first demonstrated by Weiss and Hiscoe2, who found that if a peripheral nerve was constricted the axons proximal to the site of constriction became engorged with axoplasm. Recent variations of these experiments have shown that certain axoplasmic components (acetylcholinesterase1, catecholamine storage granules3 and labelled phospholipids4) accumulate distal as well as proximal to the constriction. This tendency for axoplasmic components to accumulate on both sides of a lesion in a nerve has been interpreted as support for the idea that axoplasmic components move along the axons both from the cell body toward the axon terminals and back in the direction of the cell body.

I have studied the effect of transecting the sciatic nerve of a cat on the distribution in it of labelled axoplasmic proteins. If L-leucine-3H was injected into the vicinity of neurone cell bodies connected with axons in the sciatic nerve, the proteins within those axons were labelled, while the connective tissue surrounding the axons remained unlabelled^{5,6}. With this method the distribution of labelled axoplasmic proteins along the sciatic nerve can be analysed by measuring the amount of radioactivity present in segments of the nerve.

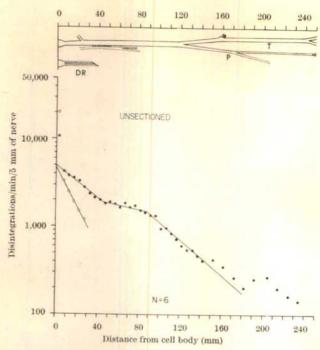


Fig. 1. The amount of radioactivity in each 5 mm segment of peripheral nerve (•) and dorsal root (○) in relation to distance from the injection site in the ganglion. The cats were killed 6 days after injection. A schematic representation of the sciatic nerve, its branches, the peroneal (P) and tibial (T) nerves, and the dorsal root (DR) illustrates the position from which the points below were obtained. N is equal to the number of segments used for each point on the curve.

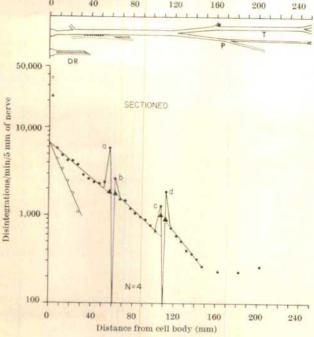


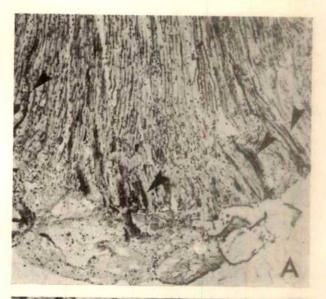
Fig. 2. Amount of radioactivity in each 5 mm segment of peripheral nerve (•) and dorsal root (○) in relation to distance from injection site in the ganglion. Sciatic nerves were sectioned bilaterally 24 h before the casts were killed 6 days after the injection of the label. The triangles represent the control value in the homologous segments of the unsectioned preparations taken from Fig. 1. The amount of radioactivity in the nerves was extrapolated to zero at the point of section.

The lumbar-7 dorsal root ganglia of five cats were bilaterally exposed by aseptic surgery and two injections of 1 μl. isotonic saline containing 5 μc. L-leucine-³H were made into each ganglion. In two cats, the sciatic nerve was severed bilaterally 60 and 110 mm from the ganglion, 24 h before they were killed. All the cats were killed 6 days after injection of the labelled compound. The

lumbar-7 ganglia and attached nerves were dissected out and cut into 5 mm segments which were then prepared either for liquid scintillation counting or for autoradiography⁵

In the unsectioned control preparations (Fig. 1), the incorporated L-leucine-"H present in the axons was distributed in the nerves along a decreasing gradient centrally and peripherally from the ganglion. In the experi-mental preparations in which the sciatic nerve was severed (Fig. 2), the decreasing gradient was interrupted by peaks of radioactivity both proximal and distal to the sites of transection. The curves from the individual sites of transection. preparations were identical to the mean curves in Figs. 1 and 2. Similar alterations in the distribution of radioactivity were also found if the sciatic nerve was severed 1 or 2 days after injection of L-leucine-3H and the animal killed 24 h later. Autoradiographs of the stumps in which increases of radioactivity occurred confirmed that the label was localized within the axons and that little or no label was present in the surrounding connective tissue (Fig. 3).

Axoplasmic proteins labelled with L-leucine-*H seem to be synthesized primarily in the neurone cell body*-7, and very little free leucine-*H is carried from the cell body into the axon*. This evidence suggests that the



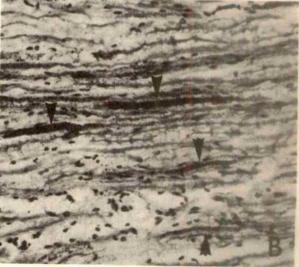


Fig. 3. Autoradiographs of the severed ends of labelled axons (arrows) in the proximal stump of the sciatic nerve, that is, equivalent to segment a in Fig. 2. A, \times 60; B, \times 200.

accumulation of label at the points of transection probably resulted from translocation of the labelled proteins which were present in the axons at the time of transection. Translocation must have occurred over a distance of several mm because the segments immediately adjacent to points a, b, c and d in Fig. 2 did not show a decrease in radioactivity which could account for the accumulation in segments a-d. Accumulation of radioactivity on both sides of the transection may indicate that some axoplasmic components containing protein are conveyed along the axon both towards and away from the cell body. It lends further support to the concept of bidirec-

tional axoplasmic transport1,3,9.

The amounts of radioactivity in the segments adjacent to the sites of transection were compared with the homologous control The mean percentage increase segments. of radioactivity in the transected segments over the control segments was: a = 211per cent; b=54 per cent; c=31 per cent; and d=117 per cent. These increases in the amount of radioactivity proximal and distal to the sites of transection are similar to those found by Miani⁴ for labelled phospholipids in the rabbit vagus nerve. Similar increases of proteins and phospholipids in the axons both proximally and distally to the lesion suggest that part of the label which accumulated in the axon stumps was bound to membranous organelles within the axoplasm. This possibility is sup-ported by the demonstration that in constricted nerves mitochondria accumulated in axons proximal to the site of constrictions, and that axoplasmic components with the dimensions of mitochondria move bidirectionally in the axons

of cultured chick spinal ganglion cells. The shape of the curves for distribution of label along the nerves in the sectioned and unsectioned preparations (Figs. 1 and 2) is similar except for the isolated segment of nerve between the points of transection. This may indicate that during the first 24 h after transection of the nerve the distribution of labelled components which are conveyed in the central or peripheral processes of the ganglion cells remains unaltered. Miani's results with labelled phospholipids support this possibility.

Bidirectional streaming in axons may be a mechanism for conveying biochemical information from the cell body into the axons and back into the cell body. Thus bidirectional axoplasmic movement could represent a feed-back loop through which the neurone cell body monitors the biochemical profile of its long cytoplasmic

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Size Relation between a Chromatographic Spot and its Autoradiogram

If a chromatogram has at the origin four spots, constituting a series of concentrations of a solute $(C_1, C_3,$ C_5 , C_7) with each member of the series containing an identical amount (C*) of radioisotopic solute (radiomer), colour development after chromatography gives the expected1 near-linear relation between the area of the spot and the logarithm of its concentration, but the corresponding autoradiogram shows spots of equal size (Fig. 1). This phenomenon, which seems to oppose what might be expected intuitively2,3, has a simple explanation.

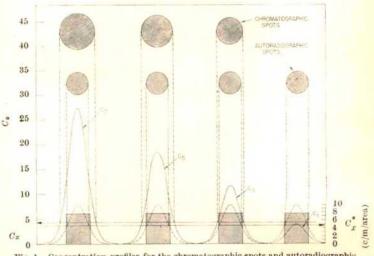


Fig. 1. Concentration profiles for the chromatographic spots and autoradiographic spots.

Consider each solute as having been spotted initially as a disk with uniform dispersion, all the disks (for each of the diverse concentrations) being of identical radius. After development for a time t, the concentration profile of solute along a diameter is given by the relation4,5

$$C(r,t) = C_0 r_0 \int_{u=0}^{\infty} J_1(r_0 u) J_0(r u) e^{-Dt u^2} du$$

where J_1 and J_0 are first and zero-order Bessel functions of the first kind, respectively, with argument u, and where ro is the initial radius of the disk, r is the radius of the spot at time t, and D is the diffusion coefficient of the solute. In our example, the corresponding profiles are shown in Fig. 1. For convenience, we consider the normal, that is, non-radioactive, solute (normer) and the radiomer as moving independently. We may then describe an independent profile for the radiomeric population, assuming its diffusion coefficient identical with that of the normeric.

We determine experimentally a concentration $[C_x]$ of the solute which provides the visual limit of detection following colour development, and similarly the concentration of radiomer $[C_x^*]$ necessary to produce visible film blackening within a time period t_x . Then $[C_x]$ and $[C_x^*]$ define the "edges" of the spot.

Because of the initial concentration differences in each C_1 , the radius (r_x) at which C_x is found after chromatography will usually be different for each spot, that is, $r_{c_1} \neq r_{c_2}$, etc. This merely states that the chromatographic areas of the different spots will be different. Because the radiomeric concentrations were identical, however, the corresponding r_c *s will be identical (Fig. 1).

Two corollaries follow. (1) Usually, C_x and C_x * will not be identical, that is, the sensitivities for recognition of colour in the development of a solute will not be the same as that for blackening of a film (the latter being a timedependent process). The autoradiogram of a radioactive solute therefore will not usually coincide in all details with its chromatogram. (2) The value of r_{cx}^* will vary as a function of time (for any particular film and condition of development). Consequently, r_c and r_c^* can be made to coincide.

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Nitrogen Compounds as Factors of Embryogenesis in vitro

In previous experiments1,2, either one of the two inorganic nitrogen compounds, potassium nitrate and ammonium nitrate, could cause the formation of embryos in tissue cultures from Daucus carota. This was most clearly demonstrated with a medium (Mw) containing White's nutrient3, 2 per cent sucrose and 5×10-8 g/ml. of 2,4dichlorophenoxyacetic acid (2,4-D). Mw could be converted from a "non-inductive" to an "inductive" form by the addition of either 4.16 g/l. of potassium nitrate or 1.65 g/l. of ammonium nitrate. The same effect, that is embryo formation, in the presence of a certain concentration of one of these compounds, was also obtained if a second medium (Ms) was used which differed from Mw only in the composition of the major and minor elements. Instead of White's salts and trace elements, MS contained those of the formula developed by Murashige and Skoog4. One of the problems left by these results is: why do the two different nitrogen compounds have a positive effect on embryogenesis in vitro?

Now and in previous experiments, freshly isolated tissues of the cambial area from two commercial varieties of carrot (cultivars: 'Rote Riesen' or 'Lobbericher Futterrüben') were used. They were always transferred after 4 weeks of culture and kept in the dark at a temperature of $28 \pm 1^{\circ}$ C. To estimate embryo formation the number of cultures producing embryos were expressed as percentage of the total number of cultures. Only tissue cultures forming mature embryos with cotyledons, that is, embryos beyond the heart stage, were recorded.

First, it was shown that the amount of nitrogen in the medium is important in the induction of embryogenesis. Although Mw contained 3.2 mmolar of nitrogen in the form of potassium nitrate and calcium nitrate, this was not sufficient. When, however, the nitrogen content of this medium was raised by addition of either larger amounts of potassium nitrate (13.8, 20.6 or 41.2 mmolar) or of ammonium nitrate (20.6 mmolar) embryos developed in approximately 50 per cent of the cultures receiving a high concentration of nitrogen (44.4 mmolar) and in 10 per cent of those receiving a low concentration of nitrogen (23.8, 17.0 mmolar, Table 1). The importance of the concentration of nitrogen in the nutrient could also be shown with Ms. In this case, a reduction from 60.0 mmoles/l. of nitrogen to 1.5 mmolar resulted in the loss of the capacity of the carrot cells to form embryos.

Table 1. DEPENDENCE OF EMBRYO FORMATION IN TISSUE CULTURES FROM Daucus carota ('ROTE RIESEN') ON THE NITROGEN CONTENT OF THE MEDIUM

Medium Nitrogen conc.	Mw	Mw+ NH ₄ NO ₃	Mw+KNO _s	Mw+KNO _a	Mw+KNO _x
(mmolar)	3.2	44-4 *	44-4	23.8	17-0
		Percentage o	f cultures pro	ducing embry	08
After 12 weeks After 16	0	28	13	3	0
weeks	0	49	40	10	10

Each value represents the result of at least two sets of experiments with sixteen to forty-two cultures. The control was White's medium (M=) with no embryo production.

Embryo formation also occurred if 41.2 mmolar glutamic acid (Fig. 1) or a mixture of amino-acids (Edamin, 60.0 mmolar total concentration of nitrogen) was added to Mw instead of potassium nitrate or ammonium nitrate. This confirms earlier reports5,6. The fact that there was no qualitative difference between the capacity of oxidized and reduced nitrogen to induce embryos (Fig. 1) demonstrates clearly that it is the concentration and not the form of the nitrogen in the medium which is important. These results are contradictory to the statement of Halperin and Wetherell⁷ that for embryogenesis in vitro ammonium is a requirement which cannot be replaced by potassium nitrate or by amino-acids.

Finally, some of our results showed that as well as the amount of nitrogen the potassium and ammonium ions might be partially responsible for the effects of potassium and ammonium nitrates. Carrot tissues grown, for example, on Ms with a low nitrogen content (6.0 mmolar) and potassium (3.1 mmolar) hardly ever formed embryos. These appeared, however, in 50 per cent of the cultures receiving the same low nitrogen level of 6.0 mmolar,

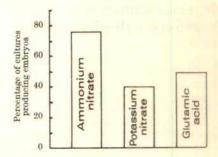


Fig. 1. Embryo formation in carrot cultures (cultivar 'Rote Riesen') on Mw (control 3-2 mmolar nitrogen) 16 weeks after addition of equivalent amounts of nitrogen (41-2 mmolar) in the form of ammonium nitrate, potassium nitrate or glutamic acid. Each column represents the average value of two sets of experiments with twenty cultures.

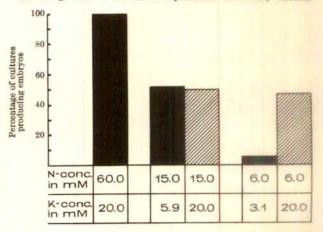


Fig. 2. Effects of different concentrations of nitrogen and potassium in Ms on embryo formation in carrot cultures (cultivar 'Rote Riesen') after 16 weeks. Each column represents the average of two sets of experiments with forty cultures. Concentrations of nitrogen and potassium were decreased by reducing both the potassium nitrate and the ammonium nitrate originally present in Ms. The concentrations of potassium were increased to the original level by adding potassium phosphate.

provided that the original concentration of potassium (19.9 mmolar) in Ms was restored by adding potassium phosphate (Fig. 2). This effect of the KH2PO4 was only evident with a low concentration of nitrogen (6.0 mmolar); it did not appear with higher concentrations (15.0 mmolar; Fig. 2). A further indication that cations can be important came from other experiments employing ammonium ions. Tissues growing on Ms without ammonium nitrate produced few or no embryos, but these were formed readily after the addition of a small amount of ammonium chloride (2.1 mmolar). This stimulatory effect of cations on embryogenesis can be causally related either to the permeability of the carrot cells to the two cations as well as to the nitrate anion, or to the activities of enzymes involved in the conversion of nitrogen in vivo, or to both of these.

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Abscission induced by Betahydroxyethylhydrazine: Conversion of Betahydroxyethylhydrazine to Ethylene

BETAHYDROXYETHYLHYDRAZINE (BOH) accelerated the abscission of detached fruits and leaves in closed containers. Evidence is presented which indicates that ethylene, formed from BOH, was the abscissing agent. Ethylene¹, BOH² and auxins³ are all active in the induction of flowering in pineapple. Burg and Burg4 have proposed that ethylene, resulting from the NAA treatment, was active in causing flowering. Our data suggest the activity of BOH and other related hydrazines is by means of ethylene.

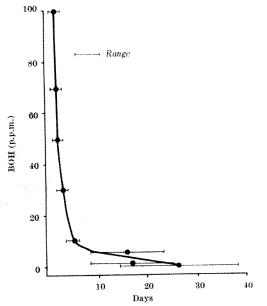


Fig. 1. Summary of abscission responses of 'Navel', 'Valencia', 'Hamlin', and 'Sweet' oranges, grapefruit and lemon fruit.

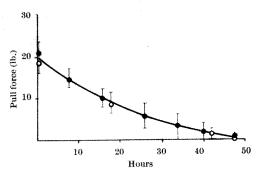


Fig. 2. Force to separate fruit from the stem, 'Navel' orange 100 p.p.m. BOH in closed chamber. Population distribution 95 per cent interval.

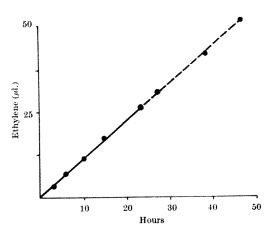


Fig. 3. Ethylene from BOH liquid (——) and 50 per cent aqueous solution (----) under reduced pressure.

For the abscission trials, eitrus fruits with about 2 in. of stem were removed from the tree, immediately brought to the laboratory and either placed in a desiccator with the stems immersed in water or set up individually in test tubes and enclosed in a polyethylene bag.

Ethylene analyses were done on a Wilkins model HY FI' gas chromatograph equipped with a hydrogen flame detector and a 1/8 in. by 2 ft. activated alumina column at ambient temperature. The sensitivity of the gas chromatograph was 1.9×10^{10} mV/mole of ethylene. The BOH samples tested were a 70 per cent field formulation solution (Olin Matheson Chemical Co.) and a research grade sample (Aldrich Chemical Co.).

Concentrations of 50-1,000 p.p.m. BOH in water induced abscission of oranges, grapefruit and lemons in less than 3 days (Fig. 1). Concentrations of 10 and 25 p.p.m. caused intermediate rates of abscission, while 5 p.p.m. or less did not accelerate abscission over the controls. The lowest concentrations showed the normal variability in abscission response which was typical of the control tests. The speed of the abscission response is shown by the rapid reduction in pull force (Fig. 2) after treatment with BOH. Within 15 h of treatment, the pull force was reduced by about 50 per cent. The force necessary to separate the stem from the fruit was measured with a constant-rate, gear driven, pull device with a meter.

When BOH was sprayed on fruits still attached to the tree and open to air circulation, there was no acceleration of abscission. This factor, plus the speed of the reaction in a closed container, made us suspect a gas effect. To check this possibility, detached fruit were placed in a desiccator with their stems in water and adjacent to an open beaker containing 1,000 p.p.m. aqueous BOH. Thus the only contact between the fruit and the BOH was the atmosphere. These fruits also abscissed in less than 60 h, and analyses of the gaseous phase confirmed the presence of ethylene. Sampling the atmosphere over a BOH

solution (from both chemical companies), contained in a test tube sealed with a serum cap, showed the presence of ethane, methane and ethylene. The identity of the ethylene was confirmed by the presence of its uniquely shaped intense absorption band at 949 cm⁻¹ in the infrared spectrum of this gas. Neither of the alkanes is physiclogically active in abscission, but ethylene is probably more active than any chemical known. It seemed logical to conclude that the ethylene responsible for abscission must have come from the aqueous BOH solution.

To confirm that ethylene was not just a soluble contaminant but was actually produced by BOH, we studied the amounts and rates of ethylene produced. The concentration of BOH in the solution did not seem to affect the fractional amount of ethylene produced in closed vessels and the reaction stopped when about 1.4 per cent of the BOH had been converted to ethylene. When the ethylene was removed by attaching gas sampling tubes under reduced pressure, ethylene production continued at the rate of 1.08 μ l./h (Fig. 3). The fact that the rate of ethylene production was constant shows that the ethylene was not a contaminant.

Table 1. EVOLUTION OF ETHYLENE FROM BOH SOLUTIONS (SEE TEXT FOR

Test DETAILS)	Ethylene evolved/24 h (moles \times 10 ⁻⁶)	Per cent conversion of BOH to ethylene
I, 50 mg/l. BOH solution I, 100 mg/l. BOH solution (pH 8·5) II, 100 mg/l. hydrochloric acid salt BOH	2-25 4-15 0-36	1·4 1·3 0·1
(pH 4-3) II, 100 mg/l.	4.61	1.4
I. Duplicate samples. II, Quadrupli	cate samples.	

An interesting biological implication of the conversion of BOH to ethylene is its pH dependence (Table 1). At pH 4.3, which would be typical of citrus fruit, the rate of conversion is only 10 per cent of the rate at pH 8.5. This would sharply reduce the ethylene production from BOH which has been absorbed by the fruit, and may partially explain the complete lack of activity of BOH

when sprayed on trees in the field.

From a physiological standpoint it seems clear that the action of BOH in citrus fruit abscission was dependent on its conversion to ethylene. N-amino morpholine, another hydrazine active in forcing the flowering of pineapple, also evolves ethylene. Because the only hydrazines which are active in inducing flowerings are those with two carbon units like BOH, it seems probable that their action is also by means of conversion to ethylene. In fact, any effect of an aqueous BOH solution becomes a potential ethylene effect. For example, Reeds clearly demonstrated that tryptamine oxidation is inhibited by BOH. Although

Fig. 4. Proposed mechanism of ethylene formation from BOH.

his data and subsequent studies7 on tryptamine oxidation inhibition by B-995 (dimethyl succinamic acid) strongly support direct action of the hydrazines, our data pose the question of the effect of ethylene on the system.

The nitrogenous products from the thermal decomposition of BOH have been identified by Howard* as methyl hydrazine, ammonia and hydrazine. (Olefinic hydrocar-

bons were not investigated.)

A reasonable mechanism for the molecular rearrangement of the BOH molecule to yield ethylene can be proposed which uses an additional carbonyl reagent such as an aldehyde or ketone as a catalyst (Fig. 4). We have tentatively identified formaldehyde as being present in these solutions by gas chromatography.

In summary, BOH has been found to induce abscission of citrus fruit explants, and this abscission has been correlated with ethylene formed from the decomposition of the BOH. A mechanism for the conversion of BOH is

proposed.

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PHYSIOLOGY

Effect of Hydrocortisone on the Closure of Palatal Shelves in vivo and in vitro

SINCE the first experiments of Fraser and Fainstati in 1951, it has become clear that cortisone induces cleft palate in mice when administered before the closure of the palatal shelves. Certain direct and indirect effects on the palatal tissue have been suggested, including the effect of cortisone on the turnover of the

amniotic fluid2-4. Harris4 postulated that oligohydramnion, resulting from maternal cortisone treatment, affects the normal development of the palatal region by constricting the embryo. Recently, however, Fraser et al. have compared the amount of amnietic fluid in embryos treated with cortisone with and without cleft palate. No differences were detected and the results seem to rule out the possibility that oligohydramnion plays a part in the genesis of cleft palate.

Fraser^{6,7} has put forward another hypothesis for the mechanism of the genesis of cleft palate and the interplay of genetic and exogenous factors in this process. The hypothesis is based on findings which show that closure of the shelves takes place later in the course of development in strains susceptible to exogenous teratogens than in those which are comparatively resistant to such factors*.

Accordingly, there might be a certain critical time (threshold), determined by the general development of the embryo, beyond which the shelves are no longer able to fuse (perhaps because of the advanced stage of development of the head and the palatal defect). An exogenous teratogen may delay the development of the palatal processes and bring it beyond this threshold. Because of the biological variation of the timing of this development within each strain, the delay of the process may bring either some or all of the embryos beyond the threshold. In susceptible strains where the normal closure takes place relatively late as compared with the general stage of development, the threshold is closer and the shift needed for demonstrable effect consequently smaller, whereas in a resistant strain the closure is usually completed well before the threshold. The hypothesis thus suggests that palatal tissues of all embryos would respond similarly to the teratogen, but as a result of genetic differences in the timing of developmental events the manifestation per cent of consequent malformations varies.

We felt that this hypothesis could be tested experimentally using organotypic tissue culture methods9-13. Accordingly, in a certain population with an in vivo manifestation below 100 per cent, an invariable effect would still be expected on all palate shelves in vitro. Instead of using an inbred strain of mice, a random-bred strain was chosen because greater variations of the indidivual responses would be expected in vivo but not in vitro. Random-bred Swiss albino mice were used. These were fed freely before and during the experiment. The in vivo experiments were performed according to the scheme of Pinsky and Digeorge¹⁴. Four milligrams of hydrocortisone ('Solo-Cortef', Upjohn) was injected intraperitoneally on days 11–14. The mothers were killed on day 19, and the embryos were analysed under a dissecting microscope. For in vitro studies, the palatal regions of 13-14 day embryos were dissected in one block, and cultivated in a modified Trowell type culture15. The culture medium was essentially a chemically defined one designed by Biggers et al.16, but earlier experience with bone culture had suggested the importance of adding ascorbic acid in a concentration of 5 µg/ml. (refs. 17 and 18). In vitro development of the secondary palate was followed under a dissection microscope by daily camera lucida drawings and through subsequent histological examinations of the explants. The quantitative calculations given in the results are based on a planimetric determination of the cleft from the drawings.

Following the experimental design, a pilot study revealed an incidence of 27 per cent of cleft palate malformation in our mice in vivo. The result of a consequent experiment is given in Table 1. Preliminary in vitro studies on palatal shelves from untreated embryos indicated that their viability was good in the culture conditions and that a complete fusion of the shelves took place in 3-4 days, depending on the age of the donor embryos. Subsequently, hydrocortisone was added to such cultures in concentrations ranging from 0.001 to 20.0 $\mu g/ml$. The hormone was added at the onset of the experiments and the same culture medium was used throughout the 3-12 day experiments. The volume ratio tissue/medium was approximately 1:50. Neither the lowest nor the

Table 1. In vivo effect of hydrocortisone on the palatal development

OF 5	RANDOM-BRED	SAISS SIET	IN OF MICE	
	No. of mothers	No. of embryos	Embryos with cleft palate	Percentage defects
Control	10	90	0	0
Cortigone-treated	10	96	34	35

Table 2. NUMBER OF COMPLETELY FUSED PALATAL SHELVES DURING 3 DAYS CULTIVATION in vitro IN THE PRESENCE OF HYDROCORTISONE IN DIFFERENT

	NCENTRATIONS	84/88
Control Hydrocortisone	0.01 µg/ml.	0/25
Hydrocortisone	0-10 µg/ml.	0/22
Hydrocortisone	1.00 µg/ml.	0/5
Hydrocortisone	$5.00 \mu \mathrm{g/ml}$.	1/20
Hydrocortisone	$10-00 \ \mu g/ml$.	2/27

Thirteen day old embryos of random-bred mice were used as donors.

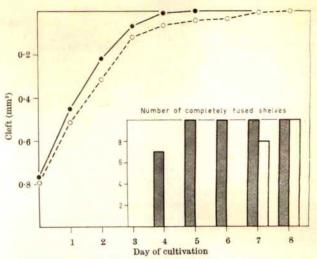


Fig. 1. The effect of hydrocortisone in a concentration of 0-01 μg/ml. on the *in vitro* development of the secondary palate of random-bred mouse embryos; • • and stippled columns, control; • - - - o and white columns, hydrocortisone.

highest concentrations of cortisone showed a uniform effect on the growth and fusion of the palates and were omitted in the final series. Concentrations between 0.1 and 0.01 µg/ml. significantly retarded the fusion of the palatal shelves as seen in Table 2 and Fig. 1. The completion of the fusion was usually postponed by 1-3 days, but it should be stressed that a complete fusion always occurred as judged from microscopic sections. So far, sixty palatal explants treated with cortisone have been followed in prolonged cultures without a single case of persistent cleft between the shelves.

The percentage of palates in which clefts were induced in vivo was approximately 35 per cent in our Yet all shelves cultivated in random population. vitro showed a tissue response to cortisone detectable as retardation of growth and fusion of the palatal processes. Despite this invariable response, none of the shelves failed to fuse in prolonged cultures. These results suggest that the end result of cortisone treatment in vivo, the palatal defect, is a summation of two factors: a direct effect of the hormone on the palatal tissue and the genetically determined developmental events of the head region. Thus the results obtained in this study can be well fitted into the hypothesis of Fraser.

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Protein Synthesis in Tonic and Phasic Skeletal Muscles

LITTLE is known of the way in which physiological function influences protein metabolism in muscle. Knowledge in this area is essential if we are to understand the biochemical mechanisms through which use and disuse alter muscle size and functional capacity. The present studies were undertaken to see if the level of protein synthesis in different skeletal muscles varies systematically with

different types of physiological activity.

It has long been known1,2 that slowly contracting skeletal muscles are darker in colour than the phasic ones. The former perform continuous, tonic work and depend primarily on oxidative metabolism3 while the faster, pale muscles fatigue more easily, are less frequently active and rely primarily on glycolytic metabolism3. muscles are of an intermediate character, being composed of both dark and light fibres in varying proportions. Henneman et al.4 demonstrated that the dark fibres are innervated by neurones with thresholds lower than those innervating the pale fibres. As a result, these dark fibres are usually more active than the larger, pale ones, which are recruited only during greater muscular efforts. average amount of electrical activity in a muscle is thus related to the proportion of dark fibres. The present experiments demonstrate that the amount of protein synthesis in a muscle is determined by the proportion of dark fibres. We have compared the ability to concentrate a non-utilized amino-acid analogue, the amount of incorporation of ¹⁴C-leucine into sarcoplasmic and myofibrillar proteins, as well as the RNA contents of red and white

In order to study protein synthesis in muscles in the absence of growth, we used hypophysectomized rats (100-120 g, Charles River strain), 4 weeks after removal of the pituitary^{5,7}. Results similar to those presented here have also been obtained with muscles from normally

growing rats.

Christensen et al. showed that the amino-acid analogue, α-aminoisobutyric acid (AIB), although not metabolized, is transported into cells by the same membrane systems which transport natural amino-acids. The relative ability of cells to take up this analogue presumably reflects differences in the rates at which they utilize amino-acids. Table 1 shows that red muscles concentrate ¹⁴C-AIB to a greater extent than the pale ones. 3 μc. of ¹⁴C-AIB-1 (2·76 mc./mg) dissolved in 0·3 ml. of physiological saline was injected intravenously into unanaesthetized rats. The animals were killed 4 h later and the muscle was excised. Tissue water was estimated as the difference between the wet and dry weights of the muscles. The dried muscles were then dissolved and their ¹⁴C-AIB content was measured by liquid scintillation.

The values presented in Table 1 are the ratios of the concentration of AIB in various muscles to that in the plasma. No attempt was made to correct these values for differences in the extracellular space in the different muscles. The extracellular component is greater in dark muscles than in pale ones, and so the data in Table 1 are probably an underestimate of the actual differences in the

Table 1. UPTAKE OF 14C-AIB BY RED AND WHITE SKELETAL MUSCLES

	Distribution ratio	Proportion of dark fibres
Soleus	$3 \cdot 4 \pm 0 \cdot 24$	Predominantly dark
Diaphragm	2.4 ± 0.19	33 33
Medial gastrocnemius	2.1 ± 0.13	Turk
Flexor digitorum longus Plantaris	1·9 ± 0·10 1·7 ± 0·09	Intermediate
Tibialis anterior	1.5 ± 0.18	**
Peroneus longus	1.4 ± 0.08	Predominantly white
Extensor digitorum longus	1.4 ± 0.07	**
Lateral gastrocnemius Semitendinosus	1·4 ± 0·06 1·2 ± 0·09	**
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Muscles are arranged according to average content of dark fibres, as measured by Streter and Woo?. Values given are the ratios of concentration of ¹⁴C-AIB in total muscle water to that in plasma 4 h after intravenous injection. Each is the mean ± S.E. of seven muscles.

ability of red and white muscles to concentrate AIB from the extracellular fluid.

Because amino-acids are not appreciably metabolized by muscles, the differences shown in Table 1 presumably reflect different rates of incorporation of amino-acids into Evidence in support of this conclusion is proteins. presented in Table 2, which compares the amount of ¹⁴C-leucine incorporated into sareoplasmic and myofibrillar proteins in various skeletal muscles. 3 μe. of ¹⁴C-leucine (1.89 mc./mg) was injected subcutaneously 24 h before the animals were killed. Samples (50 mg) of each muscle were then taken for analysis. The proteins in each fraction were isolated and their radioactivities were assayed as described previously7. The dark muscles incorporated more 14C into both soluble and fibrillar proteins (Table 2) than did the pale ones, and thus the 14C incorporation was greater in those muscles showing greater AIB uptake.

Table 2. Incorporation of Leucine-u-14c into proteins by Med and white skeletal muscles

Salubla fraction Muchbriller fraction

	Counts/min/mg	of fresh muscle
Diaphragm	4.4 ± 0.3	6-3±0-3
Soleus	3.7 ± 0.2	5-6 ± 0-2
Medial gastrocnemius	3.3 ± 0.2	5-3 ± 0-4
Plantaris	3.0 ± 0.1	4·8 ± 0·2
Extensor digitorium longus	3-0 ± 0-1	4-5±0-2
Lateral gastrocnemius	2.3 ± 0.2	3-7 ± 0-2

Values are the mean $\pm S.E.$ of at least seven observations. The involutional fraction includes some nuclear and stromal material, although at least 90 per cent of the radioactivity can be extracted as myofibrillar proteins.

To obtain further evidence for differences in protein synthetic activity, the RNA content of various muscles was also determined. Spectrophotometric analysis following reaction with orcinol was performed as originally described by Schmidt and Tannhauser¹⁰ but various suggestions of Monro and Fleck¹⁰ were incorporated. Soluble RNA prepared from yeast (Sigma Chemical Co., Type XI) was used as a standard. Red muscles have a greater content of RNA/mg than the white ones (Table 3). A similar observation was made by Margreth and Novello¹² although these workers compared muscles from different species which were growing at different rates.

Table 3. RNA CONTENT OF RED AND WHITE SKELETAL MUSCLESS

	μg KNA/mg muscle v
Soleus	7·1 ± 0·26
Medial gastrocnemius	5·8 ± 0·20
Diaphragm	5·6 ± 0·18
Plantaris	4.6 ± 0.14
Extensor digitorium longus	4.0 ± 0.18
Lateral gastrocnemius	90-0+8-8

Values are the mean $\pm S.E.$ of ten observations.

Qualitatively, the muscles with the highest RNA content are also those most active in AIB transport and aminoacid incorporation, although the exact order of the muscles in Tables 1, 2 and 3 is not identical. More than a qualitative agreement between these different approaches is not surprising, because each measures a different aspect of protein synthetic activity. Table 3 (KNA content) is, presumably, a reflexion of the amount of machinery for protein synthesis usually present in the muscles; Table 2 (14C-leucine incorporation) is, presumably, a measure of the amount of protein made in the various muscles over a period of 24 h; while Table 1 (14C-AIB) is an estimate of the extent of amino-acid uptake during a 4 h period when the animals are awake. Because the muscles of hypophysectomized animals remain constant in size, protein synthesis and protein degradation must be equally balanced. The present findings therefore also demonstrate that the amount of protein usually catabolized for every mg of muscle is greater in red muscles than in white ones. In other words, the average half lives of skeletal muscle proteins, both soluble and myofibrillar, vary in different muscles, and are smaller in the tonic muscles than in the phasic ones.

Greater protein catabolism may be a fundamental characteristic of the tonic muscles, such that greater protein synthesis is secondarily necessary for the cell to

Theoretically, the different maintain constant size. average rates of protein degradation could arise in two ways. On the one hand, certain proteins found only in the red muscles could be very short lived and therefore the average amount of protein catabolized at any time would appear greater in these muscles. On the other hand, the same proteins may turn over at different rates in the different muscles, being more labile when the muscles are used tonically than when used phasically. Measurement of turnover rates of specific proteins in different skeletal muscles would help one to decide between these alternatives.

Recently, Peterson¹² found by autoradiography that small motor neurones incorporate more amino-acids into proteins per unit volume than large ones do. This finding on spinal neurones is very similar to the results reported here. The small motor neurones and the tonic muscle fibres which they innervate are both used more frequently and seem more active in protein synthesis than the large nerve and muscle fibres4. Thus an association between physiological activity and amount of protein metabolism may be quite general. Additional studies (ref. 13 and my unpublished work) further demonstrate that the parameters of protein synthesis measured here can change rapidly in response to changes in functional demand.

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Inhibition of Egg Implantation and Induction of Abortion in Mice by Heterologous Immune Serum

IMMUNOLOGICAL reactions between mother and conceptus play an important part during pregnancy. It has, for example, been shown that placental size¹⁻³, foetal size⁴ and length of gestation4 can be influenced by the immunological relationship between mother and foetus. As early as implantation the mouse embryo is capable of expressing transplantation antigens and the mother can react to them^{5,*}. Mouse eggs which are genetically most dissimilar from the mother are more readily implanted. This is also true of the rat (personal communication from W. K. Silvers and D. Wilson). It has been suggested that immunological selection of eggs at implantation might be a contributory determinant of sex ratio8.

In view of the antigenicity of the embryo and the immunological competence of the mother it is not surprising that there have been many attempts to prevent

or to terminate pregnancy by specifically immunizing the mother against paternal transplantation antigens present in the foetus. Without exception all these measures have proved ineffective. Indeed, there is evidence which may be interpreted as suggesting that immunizing procedures of this sort increase fecundity rather than the converse.

The experiments described here were performed to investigate the effect on pregnancy of an immune serum raised in rabbits by intravenous injection of living mouse thymocytes by the method described by Levey and Medawar¹⁰. The serum was not absorbed and in addition to its antithymocyte immuno-suppressive activity it would contain a range of antibodies including haemagglutinins. Control serum was obtained from rabbits not previously injected with thymocytes. The serum was injected subcutaneously, 0.5 ml. in each injection.

The mice were from a heterogeneous colony. They were sexually mature and weighed 25-30 g. The appearance of the copulation plug was considered as day 0 of pregnancy. Egg implantation occurs on the fourth day.

In all the experiments equal numbers of mice were injected with control serum. Approximately 90 per cent of these mice delivered a normal litter 18 days after mating. This does not differ from the breeding performance of untreated stock mice, and so it was assumed that control serum in no way affected the normal course of pregnancy and the individual results will not be included.

The results (Table 1) show that the serum causes involution of the corpora lutea and the immediate termination of pregnancy. The action can be reversed by the simultaneous administration of progesterone (experiments 4a, 4b and 5). The mechanism of action of the serum in bringing about these changes in the corpora lutea cannot yet be explained. It is a short lived effect; mice will remate shortly after abortion induced by serum and carry the litter to term (experiment 4c).

Serum given soon after mating prevents egg implantation, even when exogenous progesterone has restored the sensitivity of the uterus to corn oil, an artificial deciduogenic stimulus (experiment 2). Finn¹³ considers that this stimulus can produce decidual reaction only when the hormonal status of the uterus will allow an egg to implant. Blastocysts taken from mice treated with serum fail to

Table 1. EFFECT OF CRUDE RABBIT ANTI-MOUSE THYMOCYTE SERUM ON PRE- AND POST-IMPLANTATION STAGES OF PREGNANCY IN THE MOUSE

experi-

Treatment

Time

Result

	filminic iii	ments	autopsy	
1 <i>a</i>	Serum injected on day 0 p.c. and day 3 p.c.	24	Day 7 p.e.	Uterus atrophic, corpora lutea regressed. No ovo- implantation sites. Free floating blastocysts in 20/24 mice
1 <i>b</i>	Transfer of unimplanted blastocysts from serum treated mice (1a) to the uteri of untreated pseudopregnant hosts		Day 7 p.c.	No ovo-implantation sites
1 <i>e</i>	Transfer of unimplanted blastocysts from serum treated mice (1a) to the testis of untreated host	! !	Day 8 after transfer	Six blastocysts developed normally ¹¹
3	Serum injected on day 0 p.c. and day 3 p.c. Pro- gesterone (2 mg/day) in- jected daily from day 0 p.c. Corn oil (0-01 ml. injected into left uterine) 10 - -)	Day 7 p.c.	Decidual swellings in left uterine horn of seven mice. Free floating blasto- cysts in right uterine horn of each of these seven mice
3	horn on day 4 p.c. Serum injected on day 6 and day 3, blastocysts transferred under kid ney capsule on day 3	3	Day 7 after transfer	Six blastocysts developed normally ¹²
4a	Serum injected on day	3 4	Day 9-12 p.c.	All mice aborted or resorbed conceptuses
4b	Serum injected on day 1: p.c.	2 12	Day 9-12 p.c.	All mice aborted or resorbed conceptuses
4 <i>c</i>	Mice from 4b recaged with males immediately after abortion	n 5 r	Inspected at term	Four mice remated within 9 days of aborting and carried litters to term
5	Serum injected on day p.c. Progesterone (mg/day) injected on day 8 p.c. and daily for days	2 V 7	Inspected at term	No mouse aborted

p.c., post coitus.

implant when transplanted to the uterus of an untreated pseudopregnant foster mother (experiment 1b).

Although these results suggest that the serum impaired the viability of the eggs, further experiments showed that this was not so. Blastocysts from serum-treated mice will develop satisfactorily when transplanted to the testes of untreated hosts (experiment 1c). Furthermore, blastocysts taken from untreated donors will develop beneath the kidney capsule of mice treated with serum (experiment 3).

It seems then that neither the sensitivity of the uterus nor the viability of the egg is adversely affected by the treatment. Presumably the serum has interfered with the mechanism whereby the blastocyst initiates the decidual response, a sine qua non of implantation in the uterus but not in extra-uterine sites. Eggs transplanted from treated donors to the pseudopregnant uterus of nontreated hosts (experiment 1c) must be explained by a local action of the serum carried across on the surface of the egg. A similar argument has been used by Guttmann et al.14 to account for the prolonged survival of renal allografts in rats when only the donor had been treated with anti-lymphocytic serum.

The way in which the serum inhibits the induction of a decidual reaction by the egg is unknown. Its action may be non-specific; it may damage the uterine tissues or interfere with the hormones required for implantation. The findings are also consistent with the view that the serum has inhibited an immunological interaction between

blastocyst and uterus.

Tyler¹⁵ first postulated that "implantation itself may involve interactions that are of antigen-antibody type" The following recent findings may be interpreted as lending support for this idea. (a) The mouse egg expresses transplantation antigens. (b) There is selection of blastocysts of particular genotype at implantation in the mouse? (c) Antigen contained in the blastocoelic fluid of the rabbit blastocyst is also found in the uterine lumen and uterine stromal cells just before implantation16. (d) At implantation the antimesometrial uterine epithelium cells in the rat show a marked ability to transfer substances even of high molecular weight to the subepithelial space 17. (e) In the sub-epithelial space in the mouse there is an accumulation of lymphocytes and other white blood cells around the site of, and at the time of, egg implantation 18. (f) Only the blastocyst, not other objects similar in size, is able to induce a decidual reaction in the mouse¹⁹. This suggests that the reaction is induced by something more than the physical presence of the blastocyst. If implantation requires the recognition of antigens displayed by the egg, there is some evidence to suggest that they may be tissue specific20 and/or strain specific7.

Further work is needed using purified haemagglutinating and thymocyte antisera to determine the cause of these

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Pharmacology of the Mechanism of Certain Effects of Reserpine in the Rat

TWENTY minutes after receiving large doses of reserpine (5 mg/kg, given intraperitoneally), rats develop a myohypertonic-hypokinetic-cataleptic state, showing generalized tremor, piloerection, diarrhoea, ptosis and cutaneous hyperalgesia. In such a state rats do not move, but allow themselves to be dragged by the tail. The ptosis is reversible during the first 40 min after its appearance and then irreversible (compare refs. 1 and 2). We have found that the symptoms remain unaltered for 24 h, and then gradually subside to a practically normal state after 48 h. When the forelimbs of the animal under the influence of reserpine were placed on the edge of a table, and the rat held by the tail, it remained still in this position, unlike normal animals which would climb quickly onto the table. Rats placed on the edge of an open cage on their abdomen behave similarly; they do not climb or jump down as normal rats would do. The animals treated with reserpine find it difficult to swim in water and do so in an oblique (or sloping) position, and they cannot get themselves out of the water bath. Normal rate swim quickly in a near horizontal position and get out of the water easily.

An identical state can be produced by injecting the rats intraperitoneally with large doses of serotonia or tryptamine (30 mg/kg). In the case of serotonin, however, such a state is observed only in a second phase. During the first phase (between 2 and 40 min after injection) rats show total flaccid paralysis with analgesia, but with the rest of the sensory system apparently undisturbed. The second phase after injection of serotonin (after 40 min) is identical with the state observed after an injection of reserpine. The serotonin effect disappears gradually after 2 h. When tryptamine is given there is only one phase of reaction; the animals show the same symptoms which reserpine produces immediately (2 min) after injection and these effects last for 1 h.

Doses of 2 mg/kg of adrenergic agents (adrenaline and noradrenaline) produce a different depressive state, with myohypotonia, analgesia and semiptosis.

Pharmacological assays have shown us that there is more than a casual similarity between the effects of reserpine and those of serotonin and tryptamine. We can summarize the results of our experiments as follows. (a) The antiserotoninic drug methysergide injected intraperitoneally in a dose of 5 mg/kg 50 min before the administration of reserpine postponed the appearance of its effects by 45 min. A second dose of methysergide if given 10 min after reserpine completely blocked the effects of the reserpine. The effects of serotonin and tryptamine were inhibited by one dose of methysergide injected 50 min before the administration of these drugs, and the animals remained in a completely normal state. LSD and BOL (bromolysergic acid diethylamide) (2 mg/kg) only delayed the appearance of the effects of reserpine for 40-60 min. None of these drugs depress catecholamine.

(b) Adrenergic blocking agents (10 mg/kg of phentolamine, 5 mg/kg of dihydroergotamine and 1 mg/kg of propanolol) administered in the same way did not affect the symptoms produced by reservine and serotonin. They also had no effect on catecholamine depression.

(c) Centrally active anticholinergic drugs (atropine and benactyzine, in doses of up to 30 mg/kg) showed no effect on the symptoms produced by reserpine and serotonin, nor did they affect catecholamine depression.

(d) The antihistamine drugs ('Phenergan' and 'Benadryl', in doses of up to 30 mg/kg) had a sedative effect, but were without effect on the symptoms produced by

reserpine, serotonin or catecholamines.

(e) The inhibitors of monoamine oxidase (iproniazid in a dose of 100 mg/kg, nialamide in a dose of 50 mg/kg or tranyleypromine in a dose of 30 mg/kg) delayed the second phase of the effects of serotonin and reserpine, but did not inhibit them. Iproniazid injected 24 h before reserpine and serotonin delayed their effects for 4 h; nialamide injected 24 h before, and tranycypromine injected 1 h before, delayed the appearance of the effects for 35 min. The serotonin paralysis of the first phase was markedly reduced by these drugs; the flaccidity was less pronounced and the animals tried to move with some No effect on catecholamine depression was success.

(f) Injection of 500 mg/kg of dopa 20 min earlier postponed the effect of reserpine by 4 h, inhibiting the action of serotonin and tryptamine completely; 200 mg/kg had no effect on the effects of reserpine, but blocked the tryptamine symptoms and the second phase of the

serotonin effects.

(g) Clinical antidepressive drugs, such as imipramine, desmethylimipramine, amitryptiline and nortryptiline, in doses of up to 50 mg/kg similarly retarded the appearance of the effects of serotonin and reserpine without blocking them. Catecholamine depression was not modified in any way by these drugs.

(h) Antiparkinson drugs ('Tremaril', 'Diparcol' and 'Parsidol'), in doses of up to 30 mg/kg, completely inhibited the effects of reserpine and serotonin. 'Aturban' had no effect. None of these drugs had any action on the

symptoms produced by catecholamines.

(i) Phenylethylamine and methylamphetamine, in a dose of 10 mg/kg, inhibited the effects of reserpine and serotonin and those of catecholamines (compare

ref. 3).

It is interesting that dopa postponed the effects of reserpine and inhibited those of serotonin and tryptamine, but it is difficult to see this as evidence that a depletion of catecholamines is the cause of the effects of reserpine, unless the same mechanism also applies to the effects of serotonin and tryptamine. The situation is similar with regard to the temporary blocking action of inhibitors of monoamine oxidase.

The inhibition of the effects of both reserpine and serotonin by the antiserotoninic drug methysergide argues in favour of the hypothesis of Brodie and Shore4 that reserpine acts by releasing serotonin, at least as far as the Nevertheless, effects observed by us are concerned. tryptaminic mechanisms would also fit in with our results. There are further implications in the complete inhibition of the effects of reserpine and serotonin by antiparkinson drugs while other central anticholinergic drugs were inactive in this respect.

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Neuro-interstitial Junction in a Gastropod, Glossodoris

On the basis of ultrastructural observations, we have suggested a unified "glio-interstitial" cell-system in the Mediterranean sea-slug Glossodoris1. This system includes the glial cells described in the central nervous system of several gastropods²⁻³ and the "interstitial cells" associated with the peripheral nervous system. The later non-neuronal elements resemble the "interstitial cells of Cajal" in the peripheral nervous system of vertebrates4 in their relationship with nerve plexuses and smooth muscle fibres, in their morphology and in their staining properties (ref. 5 and my unpublished results). A special type of junction between nerve fibres and interstitial cells has been found in an electron microscope study of the foot of Glossodoris tricolor (Cantraine).

This junction is formed by an apposition of membranes with morphological polarization and differentiation, as in a regular nervous synapse. Fig. 1 shows nerve fibres lying in close contact with the interstitial cell. In two places the membranes are thickened and densely stained (differentiation) and the intercellular cleft is reduced to 120 A. The nervous side of the contact shows a striking accumulation of vesicles (polarization) which are the same size as "synaptic vesicles" (300-800 Å), but some of them have an electron-dense core (200 Å) separated from the outer membrane by a clear space. This second type is similar to the vesicles in the vertebrate nervous system6 which are thought to contain catecholamines.



Arrows indicate synaptoid contacts. c, Collagen; g, interstitial granules; n, neurite; nu, nucleus of the interstitial cell.

Both the differentiation and the polarization suggest that these contacts represent the site of special exchange between the nerve fibre and the interstitial element, apparently directed from the nerve process toward the interstitial cell. The structure of the neuro-interstitial junction is similar to that of the neuromuscular junction

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in Glossodoris (my unpublished results) which suggests that the interstitial cell itself may be an effector. We have not yet seen a similar synaptoid association of nerve fibres with glial elements in the central nervous system of Glossodoris, but a parallel description of a neuro-interstitial junction in man has been published?, and there have been indications of neuro-ependymal connexions in other vertebrates*,

Polarized and differentiated contact may permit neuronal input to the glio-interstitial system. There are also three possible outputs from the system: (1) a localized action on muscle, suggested by the close contact between interstitial elements and muscle fibres (unpublished results); (2) a direct effect on neurones, where the relationship of interstitial cells with peripheral neurones parallels that of glial cells with central nervous elements, which is most conspicuously developed in the trophospongium; (3) a diffuse humoral influence on the surrounding tissues, suggested by electron micrographs which show the extrusion of granules at random points on the interstitial cell surface.

Further physiological information is necessary to establish the function of the glio-interstitial system, but the morphological data suggest that it plays an active part in nervous and neuromuscular physiology.

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State-dependent Learning produced with Steroids

THERE have been three sets of discoveries on the effects of chemical substances on behaviour. First, it has been shown that chemical agents, especially endocrine hormones, can predispose animals to display particular species-typical ("instinctive") actions such as mating, nest building and care of the young1,2. Second, it has been found that generalized stimulating, anti-depressant, tranquillizing or psychotomimetic effects on behaviour can be produced by certain chemical agents sometimes referred to as "psychotropic" drugs3. Third, it has been established that a response learned in a given chemical state is more likely to occur when the subject is in that particular state than when he is not; this phenomenon is called state-dependent learning or "drug dissociation". Only the third discovery indicates that it may be possible to obtain specific or discriminative chemical control over any chosen response through learning. It provides a means for understanding how the specific idiosyncratic response patterns comprising "personality" could come under chemical control.

Evidence that drugs can be made to have discriminative control over learned responses was first provided by Girden's experiments with raw curare⁵⁻⁷. His work has recently been corroborated and extended by other investigators using different drugs and responses 8-13. In a typical experiment, animals are rewarded for making one of two alternative responses (for example, turning right in a T-maze) while in one drug state, and for making the

other response (turning left) while in a second—usually a non-drug-state. They learn to go to the correct side on every trial.

Drugs used in recent experiments have included a variety of depressant and hypnotic agents such as barbiturates, ethyl alcohol, chlorpromazine and atropine sulphate, but state-dependent learning has not yet been demonstrated with chemicals usually produced in the

We have investigated whether steroids known to affect the central nervous system can be used as a basis of statedependent learning. Progesterone was chosen because it has hypnotic properties as have many drugs known to be capable of producing such learning14. Hypnosis is induced only with high doses of progesterone, so we started the investigation with hydroxydione sodium ('Viadril'), a synthetic compound with strong hypnotic properties15 which has a close chemical resemblance to progesterone. experiment with 'Viadril' was carried out first with male rats. When we had shown that state-dependent learning could be obtained with this compound, we changed to ovariectomized female rats for the progesterone study.

Two groups of adult hooded rats, fifteen males and eleven ovariectomized females, were trained to escape from a 0.9-1.6 m.amp scrambled electric shock in a T-maze. Each arm of the maze was 24 in. long, 8 in. wide and 8 in. high. Uniform light was provided through a translucent roof, which eliminated extra-maze visual enes. The animals were observed through one-way vision glass in the side-walls of the maze. All the floor could be electrified but the last 12 in. of the right or left arm of the T could be made "safe" by disconnecting the relevant circuit.

On each trial the floor of the maze was electrified and the rat was dropped into the starting area, facing away from the alley. It was allowed to run freely until it reached the safe goal-box. Each rat was trained to run to one arm of the T while in a "drug state" (DS), and to the opposite arm while in a "non-drug state" (NDS). In each group some rats were trained to go left in DS and right in NDS, while others were trained to go right in DS and left in NDS. Both the chemical state and the direction of turn required alternated on successive days: animals from each group were given DS trials on days 1, 3, 5, etc., while others were given DS trials on days 2, 4, 6, etc. An injection of 0.5 ml. of isotonic saline constituted NDS. The dose of 'Viadril' given to each male animal (Group I) on DS days was $25~\mathrm{mg/kg}$ ($10~\mathrm{mg/ml}$, distilled water). For female rats (Group II) the dose of progesterone, chosen on the basis of prior tests, was 100 mg/kg (100 mg/ml. in a 2 per cent solution of carboxymethyl cellulose). All injections were given intraperitoneally 15-30 min before the test. Drugged animals were tested as soon as signs of sedation (ataxia, drowsiness) were evident; occasionally, a supplementary injection was given because signs of sedation did not appear after the first injection.

The daily training of each animal consisted of five or ten trials about 2 min apart. The first arm of the maze entered by the rat was recorded. If the entire body of the animal moved into an arm from the choice area, an "entry" was scored. The direction of turn made by the animal on the first trial of each daily session indicated the degree of control the chemical states (DS and NDS) had over response choice. The correct first trial responses for two sessions (one DS day and one NDS day) are plotted as percentages in Fig. 1. (Almost all choices made after the first trial were correct.) The learning curves represent the combined results of all animals tested. In each group there was at least one animal which made no more than three incorrect choices in all trials, but there were two animals in each group whose first trial performance indicated no state-dependent learning. Animals given saline injections daily and trained to change direction on alternate days performed in a way which could be expected

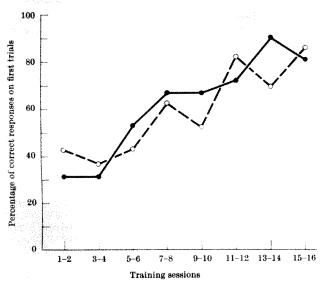


Fig. 1. Percentage of correct choices made by groups of rats on the first trials of two successive sessions, one after drug treatment and one after saline, \(\int ---\), Group I ('Viadril' versus saline), fifteen males; \(\int ---\), Group II (progesterone versus saline), eleven ovariectomized females.

by chance, indicating that the animals in the two experimental groups learned to make the correct choice on the basis of the drug state alone. This demonstration by no means proves that substances such as progesterone usually determine idiosyncratic response tendencies, or changes in response with variations in their blood con-The amount of progesterone used in this centrations. study greatly exceeds normal blood levels. Changes in complex behaviour patterns are sometimes closely associated with changes in blood chemistry and these changes are often idiosyncratic, being variable from individual to Some responses (for example, behaviour individual. during premenstrual depression) are persistent as long as certain humoral states obtain, but they are quickly disrupted by changes in those states. The persistence of responses can occur in spite of changes in the external environment, whereas their elimination can occur even when the environment appears to be constant. We should perhaps consider the possibility that through learning the responses may have become tied to certain chemical

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Control of Muscular Contractions by Spinal Neurones in Amphioxus (Branchiostoma lanceolatum)

A VARIETY of electrical events can be demonstrated in the nerve cord and myotomes of amphioxus by conventional electrophysiological methods, following electrical, mechanical or photic stimulation. Fine silver wire electrodes (20–30 μ) or tungsten micro-electrodes (2·0–0·5 μ) proved more satisfactory than glass capillaries. Recordings were made from the intact animal, from preparations stripped off muscles and from isolated nerve cords. Further details of the experimental procedure will be

published elsewhere.

Afferent volleys seem to be localized, and at recording points distant from the stimulus only interneurones respond. Photoreceptor potentials were only detectable by microelectrode penetrations and do not differ greatly from interneurone signals. Both photic and mechanical stimuli result in the appearance at different levels in the cord of brief trains of between one and five impulses the spacing of which increases from 100 to 480 msec in a typical series (Fig. 1A). The duration of each impulse is about 3.5 msec. The magnitude is constant for any electrode position and is always relatively large (over 10 µV). When two recording points on the nerve cord were employed to pick up trains of such potentials the decline in frequency was more pronounced at the electrodes furthest from the stimulus point, suggesting the involvement of a series of elements rather than continuous axons. These observations suggest that the elements involved are the large Rhode interneurones described by Bone¹ and others. The maximum rate of conduction in these fibres is between 1.5 and 2.0 m/sec compared with 0·3-1·0 m/sec in the smaller elements. Following the Rhode cell potentials in a response series are signals varying a good deal in number, magnitude and spacing. They usually seem to form compound potentials (Fig. 1B). Their total amplitude almost always exceeds that of Rhode cell impulses, but the form that they take varies considerably with the position of the electrodes. A single Rhode cell potential precedes one to five potentials of this kind, the first following the Rhode impulse by 50-100 msec. Given a sufficiently high level of stimulation several muscular waves pass down the animal and the activity of Rhode and post-Rhode elements is repeated at 2-3 c/s. Other low value signals also appear and are perhaps involved in this maintained activity. disappear as the swimming movements slow down. The compound nature and localization of the post-Rhode cell activity point to its origin in the somatic motor cell clusters shown by anatomists.

Recordings from the myotomes have so far given rather variable results, but in ideal conditions brief bursts of potentials can be seen to accompany muscular twitches

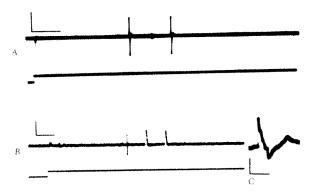


Fig. 1. A and B, Responses from the nerve cord (upper traces) to a beam of light directed against the flank (step on lower traces). C, A compound potential similar to that shown in B. Vertical scale: A, 15 μ V; B, 15 μ V; C, 10 μ V. Horizontal scale: A, 0-2 sec; B, 0-1 sec; C, 50 msec.

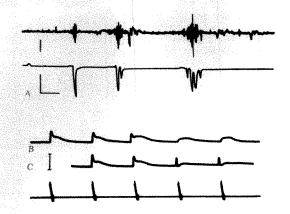


Fig. 2. A, Electrical record (upper trace) and mechanical responses (lower trace) from the thirty-fifth myotome, following mechanical stimulation of the head. B and C, Mechanical response from a myotome resulting from electrical stimulation within the adjacent nerve cord at 1 c/s. B illustrates the effect of altering the position of the electrode, and C the result of lengthening the duration of the stimulus pulse from 4 to 9 msec. Vertical scale: A, upper trace 20 μ V, lower trace 3 g; B and C, 2 g. Horizontal scale: A, 1.0 sec. In B the scale is given by the stimulus marker on the lowest trace.

(Fig. 2A). These often occur against a background of larger single potentials (100 μ V) repeated at 1–2/sec. The latter may be tonic in nature but further evidence is necessary.

Direct electrical stimulations of the muscle fibres reveal a rapidly fatiguing system the frequency discrimination of which declines rapidly above 10 c/s, a fused contraction increasing as repetitive twitches decline. The dual nature of the contractual response is much more clearly demonstrated by stimulation of the myotomes from sites within the nerve cord, when a fast and a slow component can be separated by electrode localization (Fig. 2B) or duration of stimulus (Fig. 2C). This is in accord with the separation of fast and slow muscle contacts with the nerve cord demonstrated anatomically by Flood².

The general impression of the nature of spinal neurone activity in amphioxus is of a system usually working at a low level, but capable of being driven into a state of much higher and more continuous activity by stimulation. The rate of spontaneous activity is less than two impulses, see at 20° C in the quiescent animal. When few neurones are active, responses decay rapidly. A sufficiently high level of stimulation results in many more neurones becoming active and the animal is able to swim rapidly and accurately at speeds up to 40 cm/sec.

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Effect of Promethazine Hydrochloride on the Release of Gastrin in the Dog

Antihistaminic drugs do not inhibit acid secretion stimulated by histamine or insulin in the dog, but given intravenously they do suppress the secretory response to endogenous gastrin^{1,2}. Payne et al.², after testing several antihistamines, suggested that the specific pharmacological mechanism of this phenomenon was their atropine like (anticholinergic) effect. It is, however, difficult to

reconcile this explanation with the fact that t phase of acid secretion, stimulated by insul glycaemia, was apparently unaffected by t histaminics¹.

Atropine, used systemically^{2,3,5,7} or applied to to the antrum, suppresses the secretory resendogenous gastrin, and has been suggested to this effect by blocking the release of gastrin froundetermined cell of origin. We have thought gastrin mechanism is suppressed differently by and by promethazine.

The availability of pure gastrin and the "gas polypeptides has made possible further investithis problem. The present experiments confered of promethazine with that of atropine on tory responses to both endogenous and exogenous

Intravenous promethazine (1 mg/kg) has be to diminish markedly the acid secretion induced stimulation with liver suspension. We have no out similar experiments to show that endogenor released by topical stimulation of the antiacetylcholine chloride is also inhibited by pror A dog, prepared with denervated antral as pouches and also a gastrostomy, was fasted Then acid secretion was stimulated by perfus antral pouch with a 0.5 per cent solution of acc at a rate of 0.43 ml./min and at a pressure of water. Gastric juice was collected at 10 min from both the fundic pouch and the main storms the rate of secretion reached a plateau, a six venous injection of promethazine (1 mg/kg) during 3-4 min and collections were continu acidity was titrated to the Töpfer's reagent (pH 3.5). Three experiments, comparing ac from the hour before and the hour after the is promethazine, were carried out with this pr The promethazine caused a 72-91 per cent in acid secretion presumably by either (a) prevent release, (b) blocking the action of gastrin after or (c) both of these mechanisms.

In order to elucidate this action of protwelve further experiments were carried or dogs with gastrostomy cannulas (and in ordenervated fundic pouch as well). Contine venous infusions of gastrin pentapeptide* (1 p. 1.5-2.5 h resulted in a plateau of acid outputively similar to that produced by endogens released by the topical application of acetylch was followed by a single slow intravenous i promethazine (1 mg/kg). There was no signiff of promethazine on the exogenous "gastrin" secretion (Table 1).

Table 1. EFFECTS OF PROMETHAZINE AND ATROPINE ON SECLATED BY GASTRIN PENTAPEPTIDE

	Stomach	
Atropine	$91.7 \pm 1.4 (10)$	57- <u>%</u>
	P < 0.001	F
Promethazine	$6.8 \pm 9.1 (12)$	5.
	P > 0.1	.0

Values given are means of percentage inhibition ± standa; bers in parentheses are the number of experiments carried of P represent the probability that inhibition observed was risgnificant compared with no inhibition.

In similar experiments using intravenous at dosage of 0.02 mg/kg (said to have equivibrilarly cholinergic potency to promethazine in a dose (ref. 2), there was marked inhibition of the "gastrin" (Table 1). The range of reduction of the was 82-96 per cent in the stomach and 50 in the denervated fundic pouch.

* Because of its availability, gastrin pentapeptide (N-i-but B-alanyl-tryptophanyl-methionyl-aspartyl-phenylalanine am was used. It possesses secretory effects qualitatively similar tatively less potent than gastrin. Imperial Chemical Indus England, and Ayerst Laboratories, New York, NY, supplied t nism. Atropine blocks the effects of and exogenous gastrin, while proly endogenous gastrin. In other mine seems to work by inhibiting 1 from its cell of origin, but, once its secretory effect is unaltered by

pported by a grant from the US f Health. We thank Irwin Lee and echnical assistance.

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ling Capacity related f Histamine

ve indicated that an increase in the ımine can be regarded as an important esensitization. In these experiments, bound histamine was found in the er repeated injections of histamine. teresting to look for a relationship of histamine to the tissues and their s substance.

carried out with male albino mice An intraperitoneal infusion of histacarbon-14 was given over 24 h. Fine long enough to allow the mouse access s well as freedom of movement in the nto the peritoneal cavity. Histamine) and 100 µg/g of mouse (containing nistamine) were prepared in 1.3 ml. of volume infused over 24 h by means of a motor. The mice were then kept conditions to allow unbound histamine urine and faeces. Forty-eight hours infusion, each mouse was homogenized with 200 ml. of 10 per cent trichlorod. of histamine-histidine carrier (conhistamine dihydrochloric acid and 40 drochloride/ml.). Labelled histamine ording to the method of Waton2. The of untreated mice was measured ribed by Parratt and West³. Briefly, rogenized in 10 per cent trichloroacetic o stand for not less than 24 h. The extract was filtered, the excess acid nd the solution assayed on the isolated pig ileum. Specificity for histamine the mepyramine test⁴. All values of

the base. the mice during the infusion did not ed by the histamine, but a toxic effect pecause the LD_{50} of histamine given to illy, according to Angelakos and Loews.

histamine found 48 h after the end of the infusion are given in Table 1. These show that for the smaller doses $(0.1, 1.0 \text{ and } 10 \,\mu\text{g/g} \text{ of mouse})$ the percentage of histamine taken up in relation to the histamine supplied is similar in each case. For the larger dose (100 µg/g mouse) the value obtained is approximately seven times greater.

Table 1

No. of mice used	¹⁴ C-Hist- amine infused (dose/g of mouse/24 h)	¹⁴ C-Histamine bound, values/g of mouse with standard error of the mean	Percentage of histamine taken up in relation to histamine supplied	Percentage of histamine taken up in relation to the original histamine content $(9.8 \ \mu g/g)$
13	0·1 μg	0.033 ng ±0.002	0.033	3.4×10^{-4}
12	1·0 μg	0.38 ng ±0.05	0.038	3.9×10^{-3}
9	10 μg	3.5 ng ±0.38	0.035	3.6×10^{-2}
13	100 μg	258 ng ±58	0.258	2.6

In order to compare the amounts of bound histamine with histamine already present in the mouse, the histamine content of untreated mice was determined. The mean value for twelve mice was $9.8~\mu g/g$ of mouse with a standard error of the mean of $0.6~\mu g/g$. The uptake of histamine in each dose was expressed as a percentage of the original histamine content of the mice (Table 1).

These experiments do not indicate in which tissue of the mouse the histamine is bound. According to Beaumariage⁶, the skin of the mouse contains a large amount of histamine. Schayer, has shown that when 14C-histidine was injected into the mouse, histamine was formed and bound largely in the skin. Binding therefore probably

took place chiefly in the skin. The present results demonstrate that the binding of histamine is related to the supply of histamine. For a wide range of doses (0·1-10 µg of histamine/g) this relationship was consistent. When the dose of histamine was greater than the content of histamine already present in the mouse $(9.8~\mu g/g)$ this relationship changed, and the percentage of bound histamine increased. These results indicate that new histamine binding sites were formed after stimulation by the increased supply of histamine. If there was only a limited number of binding sites available, the percentage of bound histamine would decrease

however, do not support such an assumption. The data for histamine binding agree with earlier results¹. In these experiments, $10~\mu g$ of ¹⁴C-histamine/g of mouse was injected daily over 28 days. Three days after the end of the treatment, 109 ng of histamine/g of mouse was found; this value of daily histamine uptake (3.5 ng/g) was similar to that now obtained by the 24 h infusion of 10 µg of 14C-histamine/g of mouse.

as more histamine was administered.

Thus the provision of new histamine binding sites where the body can store histamine in a non-toxic, bound form may be regarded as an important factor in the mechanism of histamine desensitization.

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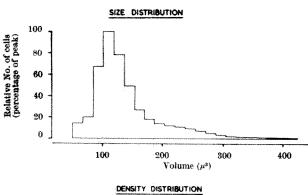
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IMMUNOLOGY

Density Distribution Analysis of Lymphocyte Populations

DESPITE their suspected heterogeneity in origin, life span and precise function, lymphocytes are usually considered to be a single group because of the rather negative morphological criteria available. One morphological division of lymphocytes into immature (large and medium) and mature (small) cells is physiologically relevant, because the larger cells represent a population which synthesizes DNA and which divides, while the smaller cells are not usually engaged in DNA synthesis. Morphological criteria are as yet, however, inadequate to make possible identification of the classes of lymphocytes which are now studied by such functional tests as the ability to respond to antigen and to produce a colony of cells which form antibody, the ability to produce various classes of antibody or the ability to initiate a graft-versus-host reaction. This communication suggests equilibrium density gradient centrifugation as a technique which may prove valuable, not only in defining lymphocyte classes, but also in separating them from each other.

The procedure for separating lymphocytes on the basis of fine differences in their buoyant density is given in detail elsewhere1. The procedure reduces the effects of cell-to-cell interaction and maintains cells close to their original volume and density. Briefly, 107-109 lymphocytes are dispersed in a linear gradient of bovine plasma albumin (14-28 per cent w/w) in balanced salt solution at pH 5.1. To bring the cells to the position of their buoyant density, the tube is centrifuged at 3,700g for 45 min in the swingout head of a refrigerated centrifuge. About thirty fractions are collected by upward displacement of the gradient out of the top of the tube. After density determinations on each fraction have been made, the cells are recovered and their number, size/range distribution, morphological type and biological activity are accurately determined. From these data, a density distribution profile for each cell type can be computed and all results expressed in this form (cells/density increment



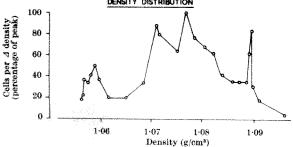


Fig. 1. Volume and density distribution curves for rat thymus cells. Both analyses were performed on the same preparation of 8 week old male Wistar rat thymus cells. The volume distribution was obtained with a model B Coulter cell counter equipped with a model J particle size distribution analyser.

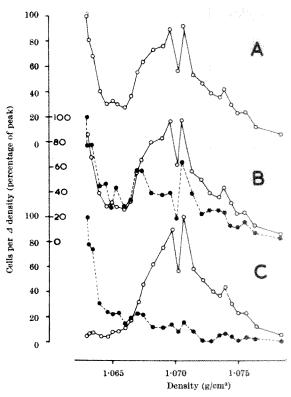


Fig. 2. Density distribution of cells of different morphological of and of different sizes. All curves are from a single run on 8 wes male rat thymus cells. On each density fraction, total cell counts az range distributions were performed using a model B Coulter cell counts and smears were prepared and stained with Giemsa for assessm morphological categories. From these data the density distribut the following types of cells was calculated. A, All cells; B, lymphocytes (\(\cdots --- \)) and medium and large lymphocytes (\(\cdots --- \)) as defined by morphological criteria; C, cells of volume 102-3 (\(\cdots --- \)), and of volume 325-342 \(\theta^2 \) (\(\cdots --- \)).

against density). The overall cell recovery is about cent and recovery of biological activity in a wide * of tests is in the range 60-90 per cent. The positive peak of cells is reproducible to ±0.0003 g/cm3 sta deviation, or to about one quarter of a fraction.

The method was originally designed to separate medium and small lymphocytes from each other. E the density of the cell should reflect the ratio between dense nucleus and lighter cytoplasm, small cells, the nucleus predominates, should be denser than large Instead of a simple separation by size alone, he lymphocytes from the thymus, lymph nodes, to duct lymph, blood and spleen all gave a much complex pattern composed of many discreet per shoulders. Fig. 1 shows the contrasts in the distr of size and range of rat thymus cells with the dens tribution of the same preparation. Further resolu this pattern with shallow gradients covering only a density range has revealed the presence of furth defined density populations.

Some indication of the nature of cells in various of the gradient is given in Fig. 2. Here the dense tributions of cells of a given morphological class, precisely defined volume, are plotted independe each other. As predicted, the upper regions of the are enriched for larger cells, the lower regions for cells. Some types of small lymphocytes, however the lighter zones and some types of larger cells in the Each morphological or size category separated into a series of density peaks. Such a tion may reflect the presence of several indelymphocyte populations, each with its own salarge, medium and small cells. Alternatively, reflect a series of definite metabolic stages through any one lymphocyte may pass in its development

This separation procedure has been applied to cells forming 19S haemolytic antibody directed against sheep red cells, as assayed by the Jerne plaque technique². In this case it is already known that a wide variety of cells may produce antibody, ranging from cells which might be classed as small lymphocytes, through large, dividing cells to the classical plasma cell^{3,4}. A typical density distribution pattern for tissue cells which form antibody is given in Fig. 3, the data in which were obtained with rat spleen 3 days after stimulation with antigen. pattern shows marked heterogeneity, with several distinct peaks which could be resolved more clearly with gradients

covering only a narrow density range.

To determine which part of the curve represented 'Vinblastine' was dividing cells, the mitotic inhibitor administered to the animal 5 h before analysis was made of density distribution. Because the drug did not seem to inactivate the cells which form antibody, it should arrest or cause a build-up of cells in the process of division and a relative depletion of product cells, compared with the untreated control. Fig. 3 shows that 'Vinblastine' caused a build-up of cells in certain regions and marked depletion in others. In general terms it seems that dividing cells are concentrated in the lighter regions of the gradient, and product cells in the denser regions.

The antibody forming cells circulating in the blood and thoracic duct give a quite different density distribution pattern from that of fixed tissue cells. This is in accordance with electron microscopic studies showing differences between fixed and circulating antibody forming cells3,5. An example is shown in Fig. 4. There is clearly a restriction

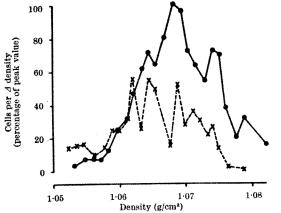


Fig. 3. Density distribution from rat spleen of haemolytic cells which form antibody. The analysis was performed 3 days after intravenous injection of a 9 week old male Wistar rat with 10° sheep crythrocytes. A separate animal was, in addition, injected intraperitoneally with 4 mg Vinblastine', 5 h (approximately one doubling time) before analysis. The areas under the two curves represent the relative numbers of antibody producing cells/spleen.

———, Control; × - - ×, treated with 'Vinblastine'.

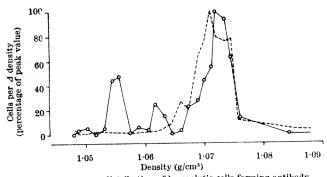


Fig. 4. The density distribution of haemolytic cells forming antibody, from the thoracic duct of a rat. A 9 week old male Wistar rat was injected with 10° sheep erythrocytes, half intravenously and half intraperitoneally, and cannulated 4 days later to collect lymphocytes from the thoracic duct. The analysis is on a 3 h collection of cells 4.5 days after antigenic stimulation. O. Antibody forming cells; . . . , total cells.

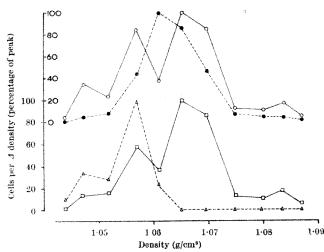


Fig. 5. Density distribution of graft-versus-host activity. The buffy-coat fraction from the blood of a single adult fowl was separated on the gradient and fractions assayed for pock forming ability on chick embryo chorioallantoic membrane, for total cell number using the model B Coulter cell counter and for morphological type using smeared and stained preparations. O—O, All lymphocytes; • --- •, pox forming cells; ——, small lymphocytes; • --- •, large lymphocytes.

on the range of cells which produce antibody that enter the circulation. Some of the peaks of active cells are well separated from the bulk of the lymphocytes and up to

100-fold purification may be obtained.

Another application of the technique was to determine the nature of cells from chick blood which are capable of initiating a graft-versus-host reaction6, visualized as the formation of pocks on the chorioallantoic membrane of chick embryo7. This approach was prompted by the controversy over whether large or small lymphocytes were the effector cells in this system. Fig. 5 compares the overall density distribution of lymphocytes with the distribution of graft-versus-host activity. The activity would seem to reside in a small sub-population, distinct from the bulk of the lymphocytes. Activity cannot be attributed either to the total population of large, or to the total population of small, lymphocytes. The curves suggest that the bulk of the active cells have the morphological appearance of small lymphocytes, although a small proportion may be of the large lymphocyte type.

The general conclusion is that lymphocytes from many sources are composed not of a continuum of cell types but of a series of discreet populations, separable by fine differences in density. The possibility of artefacts is very real in such a sensitive procedure, but the control experiments we have presented elsewhere make it unlikely that artefacts significantly contribute to the overall density distribution profile. This view is supported by the differences in biological activity presented here and in the communication by Haskill⁸. It seems more likely that the density distribution profile reflects the existence of separate functional categories, and of discreet metabolic states or differentiation stages. The technique should provide a precise and objective means of studying in fine detail the immunological function of lymphocytes

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Density Distribution Analysis of Antigen Sensitive Cells in the Rat

When lymphoid cells from spleen, lymph nodes or thoracic duct lymph are transferred together with sheep red cells to heavily irradiated recipients, an immune response occurs, the magnitude of which is related to the number of lymphoid cells injected1-3. Although Playfair1 and Kennedy² have described quantitative methods for determining the number of cells which are sensitive to antigens in such an inoculum, very little is known about the nature of these cells. The indications are that they are only slowly dividing or that most of them are in a resting state in the non-immunized animal4. Morphological evidence has been presented which tends to implicate small lymphocytes as cells which are sensitive to antigen. but the data are, as yet, unconvincing 5,6. The purpose of this work has been to use a different approach in characterizing these cells. The technique is that of sedimentation to equilibrium in density gradients of bovine serum albumin 7,8

A high resolution quantitative analysis of the density profile of cells sensitive to antigen was not routinely feasible using the assay methods of Playfair and Kennedy because of the large number of recipients needed. Two alternative methods have therefore been considered in this report. Syeklocha et al.4 have utilized the total number of antibody forming cells for the recipient spleen (defined by the Jerne plaque assay)9 as an index of the number of antigen sensitive cells in the donor inoculum. Albright et al. 10 have demonstrated a relationship between the number of injected spleen cells and the resulting antibody titre. Because the coefficient of variation in haemolysin titre from recipient to recipient is much less (12 per cent) than that observed for total plaque forming cells in the spleen (68 per cent), the mean haemolysin

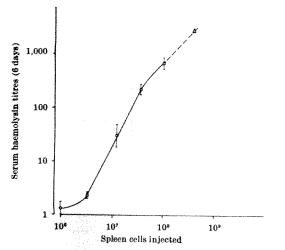


Fig. 1. Relationship between serum haemolysin titres taken on the sixth day and the number of spleen cells injected into irradiated recipients. All recipients received a dose of 850 rads of X-irradiation and 2×10° sheep red cells. The broken line extends to the maximum mean titre obtainable in non-irradiated animals. The mean variation in titre is indicated at each spleen cell concentration.

titre in recipients receiving various fractions of spleen cells has been used as a semi-quantitative assay for the measurement of cells which are sensitive to antigens. In every experiment, the total numbers of cells which form plaques have been determined and in all cases the agreement between the two methods of assay has been satis-

Spleen cells from male Lewis rats (9-10 weeks old) were injected together with 2×10° sheep red blood cells into male recipients (6-9 weeks old) which had received a dose of 750-850 rads of X-irradiation. The injections were carried out within 4 h of irradiation and the recipients were killed 6 days later to allow determination of haemolysin titres and total plaque forming cells in the spleen. In order to determine the profiles of cells which are sensitive to antigen, spleen cells were separated into ten to twenty-two fractions by equilibrium sedimentation in gradients of bovine serum albumin, before being injected into irradiated recipients.

The usual relationships between the number of injected spleen cells and the antibody titre was obtained as shown in Fig. 1. In order to calculate the relative number of cells sensitive to antigen in each density fraction, the mean haemolysin titre in each group of recipients was determined. Using this mean titre and the relationship between titre and unfractionated spleen cells (Fig. 1), the equivalent number of unfractionated spleen cells in each density fraction has been calculated. This number has been used in the determination of the density distribution profile of antigen sensitive cells. Recovery of biological activity has also been calculated from the equivalent numbers of spleen cells obtained from all fractions.

A typical profile for normal Lewis rats is shown in Fig. 2C. At least six different peaks of cells which are sensitive to antigen were observed. Although these same six components have always been observed, the relative importance of some of them has varied greatly from experiment to experiment. A possible reason for this variation will be discussed later. Recovery of biological activity in this type of experiment has been found to be 80-100 per cent.

In order to determine whether any of these peaks represented real differences in the biological properties of cells sensitive to antigens, the specific effect of antigenic stimulation was investigated. Because RNA and DNA synthesis are known to occur11 shortly after antigenic stimulation, it was expected that pronounced changes in the density profile of antigen sensitive cells would be observed on stimulation. The antigen sensitive cell profile was determined 10 h after antigenic stimulation (Fig. 2B). As expected, a profound alteration in the pattern was observed. After stimulation for 10 h, the densest components had virtually disappeared from their usual positions and in their place two major components which banded closely together in the light density region were observed. It is probable that part of the pool of circulating cells which were sensitive to antigen had lodged in the spleen during the 10 h of stimulation. Most of the cells present in the spleen at this time, however (which on subsequent transfer with antigen gave rise to an immune response), must have represented cells which had been present before antigenic stimulation and had themselves been altered directly or indirectly by contact with antigen. It was of interest to note that the position of the two chief components of cells sensitive to antigen which had been stimulated coincided with the position of a minor component in the profile derived from supposedly unstimulated donors (Fig. 2C). Because the variation which has been observed in the profiles derived from unstimulated donors (Fig. 2C) occurs in this low density regions, it is possible that this is a reflexion of varying degrees of base level stimulation with cross reacting antigens in the donor animals.

As a further study of the effects of antigen on the population of sensitive cells, an antigen sensitive cell profile was determined in animals which had been challenged with antigen 90 days before they were killed. The results shown in Fig. 2A indicate that the components present in the profile of cells sensitive to antigen were also present in the profile of unstimulated donors. The largest part, however, of the antigen-sensitive pool of the spleen seemed to be in the low density regions characteristic of stimulated cells which were sensitive to antigen. This result is taken as evidence that low levels of antigen and therefore stimulation persisted in the spleen up until this time.

RNA synthesis and cell enlargement are known to precede DNA synthesis in the graft versus host reaction¹¹. It seemed plausible therefore that the two major components observed after stimulation for 10 h represented a separation based on differences in density between cells actively involved in RNA synthesis (G₁ state) and cells sensitive to antigen which had progressed through this stage and were rapidly dividing (S-G₂-M). Because 'Vinblastine' is known to block cells entering mitosis¹², the density distribution profile of cells sensitive to antigen was determined after antigenic stimulation in the presence of 'Vinblastine'. Fig. 3B illustrates the effect of 'Vinblastine' on the density profile when the drug was injected 7 h after antigenic stimulation. The important alteration in this profile compared with that of the control stimulated animals in Fig. 3A is a significant

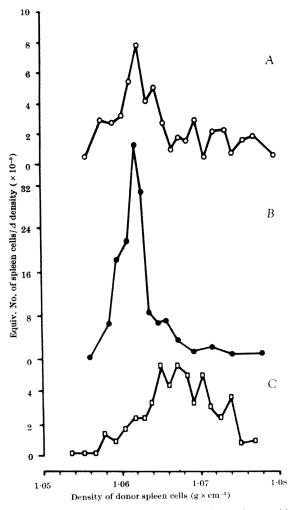


Fig. 2. Equilibrium density gradient profiles for spleen antigen sensitive cells: A, 90 days after stimulation with $2\times 10^{\circ}$ sheep red cells; B, 10 h after injection with $2\times 10^{\circ}$ sheep red cells; C, normal donor animals. From the mean titre derived from recipients of any density fraction, the equivalent number of normal spleen cells has been calculated with the aid of Fig. 1. This number has been used as a reflexion of the presence of antigen sensitive cells in each density fraction.

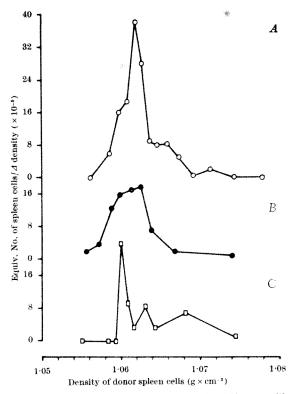


Fig. 3. Equilibrium density gradient profiles for cells which are sensitive to antigen, in the spleens of animals stimulated with 2×10^9 sheep red cells 10 h before they were killed. A, Control donors stimulated for 10 h; B, donors stimulated for 10 h but given 3 mg of 'Vinblastine' for the final 3 h; C, donors stimulated for 10 h but given 3 mg of 'Vinblastine' along with the antigen.

decrease in the major component at density 1.062. This probably reflects the elimination of cells sensitive to antigen which had entered mitosis between 7-10 h after antigen stimulation. When 'Vinblastine' was injected together with the antigen (Fig. 3C) this main component of stimulated cells which were sensitive to antigen is virtually removed, indicating that most of the antigen sensitive cells banding in this region have been stimulated into division within 10 h of injection of antigen.

Because the other stimulated component (density 1.060) did not disappear in either of these experiments with 'Vinblastine', it is concluded that this component represents cells sensitive to antigen which have been stimulated into RNA synthesis and cell enlargement but not into DNA synthesis within 10 h of stimulation.

The preliminary results presented here have indicated the usefulness of equilibrium sedimentation in analysing the lymphoid cells involved in the immune response. Although the purification achieved in Fig. 2B was fiftyfold, it was still insufficient in itself to allow a morphological identification of these stimulated cells, but it has allowed a functional identification which is probably of greater importance. Because the dual markers of function and buoyant density are used simultaneously, an absolute purification of these cells has not been needed to follow the progress of the cells which are sensitive to antigen through the various early stages of the immune response. In addition, the results have provided strong evidence for the validity of the many components which are observed on equilibrium density sedimentation not only of the antigen sensitive pool but presumably also of the various cell sources illustrated in the preceding paper.

The results presented here have been based on the assumption that only one cell population in the donor inoculum is involved in initiating and maintaining the immune response. The interpretation of the results would, of course, have to be altered if in future it is shown that two or more cell lines interact in this process.

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Study of the Ag(z) Factor

The existence of a genetically determined β-lipoprotein polymorphism in human serum was detected by Allison and Blumberg¹ and Blumberg et al.², employing a precipitating antibody which was present in the serum of a repeatedly transfused patient (C. de B.) The same antiserum was investigated by Hirschfeld et al.2 and was shown to contain at least three different anti-Ag antibodies against β-lipoprotein factors which were called Ag(a₁). $\overrightarrow{Ag}(x)$, and $\overrightarrow{Ag}(z)$. Data on the genetics of the Ag(x)and Ag(a1) factors were reported by different authors4-6 who used monospecific anti-Ag(x) and anti-Ag(a₁) sera. Only one other serum (C352) was found to possess anti-Ag(z) but it also had other anti-Ag specificities7. At present, the only source of anti-Ag(z) consists of serum from C. de B. absorbed with an $Ag(x+a_1+z-)$ serum. The complicating fact is that C. de B. seems to possess a fourth antibody, so that absorption with $Ag(x+a_1+z-1)$ sera may be unsuccessful in producing specific anti-Ag(z) antiserum. It can therefore be used in immunogenetic studies only with considerable caution.

The present study is concerned with the detection of the first two examples of monospecific anti-Ag(z) sera, the distribution of the Ag(z) factor in a United States white and in an Italian population, and its segregation in families. A precipitating antibody was found in the serum of a 6 yr old thalassaemic girl (T. I.) who had received more than fifty blood transfusions (Pediatric Clinic, University of Siena, Italy). Her serum reacted with a β-lipoprotein factor which was different from Ag(a₁). Ag(x), Ag(y) and Ag(t). Sera from patients T. I. and C. de B. absorbed with an $Ag(x+a_1+z-)$ serum were tested in parallel against 250 randomly selected individuals from Philadelphia. Only two cases of discordance were observed; these may be explained on the basis of the postulated fourth antibody present in the C. de B. antiserum. A second example of apparently monospecific anti-Ag(z) was found in a 12 yr old girl with thalassaemia (C. M.), who had received more than two hundred transfusions (Children's Hospital, Philadelphia). Both these antisera were found to give precipitin bands of identity with the anti-Ag(z) contained in C. de B. when tested against Ag(z +) sera. The phenotype of T. I. and C. M. was $Ag(a_1+x+y+t+z-)$; this would permit isoimmunization to the Ag(z) factor.

ble 1. distribution of the ag(z) factor among 307 individuals living in philadelphia and 162 individuals living in northern staly

Pheno-		ales		adelphia males	Males	+ females	Northern Italy* Males + females
types	No.	%	No.	%	No.	0/ /0	No. %
Ag(z+)	39	24.68	35	23.49	74	24.10	42 25-93
Ag(z-)	119	75.32	114	76.51	233	75-90	120 74 07
Totals	158	100.00	149	100-00	307	100-00	162 100-00

* Information on sex was not available for this sample group. Comparison: Males/females, $z^2=0.059$; d.f.=1; 0.90>P>0.80. Philadelphia/Northern Italy, $z^4=0.189$; d.f.=1; 0.70>P>0.50.

The serum of the patient T. I. has been tested every 3 months for the past 2 yr for the presence of antibodies. The Ag(z) precipitin has been found in some but not all samples collected in that period. This could be a result of the fact that the Ag(z) antigen has a frequency of 25-9 per cent in the Italian population (Table 1) so that the chance of receiving transfusions of Ag(z+) blood is one in four. We observed that, in general, if a patient with iso-precipitins does not receive an incompatible blood transfusion at least every 4 months, the antibody could disappear.

The serum T. I. was employed in the Ag(z) typing of 307 non-hospitalized, unrelated, United States whites from Philadelphia, 162 Italians living in northern Italy, and 42 families comprising 84 parents and 130 children. from Seattle, Washington, who were originally collected for a study of the families of Down's syndrome patients. The frequency of Ag(z) in Down's syndrome is not different from its frequency in the general populations. (These results are now in preparation.) Table I shows the frequency of the factor in the sample groups from Philadelphia and northern Italy.

No association between sex and Ag(z) factor was observed; it does not therefore seem to be either sexlinked or sex limited in its mode of inheritance. Assuming random mating among the populations of Philadelphia and northern Italy, the gene frequencies may be obtained by the following formula: $\overline{Ag^z} = 1 - \sqrt{Ag(z-)} = 0.129$ and 0.140 respectively for the two groups.

2. COMPARISON BETWEEN THE OBSERVED AND EXPECTED PHENO-TYPIC RATIOS IN THE OFFSPRING OF THE VARIOUS MATINGS

	Mat	lings			Offsprin	g.	
Types	Nu	mber	A #	(z+)		z-)	Totals
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
$Ag(z+) \times Ag(z+)$	4	2.5	7	9-4	5	2-6	12 47 71
$Ag(z+) \times Ag(z-)$	15	15.3	24	25.1	23	21.9	47
$Ag(z-)\times Ag(z-)$	23	24.2	0	0	71	71.0	71
Totals	42	42.0	31		99	V # 42	1.30
Comparisons: Offspring mating	re.	$\chi_{_{5}}$	d.f.	P			
Ag(z+) × Ag(z Offspring mating	+)	0.705	1 0.50	> P > 0.3	30 (Fish	er's ex	act test)
$Ag(z+) \times Ag(z)$	(—)	0.170	1 0.70	> P > 0.5	เก		

The segregation of the trait in families is shown in Table 2. The observed frequencies in the offspring of the different matings are close to those expected on the assumption that Ag(z) is inherited as a dominant autosomal character. This genetic hypothesis was also tested using Smith's method⁸ for the analysis of the families where one or both parents were of type Ag(z+) and had at least one recessive child (Table 3). In both cases, the deviations (R_1-E_1) and (R_2-E_2) are less than the standard errors S_1 and S_2 , and χ^2 is < 1.

The only other available data on the distribution of the Ag(z) factor are those provided by Hirsehfeld et al.*, who reported a frequency of 22.45 per cent in a population of 245 individuals from Stockholm. This frequency is not significantly different from the frequency in the Philadelphia population ($\chi^2 = 0.208$; 0.70 > P > 0.50) and in these from northern Italy populations ($\chi^2 = 0.649$; 0.50 > P >0.30).

Data obtained by Hirschfeld suggest that the Ag(z) factor may be controlled by a gene $\bar{A}g^z$ which is an allele of Aqt.

We are grateful to Professor F. Ragazzini (Pediatric Clinic, University of Siena) and to Dr I. J. Wolman (Children's Hospital, Philadelphia) for serum samples of

Table 3. Segregation of the $\operatorname{Ag}(z)$ factor in families where one or both parents were $\operatorname{Ag}(z+)$ and had at least one recessive child*

No. of children in family	No. of families	Observed No. of recessive children	Expected No. of recessive children	Variance
Mating type, As	$z(z+) \times Ag(z-$	-)		
C	me		meac	mebe
1	1	1	1	0
2	1	1	1	0
3	3	5	5.142	1.470
4	4	11	8.532	3.128
5	1	3	2.581	1.082
6	î	2	3.048	1.379
Total	11	$R_* = 23$	$E_1 = 21.303$	$V_1 = 7.059$
S	1 = 2.657; x2	$R_1 = 2\overline{3}$ = 0.407; d.f. = 1;	0.70 > P > 0.50	
Mating type, A	$z(z+) \times Ag(z)$	+)		
C	Me	270	McAc	M-B:
9	1	1	1.143	0.122
2	i	1	1.297	0.263
5	î	3	1-639	0.592
Total	3	$R_0 = 5$ = 0.868; d.f. = 1;	$E_{2} = 4.079$	$V_* = 0.977$
Lotal	-0.000- 41	-0.888: df-1:	0.50 > P > 0.30	

^{*} Computed by the method of Smith*.

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Subcellular Localization of Entamoeba histolytica Antigen

SERA from patients with amoebiasis have been shown, by immunofluorescence, to react with smears of Entamoeba histolytica and the reaction can be prevented by absorbing the sera with centrifuged sediments of E. histolytica cultures. This investigation was designed to determine the antigenically active part of the amoeba in this connexion and the results suggest that the activity resides in subcellular cytoplasmic particles of microsomal density, which is in accord with the cytoplasmic distribu-

tion of the immunofluorescent staining.

E. histolytica, cultured in 'Bacto' endamoeba medium (Difco) with 10 per cent horse serum, were separated from most of the rice powder in the cultures by layering on 15 per cent (w/v) 'Ficoll' (Pharmacia) in mammalian Ringer solution and centrifuging at 70 g_{av} for 10 min. The lower layer was discarded and the supernatant was recentrifuged to obtain a sediment of amoebae which was washed once with phosphate-saline (0·15 molar sodium chloride, 0·01 molar phosphate; pH 7·1) and recovered by further centrifuging. In supplementary experiments, the layering on 'Ficoll' was omitted without any apparent effect on the end result. Washed, packed amoebae were broken up in a tissue grinder with a 'Teflon' pestle in tris sucrose buffer (0.02 molar tris, 0.25 molar sucrose, 0.01 molar potassium chloride, 0.005 molar magnesium chloride, hydrochloric acid added to pH 7.4) and submitted

for 1 min to ultrasound at 20 ke/s by the MSE ultrasonic disintegrator, operated at 500 W. Disruption of amoebae was confirmed by light microscopy, and fractionation was carried out by differential centrifugation. The first sediment, collected after centrifuging at 350 gav. for 15 min, contained nuclear material and coarse cellular debris, together with clumps of bacteria and rice grains from the original culture medium. The supernatant, centrifuged at 6,500 g_{av} for 30 min (Servall $R\hat{C}$ -2, SS 34 rotor), yielded a second sediment consisting largely of membranous material and bacteria. Centrifugation of the second supernatant was carried out at 105,000 gav. for 60 min (Spinco L-2, type 50 titanium rotor) to yield a third sediment ("microsomal") and the final supernatant constituting the cell sap. These procedures were carried out at 0°-3° C and all sediments were washed once by resuspending in the tris buffer and recentrifuging.

Table 1. IMMUNOFLUORESCENT STAINING OF AMOEBIC SMEARS BY ABSORBED AND NON-ABSORBED ANTISERUM DILUTED 1:32

Absorptions	Staining
Nil	++
Tris sucrose	++
Packed amoebic culture	-
Homogenized amoebic culture	-
First sediment*	++
First sediment concentrated × 10	++
First sediment concentrated × 20	+
Second sediment*	++
Second sediment concentrated × 10	++
Second sediment concentrated × 20	+
Third sediment*	+
Third sediment concentrated × 10	-
Third sediment concentrated × 20	
Supernatant	++

Suspended in original homogenate volume.

Serum absorption was carried out by incubating a volume of serum with an equal volume of final supernatant cell sap, or of sediment suspended in tris buffer to one-twentieth of the volume of the original homogenate: 1 in 2 and 1 in 20 dilutions of this suspension were also tested, the latter dilution making the same volume as the original homogenate. One absorption was carried out at 37° C for 1 h and a second was carried out overnight at 2° C: the serum was recovered after the absorptions by centrifuging at 7,000 gay. for 30 min. The results of absorptions on the capacity of the sera to produce immunofluorescent staining of amoebic smears, summarized in Table 1, show that the antigenic part of the homogenate is very largely confined to the third sediment, which is of microsomal density. The other two sediments and the cell sap possess little antigenic activity.

Samples of the sediments and of intact amoebae were examined by electron microscopy. Pellets of material

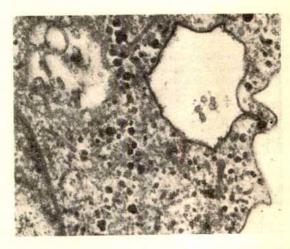


Fig. 1. Electron micrograph showing part of intact E. histolytica including quadrant of nucleus (bottom left), phagocytic vacuole (top right) and plasma membrane (extreme right). The black bodies in the granular cytoplasm have the appearance of glycogen; granular rod-like structures and (top left) small membranous vesicles are also present. ($\times 24,000$.)



Fig. 2. Subcellular fraction (third centrifuged sediment) of E. histolytica culture showing microsomal vesicles among the granular cytoplasmic material. ($\times 24,000$.)

were fixed in 2.5 per cent glutaraldehyde in sodium phosphate buffer (0.1 molar, pH 7.2), fixed in 1 per cent osmium tetroxide in the same buffer, dehydrated in acetone and embedded in 'Araldite'. Sections about 500 Å thick were mounted on carbon coated grids and stained successively with uranyl acetate and lead citrate. The antigenically active third sediment consisted of granular material and membranous vesicles corresponding to similar material in the cytoplasm of the intact organism (Figs. 1 and 2). Ribosomes could not be positively identified; structures resembling rods found in the intact organism could not be found after fractionation. Electron microscopy of the antigenically inactive first and second sediments confirmed the appearances seen by light microscopy. Among the bacteria and membranes of the second sediment there were many large vesicles, although mitochondria and Golgi bodies were not identifiable, which is in accord with observations on the intact organism by ourselves and

The exact nature of the antigenically active material is uncertain, but it is not nuclear and does not seem to be associated with surface membranes or large vesicles; it includes small vesicles and corresponds to the microsomal fraction of mammalian cells. If it can be concluded that intracytoplasmic material rather than surface membrane plays the principal antigenic part in amoebiasis infection, it could be inferred that immunological stimulation by disrupted organisms is the chief source of the serum antibody in human infection. Relative absence of antibody against surface components could be one explanation of the failure of an otherwise active immune response to prevent local invasion by the parasites in the disease.

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Number of Antibody-forming Cells in Primary and Secondary Reactions after Administration of Antigen

When newborn animals are immunized with a bacte antigen, large quantities of the antigen must be injecto elicit a primary antibody response1,2, Similarly, primary antibody response is induced in newborn rabl or in sterile piglets by sheep erythrocytes, the amount antibody in the serum and the number of antibody-fo ing cells in the spleen are greater the more antiger used3,4. In the true primary response, administration a 75 per cent lethal dose (100 ml. of concentrated susp sion of sheep erythrocytes given intraperitoneally sterile piglets, that is, 2×10^{12} cells) did not immedia inhibit antibody formation. On the contrary, number of cells producing haemolytic 19S and 7S a bodies is estimated after the intravenous administra to sterile piglets of very large quantities of the anti (20 ml. of a concentrated suspension of sheep erythrocy that is, 4 × 1011 cells), the number of antibody-forn cells in the spleen, where antigen is predominantly lo ized, is greater than when a similar dose is given i cutaneously or intraperitoneally (Fig. 1). shows that the inductive phase—when no antibody-fe ing cells are detected—lasts 36 h. After this period, t is a rapid increase in the number of antibody-form cells, at first with a doubling time of 2.6 h, later of 3 This cannot be considered to result from cell divi only. As suggested earlier4, it is supposed that the antibody-forming cells are thought to be formed differentiation of preformed competent cells and that rapid increase in the number of antibody-forming results from direct asynchronous differentiation of pre-existing competent cells. The very small number immunocompetent cells in lymphoid tissue (approxima one cell/106 lymphoid cells) explains the need to use quantities of the antigen to evoke a true primary reas in newborn animals. To make possible chance cor between the few immunocompetent cells and a cri quantity of antigen, substantially more antigen is quired than in the hypothetical case when nume competent cells are present (Fig. 2). The number of reacting with the antigen during the secondary reac was found to be greater by at least two orders of magni than the number of cells involved in the primary tion6. For simplicity, it is assumed that antigen ha same role in the primary and the secondary reactions this assumption, to activate the same number of ce the secondary as in the primary reaction, the amou antigen which would suffice would be inversely pro tional to the number of reacting cells in lymphoid ti

If a constant quantity of the antigen is require activate each immunocompetent cell, the probabili

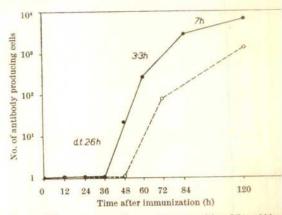


Fig. 1. Primary response after very large quantities of the red bloo antigen (20 ml. of concentrated suspension of sheep erythrod 4×10¹¹ cells) by intravenous (●) and subcutaneous (○) injection

 a_t is the concentration of the antigen at time t, and C is a constant (given by the experimental system used).

If N immunocompetent cells are present at immunization, then $n_t = N.P_t$ cells from these N cells will have contact with the antigen during time 0 to t. For a sufficiently large value of t, and on the simplified assumption that the concentration of the antigen decreases exponentially with time, Fig. 3 shows how the average number of antibody-forming cells will depend on the quantity of the antigen injected for three populations of immunocompetent cells of increasing size $(N_1 < N_2 < N_3)$. The number of antibody-forming cells increases linearly with the increasing dose of the antigen; only at very large doses of antigen does the rate of increase of antibody-forming cells slow down;

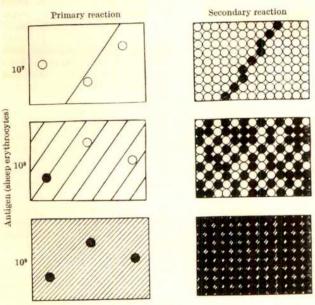


Fig. 2. Model of relationship between the number of competent cells and the quantity of antigen.

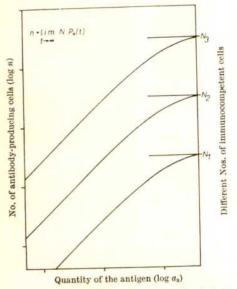


Fig. 3. Scheme of the dependence of the number of activated immunologically competent cells n on the antigen dose a_0 .

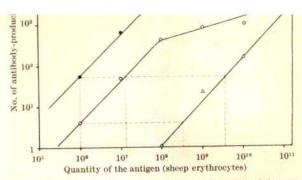


Fig. 4. Quantity of antigen necessary for the formation of the same number of antibody-forming cells during primary and secondary reactions.

4. Primary response. Secondary response: O, complement only (198); , complement plus anti-IgG serum.

it ceases when almost all immunocompetent cells are activated.

These assumptions were tested by estimating the number of cells responding after induction of primary and secondary response in sterile piglets fed a non-antigenic diet7. A group of 7 day old piglets was immunized with various amounts of sheep erythrocytes (Table 1). Only after the injection of more than 2 × 108 sheep erythrocytes can a primary response be detected; after increasing the dose of the antigen injected, more antibody-forming cells are detected during the primary response. Another group of piglets was prepared for secondary response by immunization on the seventh day of life with 2×109 sheep erythrocytes. This dose caused a low primary response, which disappeared during 3 weeks, at which time the animals were re-stimulated. Before stimulation neither antibody-forming cells nor serum antibodies were detected. The animals were stimulated again with various doses of the antigen, as in the primary stimulation. In comparison with the primary response a similar number of cells forming 19S antibodies was detected in the secondary reaction when the amount of antigen was more than two orders of magnitude smaller. A quantity of antigen three orders of magnitude smaller sufficed (Fig. 4) to detect cells forming 7S antibodies (using anti-IgG serum).

Table 1, RELATION BETWEEN THE QUANTITY OF THE ANTIGEN AND THE NUMBER OF ANTIBODY-PRODUCING CELLS IN PRIMARY AND SECONDARY REACTIONS

The dose of sheep erythrocytes injected I.p.	Primary response in newborn piglets (7 days after antigen injection)	piglets primary birth with 10° s Antibody-produ	nse of 1 month old stimulated after heep erythrocytes. cing cells detected jection of antigen By anti-IgG serum and complement (7S)
2×10^{6} 2×10^{7} 2×10^{8} 2×10^{9} 2×10^{10} 2×10^{14}	0	4	56
	0	48	640
	1	416	3,600
	22	800	8,000
	162	1,040	20,000
	1,861	3,030	30,060

These results indicate that a hundred times as many more cells producing 19S antibodies and a thousand times as many producing 7S antibodies are detected in the secondary response as in the primary response. Within certain limits of antigen dose, the results correspond to the suggested equation (1), that is, there is a linear dependence of the quantity of the antigen on the number of cells detected (one-hit system). The linear relation is valid in the primary reaction for a wide range of antigen doses, but in the secondary reaction it is valid up to the critical point of saturation only. This indicates

a difference in the capacity of antigen to bind to cells which react in the primary and the secondary reaction.

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PATHOLOGY

Facilitation of Tumour Growth by Bacillus pertussis

SEVERAL Gram negative bacteria or their components can exert a slight but significant inhibitory effect on the growth of tumour isografts1-4. Protection is obtained by administering the bacterial product at various times before the inoculation of the tumour cells. It is thought that these agents cause non-specific stimulation of the immune response against tumour-specific antigens.

On the other hand, bacterial products have been shown to inhibit manifestations of cellular immunity when applied simultaneously with the antigen. The tuberculin reaction in guinea-pigs and a normal lymphocyte transfer reaction in chickens were clearly reduced by concomitant administration of B. pertussis vaccine or extracts, and by microgram quantities of S. paratyphi B and S. typhosa extracts 5-7, whereas previous administration of these agents tended to enhance the two reactions. We have therefore investigated whether Gram negative bacteria have an inhibitory effect in a system involving transplantation immunity

against tumour isografts.

The experiments were performed with 6-8 week old inbred mice of the C57L/KL strain, weighing 16-20 g. Mice of both sexes were randomly placed in control and experimental groups. The experiments were carried out with Moloney lymphoma isografts. The tumour cells were obtained from a YLI lymphoma line propagated in C57L/KL mice at this institute. The solid lymphomas were excised, cut into small pieces, forced through a 60-mesh stainless steel screen into balanced salt solution (BSS) and filtered through gauze. The concentration of viable cells was estimated by the eosin exclusion method. Graded doses of tumour cells in 0-1 ml. of BSS were injected subcutaneously. The inoculum was placed midway between the axilla and the last rib of the mice. The development of the tumours was followed by bi-weekly measurements with a calliper in its long axis and in a direction perpendicular to this. Tumours were recorded as takes as they became and remained palpable. If the inocula took in the recipients, three different patterns of tumour development were seen. (1) Progressive growth to large-sized masses of about 30 mm average diameter. with the mice dying after about 2 months. (2) After an initial growth of up to 8-10 mm average diameter, a total regression of the local turnour could occur. The regression was considered to be complete if the mice survived until the end of the experiment at day 110 without macroscopic evidence of leukaemia at autopsy. These mice were recorded as negative when any palpable tumour nodes had disappeared. (3) In some cases, the regression of the palpable local tumour was followed by the development of generalized leukaemia. These mice became emaciated and ultimately died showing massive generalized lymphadenopathy. In these mice, the tumours were recorded as takes throughout the experiment.

Within 30-60 min after the inoculation of the tumour cells, 0.1 ml. of B. pertussis vaccine (Statens Bakteriologiska Laboratorium, No. 295) was injected intraperitoneally into the mice of the experimental groups. vaccine contained 20 × 10° bacteria/ml. of saline to which 0.01 per cent merthiolate had been added. Control mice were injected with 0.01 per cent merthiclate in 0.1 ml. of

In analogous experiments, the following tumours were also inoculated into isogeneic hosts: the Moloney lymphoma lines YAC-IS (into A/Sn mice), YBA, YBB and YBC (into CBA), the methylcholanthrene-induced sarcoma MSB (into $[A.BY \times A/Sn]$ F_1 hybrids) and the polyoma tumour line SEWA (into A.SW/Sn/Kl mice). With the latter, cells were obtained from a tumour maintained by subcutaneous transplantations for thirteen generations in A.SW/Sn/Kl mice. The polyoma tumour was cut into small pieces and incubated with 0.5 per cent trypsin for 20 min at room temperature with continuous stirring. The cell suspension was then washed with BSS and adjusted to a concentration of forty viable cells/0-1 ml., the dose which was injected subcutaneously into the A.SW/Sn/Kl recipients. One group received B. pertussic vaccine intraperitoneally as in the experiment with YLI. whereas to the second experimental group 1 mg/kg of crude extract from B. pertussis culture broth was administered by the same route. Tumour development was followed as described for the YLI lymphomas.

It can be seen from Table 1 that the administration of B. pertussis vaccine concomitantly with the inoculation of 104-106 YLI cells led to an increased incidence of turnour This increase was most pronounced about 2-3 weeks after implantation of the turnour. Subsequently, in the mice transplanted with 105 cells, the difference tended to decrease as a consequence of the more delayed occurrence of tumour in the controls and the higher rate of complete regressions in the group treated with B. pertussis. As to the final outcome, the difference was most marked after the inoculation of 106 YLI cells (8/15 takes versus 0/14 in the control group). If the cumulative incidence of tumours was considered, the difference was statistically significant in the groups inoculated with 104, 105 and 105 YLI cells. Initially, the average tumour diameters were smaller in the control groups, but the final survival time of the mice bearing tumours was not significantly different between experimental and control groups. In exploratory

Table 1. EFFECT OF B. pertussis on the incidence of YLI LYMPHOMA AFTER THE TRANSPLANTATION OF VARIOUS CELL DOSES

No. of tumour cells transplanted	Treatment (No. of mice)	Tum comple 12	our ir ete reg aft 15	ressio	ce (No ns) at nsplai 24	the f	ollowi	ing in	lo. of tervals	Days on which death occurred	Cumulative tumour incidence	No. of complete regressions
104	Controls (20)	0	0	7	4	.1	4	4	4	68, 86, 94+, 108+	Q	á
	B. pertussis (20)	1	5*	12	8	8	8	8	ŝ	55, 61, 70, 71, 74, 82, 82, 92+	17*	9
106	Controls (21)	1	2	5	6	6	7	7	7	44+, 49, 55, 68, 75+, 76, 76	10	9
	B. pertussis (21)	4	12†	16†	10	10	10	11	11	40, 48, 49, 53+, 55+, 56, 56, 58, 61, 68, 86	19*	2
10*	Controls (14)	4	1	0	0	0	0	0	Õ	10, 10, 10, 00 , 00 , 00, 00, 00, 01, 00, 00	4	4
	B. pertussis (15)	7	5	8†	84	7*	7*	8†	8†	43, 49, 50, 59, 61, 71+, 86+, 86+	13 †	ž.
107	Controls (10)	8	6	6	5	5	5	5	5	35, 48+, 57, 65, 70+	207	*/ *
	B. pertussis (9)	7	7	8	8	7	7	7	7	35, 37, 43, 50+, 75+, 82, 90	9	76 19
										* ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' '	**	u.

^{*} Mice dead with generalized leukaemia after regression of the solid tumour (counted as takes throughout the observation period). + P < 0.01.

Table 2. INCIDENCE OF SEWA POLYOMA TUMOURS AFTER INJECTION OF FORTY CELLS

Treatment	Cum	ulative	tumou at		ence (t	akes)	Days of death
(No. of mice)	19	24	28	40	49	68	
Controls (6) B. pertussis (6)	0	0	0	0	0	0 3	48, 57, 64
B. pertussis extract (6)	ŏ	2	2	2	3	3	49, 49, 52

tests with the other tumours listed, B. pertussis vaccine did not cause increased incidence of tumours, except for the SEWA polyoma tumour, where a similar trend as in the YLI lymphoma was observed (Table 2).

It is interesting that in the control groups inoculated with 104 or 105 YLI cells there was a higher incidence of tumour takes than in the group receiving 106 cells. Similar observations have been described with the transplantation of sarcomas induced by methylcholanthrene³. It is tempting to ascribe this phenomenon to the "sneaking through" mechanism, allowing small numbers of tumour cells to escape host defence reactions at a critical time, whereas medium sized inocula (106 cells in our case) would elicit a more powerful and effective immune response. With still larger cell doses (107), a breakthrough of tumour growth, overwhelming host resistance, might account for the high incidence of tumours.

To extend these preliminary observations, it will be necessary to assess the effects of bacterial products of other origin, of their dosage and of the time of application. It is conceivable that the same bacterial product might inhibit or promote tumour growth according to these criteria.

If one considers that the Moloney lymphomas in C57L/KL mice show the greatest immunosensitivity both in vivo and in vitro among the Moloney lymphomas tested 10 and also that the SEWA tumour has a relatively strong immunosensitivity11, it would seem that the observed effect occurs only if an immune response of some strength is involved. As to its mechanism, one possibility is the enhancement of tumour growth. The adjuvant effect of the pertussis vaccine on humoral antibodies might lead to their unusually early formation. assumption is unlikely, however, in the light of the high sensitivity of the YLI tumour to humoral cytotoxic action (personal communication from G. Klein). Alternatively, the transient inhibition of phagocytosis occurring after endotoxin administration¹², or, more likely, antigenic competition or "preoccupation" of some component of the immune response, ought to be considered. importance of timing and dosage is also emphasized by an observation made in another, but probably related context. If mice are treated with B. pertussis vaccine or S. typhosa lipopolysaccharide a few hours before infection with other bacteria, their susceptibility to infection is heightened, while pre-treatment at a longer interval increases resist-Furthermore, fractions of M. tuberculosis in certain doses can accelerate the appearance of isogeneic tumours if administered even some weeks before their transplantation. Thus micro-organisms, including bacteria, bacterial substances and viral agents14, may temporarily depress the immune response by a non-specific mechanism the nature of which is unknown. Perhaps it could be that a selective advantage would be conferred on micro-organisms by an inherent immunosuppressive capacity of some degree.

This is additional evidence for the inhibitory effect of B. pertussis vaccine on the outcome of a cellular mediated immune response. For practical purposes, the observation that states related to the presence of vaccines or infective agents can, in certain conditions, facilitate the growth of relatively small numbers of malignant cells clearly deserves further investigation.

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Sequential Biochemical Alteration to Antifolate Resistance in L1210 Leukaemia

Antifolate resistant, transplantable, murine tumours are most often characterized by a single change involving either the transport of drug¹ or the level of dihydrofolate reductase²⁻⁵. This is understandable if one accepts the idea that multiple genetic events leading to an involvement of more than one physiological site rarely occur simultaneously. In two cases, where both physiological properties were altered^{6,7}, the development of resistance probably occurred in a step-wise manner⁸. demonstrated this type of sequential alteration in resistance at both the physiological and cytogenetic level during repeated transplantation of a subline of L1210 leukaemia.

An antifolate resistant subline of the parental line V was selected by transplantation in $BD\hat{F_1}$ mice during combination chemotherapy with amethopterin (0.75 mg/kg), 6 mercaptopurine (6.25 mg/kg) and 5-fluorouracil (10 mg/kg). Although this line, L1210/A/MP/FU (XVI₄), was resistant to all three drugs within several transfer generations^{5,10}, maintenance under the same chemotherapeutic conditions resulted in a population shift at some time between transfer generation 153 and 191 to a cell type lacking the subtelocentric (ST) marker chromo-Preservation of frozen ascites at different transfer generations enabled us to reinitiate the subline at generation 153 and to examine it concurrently at both karyotypic stages for 3H-amethopterin transport and dihydrofolate reductase activity. Cells were collected on the sixth day after transplantation for karyotype analyses10 and on the seventh day for measurements of 3H-amethopterin uptake 12 and the preparation of enzyme extracts 12 . The determination for dihydrofolate reductase activity was described elsewhere13. For measurements of 3H-amethopterin uptake, 2×107 cells in modified Eagle's minimal medium¹⁴ (pH 7·5) were incubated with ³H-amethopterin (1.35 mc./mg) at both 0° C and 37° C. Methods used for the removal of external radioactivity by washing, the extraction of labelled drug and the counting of radioactivity have been described 15. Results are summarized in Table 1.

The level of dihydrofolate reductase in cells of subline $\mathrm{XVI}_4\left(ST^+
ight)$ at transfer generation 153 was like that of the parental line V. Following a shift in the population to the ST- cell type, the enzyme level rose twelve times. This increase was similar to that reported earlier^{5,10} for a related ST- subline. The rise in enzyme level in the altered subline XVI, was verified by titration for enzyme

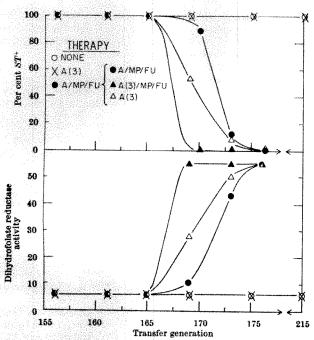


Fig. 1. L1210 leukaemia cell population change $(ST^+ \rightarrow ST^-)$ during transplantation of subline XVI, and the effect on the dihydrofolate reductase level. The subline was reinitiated at generation 153 and chemotherapy begun at generation 154; A, amethopterin, 0.75 mg/kg; A (3), amethopterin, 3 mg/kg; MP, 6-mercaptopurine, 6-25 mg/kg; PU, 5-fluorouracil, 10 mg/kg; the subline exposed to the A/MP/FU combination was divided into three branches at generation 165 and the three forms of therapy begun at generation 165 forms of therapy begun at generation 165. three forms of therapy begun at generation 166

in each of the three preparations based on the binding of amethopterin 16. The uptake of 3H-amethopterin by L1210 leukaemia cells occurs principally by active transport. An experimental description of this system will be published elsewhere12. The rate of uptake of 3H-amethopterin at 37° C by cells of all three lines was linear for at least 15 min. Cells of the unaltered subline XVI, (ST^+) transported 3H-amethopterin at a rate four times lower than cells of the parental line V. Following alteration of the line $(ST^+ \rightarrow ST^-)$, however, the rate of transport was normal. Lineweaver and Burk's¹⁷ analysis of 3H -amethopter in transport demonstrated Michaelis-Menten kinetics for all three systems. The K_m for the system in both ST^+ and $ST^$ resistant cells was three times greater than that calculated for the system in the parental cells. This is taken as evidence for a reduced binding affinity of some carrier component and explains the lower rate of transport in the ST+ cell. Because the same reduction in binding affinity was also characteristic of the carrier in the ST cell, we conclude that the system in this cell type was similarly altered (that is, the impairment is unrelated to the presence or absence of the ST chromosome). The normal rate of transport in this line was probably caused indirectly by the binding of more drug by an increased level of dihydrofolate reductase. The selective advantage determined by the increase in dihydrofolate reductase was thus diminished by an "indirect" restoration of a prior impairment.

Table 1. PROPERTIES OF DRUG SENSITIVE AND RESISTANT LINES OF L1210

		DRUMAN	MIA		
Cell	sT	*H-ametl	nopterin		rofolate ictase
line	chromosome*	trans	port	Specific	Drug bind.
		Rate	Km‡	activity§	ing sites¶
V	+ 4	1.55	0.43	1.43	2.2
XVI.		0-39	1.30	1.38	2.3
XVI		1-50	1.25	15.52	26.7

*Subtelocentric chromosome present (+) or absent (-) in 100 per cent of cells counted.

† Uptake of *H-amethopterin at 37° C, less uptake at 0° C, at a concentration of 0.22 × 10° molar; rate = moles/min/mg (dry weight).

‡ Michaells constant in moles/l. The rate of uptake was determined over a concentration range of 0.44 × 10 * molar.

‡ mp moles dihydrofolate reduced/min/mg protein at 25° C.

¶ Moles of amethopterin bound/mg protein × 10-13.

The population shift ($ST^+ \rightarrow ST^-$) during transplat of subline XVI, and the subsequent effect on the di folate reductase level were followed in variable (therapeutic conditions after reinitiating the subl vivo from ascites frozen at transfer generation 153. are given in Fig. 1. The percentage of ST^+ cells dec ten times every eight transfer generations in the e A/MP/FU combination. No ST^+ cells were obser generation 175. An increase in the enzyme concent occurred in a corresponding manner. Selection ST- cell type in this line could have occurred transfer generation 100, for the frequency of ST (assuming the same rate of increase in the percent ST- cells each generation) at that generation wo about 1/million cells, the standard transplar inoculum. This agrees with data derived from the port experiments, which show that the SToriginated after the initial selection for a cell type defective transport mechanism. The absence of a population shift in the presence of amethopterin (3: alone suggests that the presence of all three drug specific requirement for the selection of the ST- var this subline (Fig. 1). Selection of this variant by the treatment, however, initiated at a later generatio the more rapid selection in the same combination a higher concentration of amethopterin also indica individual role for this drug in the selection process data add emphasis to the idea that leukaemic cell p tion shifts within the host, mediated by a specific e therapeutic regimen, are essentially genetic pheno

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BIOLOGY

More Humane Way with Seals

From July 12 to July 27, 1967, I visited St. Paul I Pribilof Islands, Alaska, USA, to observe part annual harvest of northern fur seals, Callorhinus un and to evaluate the killing methods which were

The outdoor temperature there in mid July is about 50° F. I watched the herding and driving of non-breeding male fur seals from their hauling grounds on the beaches to the fields behind the beaches where they are killed. distance over which the animals were driven varied from a few hundred yards to half a mile or so. I observed the killing of 3 and 4 yr old male fur seals with hardwood clubs 155 cm long which weighed 2.2 kg. These clubs were wielded by five men, known as "clubbers", who were the most experienced and most highly paid members of the sealing crew. Seals in groups or "pods" of about ten would be driven from the chief herd towards the clubbers, who accurately clubbed each seal once on the top of the head. Occasionally, if the blow was not apparently accurate, the seal was hit on the head a further blow or blows. The clubbed seals were then laid in a row and a man known as a "sticker" would carefully incise the skin in the mid-ventral line to the mid-sternal region and thrust the knife through the costochondral junction into the thorax, aiming at the heart. The seal would then be skinned by another group of men.

I conducted post-mortem examinations on a sample of 1,121 fur seals treated in this manner. This sample was taken from a total number of 16,578 fur seals killed during my 2 week stay on the island. The method of examination was first to examine the skulls for signs of gross fracture and bruising and then to examine the thorax to determine the extent of haemorrhage into the pleural and pericardial cavities, and to determine whether or not the myocardium was punctured. The lungs of 418 animals were examined to determine the presence or absence of gross

emphysema.

From the accompanying table of results, it can be seen that a total of twenty-one or 1.9 per cent of the skulls which I examined showed no fracture of the cranium, and thirty-eight or 3.4 per cent of hearts were not punctured. The thorax of every fur seal carcass which I examined, however, had been opened, and this would lead very rapidly to death from respiratory failure, had the animal not been killed by either the blow to the head, or by cardiac puncture. I found no evidence that any fur seal

had been skinned while still alive.

Comparing these observations with similar observations on the Harp Seal hunt in the Gulf of St. Lawrence¹ it can be seen that whereas 1.9 per cent of 1,121 crania examined on the Pribilof Islands were found to be unfractured, 36 per cent of 154 crania examined in the Gulf of St. Lawrence were found to be unfractured. There seem to be several factors contributing to this difference. In the Pribilof Islands, sealing takes place on land, at temperatures of about 50° F. The men clubbing the seals are a small group of experienced men working under close supervision, supported by a team of workers who complete the operation. The sealers work only a few hours each day (from 2 to 6 h), have frequent breaks for refreshment, and are salaried employees. Sealing takes place over a period of about 6 weeks, and of the order of 60,000 seals are taken annually. In contrast, in the Gulf of St. Lawrence, sealing takes place on broken sea ice at temperatures below freezing. The Gulf seal hunters are untrained in the killing of animals and each man both kills and skins the harp seal pups. The annual quota of 50,000 is taken in 3 to 5 days, during which time about 600 sealers work independently with virtually no supervision. The large number of men working over a large area of broken ice presents a formidable barrier to effective control. The sealers work from 6 a.m. to 6 p.m. every day during the hunt and are paid by the piece.

In my opinion, the striking contrast between these two sealing operations, involving the northern fur seal on the

Pribilof Islands and the harp seal in the Gulf of St. Lawrence, indicates that as long as the present structure of exploitation of the harp seal in the Gulf of St. Lawrence remains, humane killing does not seem likely.

The expedition to the Pribilof Islands was financed by the World Federation for the protection of animals. thank the US Bureau of Commercial Fisheries, Department of the Interior, for assistance. Mr P. R. Simpson arranged the expedition and assisted at the post-mortem examinations.

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Oxidation of Sulphonic Compounds by Aquatic Bacteria isolated from Rivers of the Amazon Region

THE sulphonic group has been found as a component of aliphatic compounds in plants and algae1-5. The metabolic fate of sulphonic compounds in micro-organisms has been so far the only available means of studying their transformation in nature^{6,7}. As well as the importance of this information for a general understanding of the biochemical pathways of living organisms, there is also the question of the increasing use of sulphonic detergents and their release in sewages and soils. Their biodegradation depends on the ability of micro-organisms of soil and water to oxidize them. Previous work has shown that soil and water poor in vegetable residues, such as that near big cities like Rio de Janeiro, contain very few bacterial strains able to carry out this degradation. Only a few such bacteria were found in soil samples, and no bacteria able to oxidize sulphonic compounds were found in water samples7.

The rivers in the Amazon region are very rich in materials of vegetable origin, and so we have looked for these bacteria and describe our results here.

Samples were collected from the Negro River, the Amazonas River, the Guamá River and the Paracauaú River. 3-Sulpho-1,2-propanediol (SPD) was selected as a representative of a sulphonic vegetable compound. The barium salt, prepared by M. Miyano, was transformed to the sodium salt and used throughout the experiments. SPD labelled with sulphur-35 was prepared by A. A. Benson from the iodine-1,2-propanediol and monobasic potassium sulphite labelled with sulphur-35. Some synthetic sulphonic compounds were also tested; these were N-tris(hydroxymethyl)l-methyl-2-aminoethanesulphonic acid (TES); N-(2-sulphoethyl)-morpholine (MES); N-(3sulphopropyl)-morpholine (MOPS); N-(2-hydroxyethyl)-, N'-(2-sulphoethyl)-piperazine (HEPES); and N-(2-hydroxyethyl)-,N'-(3-sulphopropyl)-piperazine (HEPPS).

For the bacteria we used the enrichment culture techniques, followed by the isolation and classification of the bacterial strains by classical procedures. The bacteria were tested for their ability to grow aerobically at 30° C, on the specific substrate as the only source of earbon and sulphur. In some experiments, the synthetic compounds were also assayed as the only source of nitrogen, as well as the only source of carbon and sulphur. In one set of experiments, $1.0 \mu g$ of vitamin B_{12} was added to each 1 ml. of medium. Growth was measured by the relative increase in cell volume. Non-proliferating adapted cells, obtained by centrifugation during the exponential growth phase, were washed three times with 0.02 molar trishydrochloric acid buffer (pH 7.4) and resuspended in the same buffer. The radioactivity was measured in a Nuclear Chicago scintillation counter.

Bacterial strains growing on SPD were isolated from all the samples tested, when the cultures were kept at

Number of seals taken between July 14 and July 26, 1967 Number of seals examined at post mortem 16,578 1.121

Number of unfractured crania 21 (1.9%)

Number of hearts not punctured 38 (3.4%)

Table 1. DISTRIBUTION OF METABOLIC PRODUCTS FROM 34S-SULPHOPROPAN-

Fractions	$c.p.m. \times 10^8$	Per cent
Initial activity	2,400	100
Supernatant solution	580 1.520	24 63
Cells Alcoholic extract	1,520 55	2

30° C. At 24° C they do not grow on the selective medium after a week of incubation. The strain was tentatively classified as a species of Pseudomonas sp. and was used to oxidize labelled SPD in non-proliferating conditions. The reaction mixture contained 2 mg of cells suspended in 1.0 ml. of 0.02 molar tris hydrochloric acid buffer (pH 7.4), 20 µmoles and 2,400×103 c.p.m. of labelled SPD, and distilled water to 2.0 ml. After 4 h of incubation at 37° C and aeration, the cells were centrifuged, washed and extracted with hot 80 per cent ethanol. The radioactivity was counted in the whole cells, in the supernatant and in the alcoholic extract. The results are presented in Table 1.

The distribution of radioactivity showed that the sulphur from the SDP was accumulated chiefly in the in-

soluble fraction where proteins are found.

Two dimensional paper chromatography was carried out with the alcoholic extract on Whatman No. 1 paper, in phenol-water (100:40 w/w) and n-butanol-propionic acid-water (142:71:100, v/v). Three radioactive spots were detected: one, at the origin, was identified as free sulphate by paper electrophoresis⁶; the second was identified as SPD by co-chromatography with the authentic standard; the unknown spot is probably an intermediate and is not yet identified.

The synthetic compounds MES, MOPS, HEPES and HEPPS were able to support the growth of the species of Pseudomonas isolated from the SPD medium after 48 h of incubation at 30° C as long as there was a source of nitrogen. When the medium lacked this source there was no significant growth. The compound TES was a better source of carbon and sulphur than the others, for there was full growth after 18 h of incubation.

Addition of vitamin B₁₂ did not affect the utilization of the assayed compounds in any way. The blanks with the mineral medium and the vitamin did not show any growth.

I conclude that in the microbiological flora of waters enriched by vegetable residues, as in the rivers of the Amazon region, there are bacterial strains able to control the induction of the enzymes which catalyse the oxidation of sulphonic compounds. These bacteria allow natural sulphonic compounds, as well as synthetic ones, to participate in the natural cycle of the elements, releasing their carbon and sulphur in chemical forms available for utilization in other living processes.

This work was undertaken when I took part in the Amazon expedition on the RV Alpha Helix, operated by the Scripps Institution of Oceanography, University of California, San Diego, and was supported by grants from the US National Science Foundation and the National Research Council of Brazil. I thank Dr Andrew A. Benson, who encouraged this work, and Dr M. L. Ibanez for

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Is an Ectoproct Possible?

Mangum and Schopf1 claim on the basis of their calculations that the diffusion of oxygen into the body of an ectoproct is insufficient to supply the animal with its respiratory needs. They conclude that the periodic contraction of an ectoproet circulates its coelomic fluid. I believe their calculations are incorrect and that diffusion of oxygen into the ectoprocts is sufficient to supply their needs.

Their calculations are based on a formula from Gerard* for calculating the amount of oxygen which can diffuse into cylindrical tissue, the tentacles and the tentacle sheath. Gerard made a mistake in deriving his formula in that he used $A\pi r^2$ to represent the amount of oxygen required by a unit length of cylinder of radius r where A is the oxygen consumption of unit weight and time. To be correct, either the density of the tissue must appear in the formula or the oxygen consumption must be in terms of unit volume and time rather than weight and time. The complete formula takes the form

$$C = \frac{Ar^2}{4\overline{D}}$$

where C is oxygen concentration at the external surface. A is the rate of oxygen consumption and D is the diffusion coefficient for oxygen. This is dimensionally correct when C is ml./ml., A is ml./ml. sec, r is cm and D is cm²/sec. The density of tissue is very close to one, and so the error introduced by using oxygen consumption on a wet weight basis rather than a volume basis is usually insignificant. Unfortunately, among the papers on oxygen diffusion into cells, Fenn³, Gerard², Krogh⁴ and Harvey⁵, only Harvey makes explicit the assumption of a density of one.

Mangum and Schopf express C in atm.; A in mm³/mg dry weight min, r in cm and D in atm./cm/cm². Using these units it is impossible to make any kind of sense out of the

equation because it reduces to atm. =
$$\frac{\text{min /oss}}{\text{atm./mg/min}}$$

Part of the difficulty lies in the units assigned to D which in modern literature usually represents the diffusion Mangum and Schopf are using something coefficient. often called the permeability coefficient, the diffusion Instead of coefficient multiplied by the solubility. atm./cm/cm² as they state, their number has the dimensions cm²/atm./min. The dimensions are clear in Krogh from whom they obtained their numbers. Unfortunately, Krogh6 later used atm./cm/cm2 as if it were the units of permeability although it actually refers to the conditions of the measurement: the flux measured through an area of 1 cm² with a gradient of 1 atm./cm.

When the calculations of oxygen diffusion into the ectoproct are made on the correct volume specific basis the results are about 100 times larger than those calculated by Mangum and Schopf for the tentacles and sheath. Even their results, however, are large enough to account for the oxygen consumption of those parts of the animal entirely on the basis of supply by diffusion.

The critical part of the animal is the zoecium which they maintain needs 300 times more oxygen than can be supplied by diffusion. For the calculation they use the formula

$$C = \frac{Ad}{D}$$

in which d is the depth of the zoecium in cm and the other symbols are as before. With the correct units for permeability, D, the equation gives $C \text{ cm}^3/\text{cm}^2 \text{ min which is}$ equivalent to mm³/mm² min. Mangum and Schopf called their results mm³/mg h, with the consequence that, when the value for the zoecium as a unit was calculated, their value was 1,000 times too small. The dry weight of the zoecium was about 10-3 mg and the frontal area through which diffusion occurs was about 1 mm2.

The correct values are given in Table 1 and compared with the results of Mangum and Schopf and with their values for the oxygen consumption of the animals. (The correct values are calculated with an oxygen pressure of 0.21 atm. which corresponds to that in water in equilibrium with air, not the value of 0.29 used by Mangum and Schopf which corresponds to considerably oversaturated water. It should also be noted that this is not a measure of oxygen concentration as they state, but of the pressure which together with the solubility determines the concentration.)

Table 1. SUMMARY OF CALCULATIONS OF OXYGEN DIFFUSION IN A SINGLE ECTOPROCT

Part of animal	Oxygen supply by diffusion Calculation of Mangum and Schopf (mm²/h×10-²)	My calculation mm³/h × 10-2	Observed oxygen uptake from Mangum and Schopf mm³/h × 10-2
Lophophore	23·9	280	0.026
Tentacle sheath	0·3	3·6	0.029
Zoecium	0·004	2·0	0.37

These data are after Mangum and Schopf, who called attention to the misprint in the original table for their calculation for the zoecium.

It can be seen from Table I that the zoecium can be supplied with oxygen by diffusion with only a 20 per cent reduction in partial pressure of oxygen at the back wall, the point farthest from the water. There is no reason to assume circulation of the fluid within the body is necessary to satisfy the oxygen needs of ectoprocts.

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CYTOLOGY

Nuclear Pockets in Normal Monocytes

NUCLEAR pockets (or nuclear blebs, nuclear loops) have been described in leukaemic cells in different treated and untreated leukaemias1-4, and their specificity for leukaemic

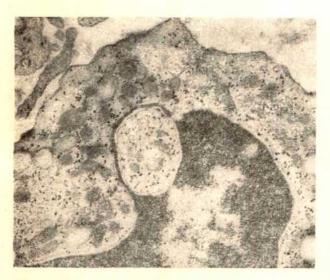


Fig. 1. Nuclear pocket in neutrophil leucocyte of peripheral blood



Fig. 2. Nuclear pocket in normal monocyte of peripheral blood (×16,000).

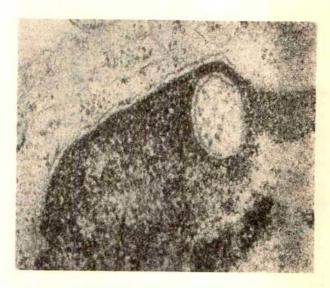


Fig. 3. Different form of nuclear pocket in a normal monocyte of peripheral blood (×70,400).

cells has been discussed. They are similar to the material joining nuclear lobes of segmented granulocytes. Smith⁵ has demonstrated nuclear pockets in non-leukaemic lymphocytes.

We have frequently found nuclear pockets in lymphocytes, neutrophils (Fig. 1) and also in monocytes (Figs. 2 and 3) of healthy subjects. Nuclear pockets can therefore be considered not to be specific for leukaemic cells. They seem to be a physiological structure in all forms of leucocytes. Nuclear pockets may have the function of enlarging the surface of the nucleus-they are not necessarily a malformation of the nuclear envelope.

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MICROBIOLOGY

Acetylene Reduction by Nitrogen Fixing Extracts of Clostridium pasteurianum: ATP Requirement and Inhibition by ADP

NITROGEN fixing extracts of Clostridium pasteurianum have been shown to catalyse the reduction of acetylene1.2. The requirements for this reaction were shown to be the same as those for nitrogen fixation, that is, a low potential electron source, ATP, an ATP generating system and magnesium ions. Acetylene reduction provides a more sensitive assay for the activity of the nitrogen fixing system than ammonium production and the measurement of rates of reactions is facilitated. The results described in this communication show that the reduction of acetylene by these extracts can be supported directly by ATP without an ATP generating system. When an ATP generating system is not used we now find that (a) magnesium ions are required for ATP utilization by the nitrogen fixing system, a fact not previously demonstrated, for magnesium ions were required for the ATPgenerating system; that (b) the acetylene reduction by the nitrogen fixing system is inhibited by ADP in a manner which suggests that ADP is a negative modifier; and that (c) the acetylene reducing system needs to interact with more than one molecule of ATP.

C. pastcurianum was grown in a nitrogen-free medium under nitrogen and extracts were prepared from dried cells in 0.05 molar tris hydrochloric acid buffer (pH 8)3; partially purified molybdoferredoxin and azoferredoxin were prepared by the method of Mortenson, Morris and Jeng. The rate of reduction of acetylene to ethylene was determined by gas chromatography using a 72 cm column of 1.5 mm diameter containing 'Poropak R' (Varian Aerograph Co., California). Nitrogen was used as carrier gas at room temperature using a hydrogen flame ionization detector. The reaction was carried out at 30°C in 10 ml. serum bottles and gas samples were taken using a gas-tight syringe. The reaction mixture (a total volume of 2 ml.) contained 115 µmole potassium cacodylate (pH 6-8), the appropriate amount of MgCl, and ATP, and 0.5 ml. extract (14-18 mg protein). When partially purified components were used the extract was replaced by 0.15 ml. molybdoferredoxin (1.5-2 mg protein) and 0-15 ml. azoferredoxin (1-1.5 mg protein). The final pH was 6.8. When an ATP-generating system was used, 55 μmole per ml. of acetyl phosphate was added. ATP:

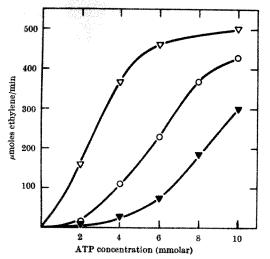


Fig. 1. Effect of ATP concentration on the rate of acetylene reduction catalysed by molyhdoferredoxin and azoferredoxin at different levels of ADP.

∇, ATP alone; O, ATP + ADP (3 mmolar); ▼, ATP + ADP (5 mmolar).

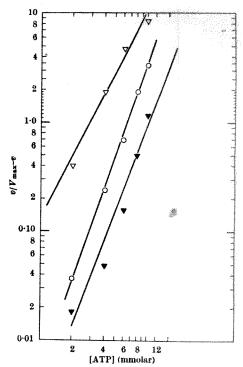


Fig. 2. A plot of log ATP concentration against log $(v_i V_{max}, v)$ at different levels of ADP when molybdoferredoxin and azoferredoxin were used to catalyse the reduction of actylene to ethylene in the presence of sodium dithionite. ∇ , ATP alone; \bigcirc , ATP + ADP (3 mmolar); \bigvee , ATP + ADP (5 mmolar).

acetate phosphotransferase was already present in the extract used. The gas phase was 0.85 atm. hydrogen and 0.15 atm. acetylene.

Early in this work when crude extracts were used, we found that ATP would support acetylene reduction in the absence of an ATP generating system. Neither ADP nor GTP would function in place of ATP. With the generating system the optimum ratio of magnesium ions to ATP was 2.5:1, but with ATP alone the optimum ratio of added magnesium ions (9 mmolar) to ATP (18 mmolar) was 0.5. The 0.5 ml. of crude extract used contained up to $2.4 \mu \text{mole}$ of magnesium ions (1.2 mmolar in the reaction mixture), all of which appeared to be bound to proteins for no activity was observed unless magnesium ions were added. The optimum ratio of magnesium ions to ATP when partially purified molybdoferredoxin and azoferredoxin were used was 0.5. At this ratio the initial rate of acetylene reduction catalysed by partially purified molybdoferredoxin and azoferredoxin (using sedium dithionite as an electron donor) was affected by the concentration of ATP (Fig. 1). A Hill plot** of log $(v/V_{\rm max}-v)$ as a function of log ATP concentration gave a straight line with a slope of about 1.9 (Fig. 2) suggesting that two molecules of ATP participate in the rate determining step or steps of the reaction catalysed by the acetylene reducing system. When crude extracts were used in these experiments in conditions where ATP was hydrolysed at a fast rate to ADP, slopes higher than 2 were obtained. These results were caused by ADP inhibition which will be shown later in this communication.

When a substrate quantity of ATP (18 mmolar) was used in place of small amounts of ATP (0.2 mmolar) and an ATP generating system, crude nitrogen fixing extracts catalysed a linear rate of reduction of acetylene, but the reaction stopped after 10-15 min although ATP was still

Table 1. EFFECT OF ADP AND AMP ON THE RATE OF ACETYLENE REDUCTION BY EXTRACTS FROM Clostridium pasteurianum

Addition	Percentage inhibiti
ADP, 3 mmolar	42
ADP, 5 ,,	53
AMP, 5	51

ATP concentration was 18 mmolar.

present. In contrast, using the ATP generating system the reaction continued linearly until all the ATP generator was consumed. ATP at the concentration used (18 mmolar) was not itself the cause of the decrease in reaction rate, for it was found that substrate concentrations of ATP in the presence of ATP generating system resulted in acetylene reduction at a rate close to that obtained when catalytic amounts of ATP and the ATP generating system were used.

The early decline in the rate of acetylene reduction when substrate quantities of ATP were used was simultaneous with the rapid disappearance of ATP even in the absence of acetylene, and this suggested that a product or products of ATP utilization were inhibiting the reaction. This was further indicated by experiments in which the crude extract was incubated in the presence of magnesium ions and ATP for 20 min before the acetylene was added. In these conditions, no reduction of acetylene occurred unless additional ATP or ATP and acetyl phosphate were added. When, however, ATP was added after the 20 min preincubation with ATP, the rate of acetylene reduction was much less than if ATP has been added initially.

The products of ATP utilization, ADP and AMP (unpublished results of Kennedy and Mortenson), produced by crude extracts during reduction of acetylene were shown to inhibit acetylene reduction (Table 1). When partially purified molybdoferredoxin and azoferredoxin4 substituted for the crude extract and sodium dithionite was used as the electron source in place of reduced ferredoxin, ADP but not AMP inhibited acetylene reduction. The result with AMP suggests that the inhibition of acetylene reduction by AMP when crude extracts were used was probably caused by the presence of adeny-

late kinase.

The effect of the concentration of ATP on the rate of acetylene reduction catalysed by partially purified molybdoferredoxin and azoferredoxin4 with dithionite as an electron source was examined at 3 and 5 mmolar ADP. The results were plotted as log $(v/V_{\text{max}}-v)$ against log ATP concentration 5,6 (Fig. 2) and the slope of the straight line obtained with both 3 and 5 mmolar ADP was about 3. With 5 mmolar ADP the line was shifted to the right which suggested that ADP, the product of ATP utilization in the nitrogen fixing system (unpublished results of Kennedy and Mortenson), may regulate the acetylene reducing system by functioning as a negative modifier. When the ratio of ATP to ADP is high as shown here with an ATP generating system, no ADP accumulates and acetylene reduction is optimal. When the ratio of ATP to ADP is low, acetylene reduction (nitrogen fixation) is inhibited and the ATP remaining can be used for more critical cell functions.

These studies were carried out using acetylene reduction as a measure of nitrogen fixation because of the ease and reliability of measurement of ethylene. This seems to be valid because all data indicate that the mechanisms of reduction of nitrogen and acetylene are similar1,2.

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Inhibition of Multiplication of Foot and Mouth Disease Virus in Adult Mice pretreated with Freund's Complete **Adjuvant**

Increased resistance has been reported in certain virus infections after stimulation of the reticulo-endothelial system (RES)1,2. We have observed that, in adult mice pretreated with complete Freund's adjuvant3, the multiplication of foot and mouth disease virus (FMDV) is inhibited4, and we report here the results of further

experiments.

Two attenuated strains of FMDV, type "O", were used. One was lethal and the other non-lethal to adult mice. The non-lethal strain induces a symptomless but regular virus multiplication in the pancreas, accompanied by viraemia, after intraperitoneal inoculation of a hundred suckling mice half lethal doses $(smLD_{50})$ (ref. 5). All suckling and adult mice were from an inbred colony of the Balb/c strain, susceptible to experimental infection with FMDV. Suckling mice were used for virus titrations, serum neutralization tests and for detection of viraemia. Adult male mice, 8 weeks old, were used for comparative study of virus multiplication. Freund's complete adjuvant containing standard amounts of desiccated cultures of Mycobacterium tuberculosis (5 mg/20 ml. of adjuvant) was prepared by the classical method3. Pretreatment with adjuvant consisted of intradermal injections at four sites, giving a total of 0.3 ml. to each mouse. The first set of injections were given 10 days and the second set 3 days before inoculation of virus. Bordetella pertussis, when used to induce the adjuvant effect, was in the form of formolized vaccine, available from the Institut Pasteur, In each experiment, thirty adult mice in ten groups served as controls and an equal number in the group pretreated with adjuvant. Virus multiplication was studied comparatively by assaying virus titres in the pancreas and testing for viraemia. For virus assay, at each post-inoculation time, mice from the control and pretreated groups were killed at intervals for virus assay. To test for viraemia all mice were bled from the orbital vein. The blood, diluted 1:10 in Earle's tissue culture medium without anti-coagulant, was tested immediately. Serum virus neutralization tests were performed on pooled samples of sera from control and pretreated mice, keeping the quantity of virus constant. The titrations of the interferon-like substance were carried out in an L cell-vesicular stomatitis virus system. The interferonlike substance is referred to as interferon in the text chiefly because of two characters: this virus inhibitor is acido-resistant (exposed to $p \to 2 \cdot 0$ for 72 h) and is active in cells of mouse origin (L cells) while inactive in cells from other species. The interferon titres reported in Fig. 2 are obtained from extracts of spleen and are expressed as reciprocals of the dilution capable of reducing the number of plaques (unit of interferon) as compared with control cultures by 50 per cent6.

Table 1, which summarizes the results of three experiments, shows that 65 per cent of pretreated mice showed no viraemia compared with 100 per cent positive results with controls. The 35 per cent of mice which were positive in the pretreated groups showed lower virus titres in the pancreas than did controls. Furthermore, these titres appeared later and disappeared earlier. With a variable virus inoculum, it was observed that in controls only $50 \ smLD_{50}$ was required to induce viraemia in $50 \ per \ cent$ of mice, while in pretreated mice 700 $smLD_{50}$ was required to induce the same effect. Inhibited virus multiplication was observed even with the lethal strain of virus. In this case, while in controls the LD_{50} for adult miceexpressed in smLD₅₀—was less than 5 smLD₅₀, in mice pretreated with adjuvant it was 300 smLD₅₀. Similar protection was observed in mice which had been pretreated with B. pertussis (twice before the inoculation of virus as in the case of Freund's adjuvant). To test whether M. tuberculosis incorporated into Freund's adjuvant was responsible for inducing the inhibition of virus multiplication, the effect of pretreatment with incomplete Freund's adjuvant was examined. In this case, no virus inhibition was noticed. Wax D (ref. 7) fractions from human strains of M. tuberculosis which contain the peptide moiety' have been shown to be capable of inducing increased immunological response. To test whether some of these fractions could inhibit virus multiplication, mice were pretreated with adjuvants containing waxes $D_{p_{15}}$ and D_s from strain Canetti. Only wax $D_{p_{15}}$, which contains the peptide moiety, could inhibit virus multiplication, while the wax D_s , which has no peptide in it, failed to do so.

Table 1. TITRES OF FOOT AND MOUTH DISEASE VIRUS IN PANCREAS AND PERCENTAGE OF MICE SHOWING VIRAEMIA IN ADJUVANT PRETREATED AND CONTROL MICE

Post-	Control m	ice groups	Adjuvant pretreated mice groups		
inoculation	Virus titres Viraemia		Virus titres	Viraemia per cent	
time (h)	in pancreas	in pancreas per cent			
6	103	0/30 0	10 ^a	0/30 0	
16	10*	4/27 15	10 ²	0/27 0	
24	10***	16/24 65	10 ^s	1/24 5	
36	10***	21/21 100	104	3/21 15	
48	10***	18/18 100	102	6/18 35	
64	104.0	13/15 85	102	0/15 0	
72	10***	10/12 80	$\tilde{10^2}$	0/12 0	
96	102	1/9 10	102	0/9 0	

The titres in pancreas are expressed in suckling mice LD_{50} . Viraemia is shown with the numerator indicating mice which were positive and the denominator shows total mice tested.

The titres below 10^{5} g of pancreatic tissue cannot be detected and so are considered as the lower detection limit.

All the mice were injected intraperitoneally with 100 suckling mice LD_{50} of foot and mouth disease virus at zero time.

The two most important factors responsible for restricting virus multiplication in vivo are antibodies and interferon8. Comparison of antibody concentrations in control and pretreated mice is shown in Fig. 1. Clearly, in the controls antibodies could be detected after the fifth day and reached 2×104 infectious dose neutralizing antibodies (abs)/ml. of serum on the seventh day and continued to show similar titres until the sixth week. In mice pretreated with adjuvant, the antibodies appeared later (by or after 7 days) and, although by the third week they had reached the same titres as in the controls, they disappeared by the tenth week when in the controls there was still a titre of 2×10^3 abs/ml. of serum.

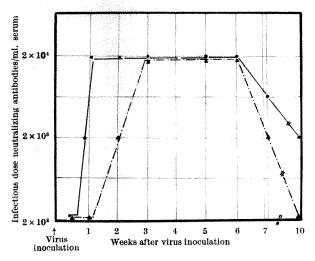


Fig. 1. Comparative antibody concentrations in pretreated and control mice. All mice were injected with 100 suckling mice LD_{50} of foot and mouth disease virus at the same time and samples of sera were tested on pooled material from each group. These serum virus neutralization tests show that antibodies in controls (———) appear earlier and disappear later than those in pretreated mice (\blacktriangle —·— \blacktriangle).

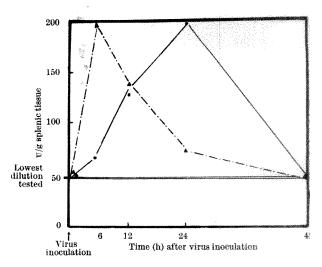


Fig. 2. Comparative concentration of interferon in pretreated and control mice. All mice were injected intraperitoneally with 100 suckling mice LD_{10} of foot and mouth disease virus. In mice pretreated with adjuvant, highest titres are observed at 6 h ($\Delta - \cdot - \cdot \Delta$); in controls ($\bullet - \cdot - \bullet$) these are seen only at 24 h.

The concentrations of interferon in mice treated wif adjuvant and in controls are shown in Fig. 2. No inte feron could be detected before virus had been inoculated either in pretreated or control mice. In pretreated mic highest titres were observed at 6 h with a progressive decline at 12 and 24 h. In controls, the opposite situatic obtained, with the lowest titres at 6 h reaching a max mum by 24 h. Concentrations of interferon decreased i less than the limit of detection in both groups by 48 h.

The experimental evidence indicates that substance with similar actions with respect to Freund's complet adjuvant can induce an effect, as a result of which virt multiplication is inhibited. The low concentrations of ant bodies in the pretreated group preclude the possibility antibodies inhibiting the virus multiplication in the present system. On the contrary, the altered productic of interferon in the two groups seems to be important virus inhibition. These results resemble those of Youngas and Stinebring, who have shown that pretreatment wit BCG results in mice being hyper-reactive to the inte feron stimulating effect of E, coli endotoxin. The absent of interferon at zero hours (at the time of inoculation virus) suggests that interferon is produced under the stimulus of the inoculated virus. In view of the previous observations that it takes at least 4 h to complete or virus multiplication cycle in FMDV, the high titres interferon, as early as 6 h after inoculation of viru followed by a progressive decrease, suggest that, in min pretreated with adjuvant, interferon is produced befor or in the initial stage of virus multiplication while controls it follows the multiplication.

The increased immunological response reported prev ously 10-12 and early production of interferon observe here suggest the possibility that these apparently differen reactions are a manifestation of enhanced functions the same set of cells, mobilized and activated by Freund After such treatment every function of the adiuvant. reticulo-endothelial system can be expected to be stime lated and the answer, evident from the response, depend only on the nature of the foreign substance injectedfor example, antigen-like human serum albumin¹¹ provoks production of antibody while the virus provokes ear production of interferon. It is interesting that there increasing evidence of the role of the reticulo-endothelia system, particularly spleen and macrophages 13-17, the production of interferon.

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GENETICS

Lactose Intolerance in Asians

This investigation was prompted by the clinical observation of lactose intolerance in five Asian students who were attending a gastroenterological out-patient department for vague abdominal pain associated with intermittent diarrhoea. Lactose intolerance is now a well recognized clinical entity, being more common in asymptomatic negroes1,2 and Australian aborigines3 than in Caucasians.

This initial investigation was carried out to determine the incidence of lactose intolerance in a group of asymptomatic Asian students. The control subjects were twelve asymptomatic Australian students of Caucasian background. The test subjects were twenty Asian students. Fifteen were Chinese from Hong Kong, Singapore or Malaysia, and five were from the Indian sub-continent. All were free of symptoms but many on specific questioning admitted to abdominal pain and diarrhoea after ingesting milk. To test for lactose tolerance, 80 g of lactose dissolved in water was given to fasting control and test subjects. Blood samples were taken after 0, 30, 60, 90 and 120 min. Total blood sugar reducing substances were determined by a "ferricyanide" method adapted to the autoanalyser. Any symptoms stated were noted. To test for glucose-galactose tolerance, the same procedure was repeated with both groups after the oral administration of 40 g each of glucose and galactose.

In the control group the mean maximum increase in blood sugar after 80 g of lactose was 34 mg per cent with a range of 20-50 mg per cent. After 40 g of glucose and galactose the mean maximum rise was 43 mg per cent

with a range of 20-105 mg per cent. In Chinese students, the mean maximum increase in blood sugar after 80 g of lactose was 8 mg per cent with a range of 0-25 mg per cent. In the Indian students, the mean maximum rise in blood sugar was 8 mg per cent with a range of 0-30 mg per cent. After 40 g each of glucose and galactose the Chinese students showed a mean maximum increase of 46 mg per cent with a range of 30-65 mg per cent; the Indian students had a mean maximum rise of 32 mg per cent with a range 20-45 mg per cent. These results are set out in Table 1. The mean results of the blood sugar estimations in the different racial groups are shown graphically in Fig. 1.

Table 1. MAXIMUM INCREASE IN BLOOD SUGAR IN CAUCASIANS, CHINESE AND INDIANS AFTER INGESTING LACTOSE, GLUCOSE AND GALACTOSE

		Maximum inci	rease in blood sugar ml. of blood)
Subjects		After 80 g of lactose	After 40 g of glucose and 40 g of galactose
A Controls 1 (Caucasian) 2		30	20
		50	45
(0 00 00 00 00 00 00 00 00 00 00 00 00	3	40	35
	4	35	30
	5	30	105
	6	20	30 45
	7	30	45 30
	in) 2 3 4 5 6 7 8 9	25	50 50
	. 9	35	40
	10	40 35	35
	11	35	50
	12	Mean 34	Mean 43
B Chinese	13	10	
	14	0	50
	15	0	40
	16	25	55
	17	5	30
	18	15	65
	19	10	99
	20	25	50
	21	15	45
	22	9	***
	23	រត្	35
	21 22 23 24 25	9 5	
	20	ň	uniters.
	26 27	ň	
	2.1	5 10 5 5 6 0 0 Mean 8	Mean 46
C Indian	28	0	20
	29	Ö	45
	30	0	35
	31	10	30
	32	30	30
		Mean 8	Mean 32

Abdominal pain and diarrhoea were experienced by patients 5 and 9 of the control group. All patients in the Chinese group and all except patient 5 in the Indian group complained of abdominal pain, borborygmi and diarrhoea.

This study of asymptomatic volunteers demonstrates that intolerance to lactose is common in a Chinese and Indian student population. It is accepted that an increase in blood sugar of less than 20 mg per cent after a loading dose of more than 50 g of lactose is associated with intestinal lactase deficiency4. Figures between 20 and 25 mg are equivocal. All Chinese developed abdominal pain and diarrhoea after lactose ingestion. This was associated with a mean increase in blood sugar of 8 mg per cent compared with a mean increase of 34 mg per cent in the control series. No Chinese experienced any symptoms after the glucose-galactose test and here the mean increase was 46 mg per cent compared with a mean rise of 43 mg per cent in the control series. Four out of five Indians experience abdominal pain and diarrhoea after ingesting lactose and this was associated with a flat lactose curve. The fifth patient (patient 32) experienced no symptoms after ingesting lactose and had a maximum rise of blood sugar of 30 mg per cent.

It is interesting that this patient has been resident in Australia for 13 yr and this in itself would argue that the

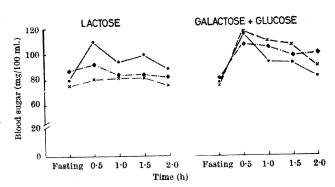


Fig. 1. Mean obtained for blood sugar values in lactose and glucose galactose tolerance tests in Caucasian ($lue{---}$), Chinese ($\times --- \times$) an Indian ($lue{---}$) subjects.

lactose intolerance common in Chinese and Indians may have an acquired rather than a genetic basis. In addition, the incidence of this enzyme deficiency seems to be too

high to be explained by a recurrent mutation.

This work has two implications. First, lactose intolerance should always be considered in Asian patients complaining of abdominal pain and diarrhoea. Second, it has a much wider socio-economic application when the part played by milk and milk products in food aid programmes to under-developed Asian countries is considered. It could be that aid in this form with the subsequent induction of diarrhoea is not the most efficient method of helping a malnourished community.

We thank Dr R. Bartholemew for carrying out the blood sugar estimations. We also thank Professor R. B. Blacket and Professor R. J. Walsh for their encourage-

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APPLIED SCIENCE

Combustion of Coal in Oxygen

DURING an investigation of high temperature flames (at more than 2,500° K), we have developed a burner system capable of burning up to 6 g/sec (50 lb./h) of coal in oxygen to form a stable, continuous flame. An integral part of this development has been a study of the mechanisms which control the rate of combustion.

A detailed account of the burner system is given elsewhere but, essentially, the coal was fed by a screw into a mixing device and then into the burner head. stabilization was achieved within the burner head by means of a water-cooled bluff-body and combustion took place at atmospheric pressure in a zirconia tunnel.

Coal (4 per cent ash, 38 per cent volatile matter) was ground and graded according to size by sieving and centrifugal classification in air to produce seven fractions in an overall size range of 12-70µ. Each fraction contained more than 50 per cent by weight within a 10µ interval. Flame temperatures were measured by the line reversal method which indicated a variation in temperature along the length of the flame from 2,900° to 3,050° K. At this temperature we concluded that the reaction temperature remained sensibly constant along most of the flame

Each size fraction was burned at two ratios of oxygen to carbon: (a) with oxygen just less than stoichiometric for water and carbon dioxide production (assuming no dissociation), that is, a ratio of oxygen to carbon atoms of 2.11; and (b) at a ratio of 1.51. Burning times for the various fractions were compared on the basis of the distances from the burner head necessary for 80 per cent by weight of the coal to be burned. The extent of com-bustion was determined by means of a water-cooled sampling probe in which not less than 50 mg of solid material was collected during a flame immersion time of 30 sec. Burning times were calculated from these measured distances and calculated values of the particle velocities. Calculation of the velocities was based on the assumption that the particle velocity was the same as the gas velocity and the latter was obtained from measured values of the

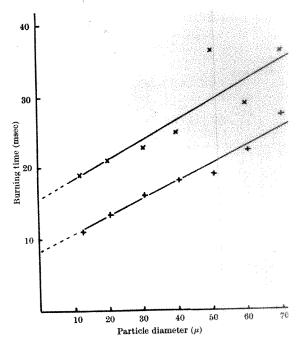


Fig. 1. Effect of particle size on burning times. × , (ratio, 1.51; +, oxygen-carbon ratio, 2.11. Oxygen-ca

mass flow rate of combustion products, flame dimenflame temperatures and the computed composition (products2. The burning time versus particle dia for both oxygen-carbon ratios is plotted in Fig. 1 two conclusions have been drawn from these data.

First, over the particle size range of 12-70μ, the bu times of coal particles in oxygen which give 80 per combustion can be described by the empirical equati

 $t = 2.8 d + 16 \times 10^{-3}$ (oxygen-carbon ratio of 1.51) $t = 2.4 d + 9 \times 10^{-3}$ (oxygen-carbon ratio of 2.11)

where t is the burning time in sec and d is the equi-

spherical particle diameter in cm.

Second, departures from the first approximation linear dependence of burning time on diameter have explained in terms of the type of mechanism exerts the predominant effect on the burning Between 12 and about 30µ the data were satisfax correlated by means of linear relationships, w between 30 and 70µ better correlation was obtained it was assumed that the burning time was propor to the square of the diameter. For single pa greater than 80µ in diameter, it has been demonst that mass transfer processes control the rate of co tion. As the particle size is reduced, a critical si be reached when chemical processes begin to) significant part in determining combustion rates. basis that the time of burning is proportional square of the particle size when mass transfer propredominate and proportional to particle size chemical processes exert the greater influence on th of combustion, it is suggested that, in the conditi these experiments, the critical size lies in the vice 30µ.

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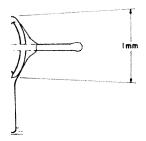
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and linear bearings is ing forces, generated by acting on the moving oted that such capillary noving element, but the being exploited until ause capillary bearings ree and astatic even at rt loads of more than



od is centralized by the drops e to rotate and translate along

sillary bearing, where a centralized by the drop two loops, is shown in , about and to translate slations can be restricted at its ends just touch the

lown in Fig. 1, was used class tube on which was ignetized steel 0.025 mm naking a compass which were filled with water, pump oil. The latter l damping. The compass ts correct position after ghts, other magnets or

ry bearings are possible. of the liquid free surface alizing loads, for, in this ree liquid surface is a nent is central. In the Boys1, Allen2 and Fig. 1 irement seems to be met e liquid free surface is a 1 the capillary lubricated ection of the free liquid th it is indefinitely close

5. 1, lubricant migration, ecause of surface leakage nould not occur because, loop by surface leakage vould require an increase uld increase the pressure the creep. This method s evidently been over-

by transfer in the vapour is sufficient, however, to ner such that the desired hat location where the urface, and therefore a fer by creep and through 78 return the lubricant to this location. A capillary lubricated bearing with this property was constructed and is shown in Fig. 2. The moving element was a tube 0.710 cm outside diameter and 0.710 cm long bored out to leave sharp edges at the ends. It was made of hardened AISI 410 ferritic stainless steel and weighed 270 mg. The displacement of the moving element was 0.280 ml. so that it could be hydrostatically floated by adjusting the liquid density. It ran in a glass tube with a bore of 0.760 cm. The glass tube was filled with a water-isopropanol mixture, which would float the steel ring best, and then sealed. The moving element was free to rotate, and behaved like a compass; it was also free to translate and made a sensitive level. The liquid always returned to the crevice of this bearing and re-centralized the moving element after shocks had sprayed the liquid randomly within the tube.

Hydrostatic support can stabilize, at most, three degrees of freedom of a moving element, the other three degrees of freedom being gravitationally equipotential. In the bearing of Fig. 2, capillary centralizing forces operate in two rotational and two translational degrees of freedom. The vertical degree of freedom is strongly biased hydrostatically because the capillary forces alone could not support the weight of 270 mg. Non-contacting and therefore stiction-free supporting forces could also be obtained electrostatically or magnetically4,5, and the necessary centralizing forces obtained without power dissipation by capillarity.

Fig. 2. A capillary lubricated bearing. The moving element is a tube, running in a sealed glass tube. With this configuration the lubricant has a minimum surface energy.

10 mm

In conclusion, capillary forces can centralize the moving element of a bearing. Such bearings can be arranged so that the liquid lubricant is stably retained against creep and vapour transfer. Steady loads can be supported hydrostatically while capillary forces centralize the moving element stiction-free and out of contact with solid supports.

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BOOK REVIEWS

HOW MEMORY WORKS

Mechanisms of Memory

By E. Roy John. Pp. xii+468. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 112s.

This is probably the first book that provides a full scale survey of the mechanism for the storage of information in the nervous system and in this sense it is a landmark of historical importance. It provides a useful review of the literature, with reasonably sophisticated ideas about the nature of the representation of information in neural populations. For all this we can be most grateful to Dr John, as also for his own contributions to several aspects of the subject. Unfortunately, although there is some caution and reservation in discussing published work, this does not go far enough. In many parts of the book we are referred to unpublished work, personal communications and ephemeral publications such as the Federation Proceedings. The subject still lacks a firm body of well established anatomical, physiological and biochemical facts. Many of the results alleged by various authors are probably wrong. The author does not always examine them and assess their weakness or strength, but uses a process like algebraic summation to arrive at a probable answer for contradictory results. This reservation does not alter the fact that this is a most exciting book on a subject of very great current interest.

The author's review wisely involves no attempt to survey the vast behavioural literature on learning and forgetting. Instead his purpose is clearly set out as "to present and discuss some of the facts and logical considerations which may guide our strategy in devising experimental approaches to the problem of the physical basis of memory". He lists four fundamental functions that a memory mechanism must perform: (1) The configuration of external and internal stimuli impinging on an organism, which constitute experience, must somehow be coded into a neural representation. (2) This must be stored. (3) There must be access to specific stored items. (4) "The retrieved data must again be decoded into neural activity, which somehow revives . . . the original experience and thus constitutes a memory".

Even this abbreviated paraphrase will indicate the magnitude of the problems to be attacked. He proposes to include the phenomena of human memory, but since his purpose is to deal with the physical basis his account is inevitably largely of animal experiment. He first deals briefly with the problem of localization, leading him to "question the validity of connectionist theories according to which memory must be localized in a region". This section consists only of a short review of mainly classical attitudes to the problem. Lashley and Kluver are expounded but not criticized. Neither Penfield nor Weiskrantz is mentioned. Indeed, we should not expect to find them, as the "behavioural literature" is to be omitted, but in discussion of this topic the omission is grievous. Of course, the difficulties of "connexionist" theories are well recognized. No one supposes that memory is stored in specific cells so isolated that "if they were to discharge due to spontaneous causes, we would expect to be continually bombarded by unrelated frag-ments of recollections". The problem surely is how whatever changes take place in cells are so related as to provide the necessary neural representation.

In the author's statistical hypothesis "the information

. . . from the activity of a group of cells (is) repres by the orderly behaviour of the ensemble, compared the random or characteristic basal discharge pa The information content of the ensemble might a of the emergence of organized patterns of reference random or rhythmic baselines".

In any case, as he puts it, "whether memory is a in a place or a process in a population . . . the sus change . . . must be mediated by some alterat matter". To find more about this change he first dis the "labile phase in memory" during which consolir is taking place—the vulnerable stage, varying from seconds to days or weeks. There is here a valuable sion of the possibilities of reverberatory activity as various difficulties in supposing that it could ca representation. This and the work with spreading c sion leads him to the conclusion that intermediate h mechanisms of two sorts may exist.

Passing to discussion of the mechanism of stable mation storage, he first reviews the evidence tha mediated by the "structure of macromolecules" a particular RNA. He and his colleagues have rej and claim to have confirmed the experiments of McC with planarians. As he points out, however, the of Hartry et al. suggest that cannibalism alone faci learning. After describing the experiments on the of inhibition of protein synthesis on learning he con that although the experimental results are not com-"there is an impressive quantity of data strongly pe towards RNA and protein synthesis as deeply impl in the function of memory".

Basing himself on this probability, he proceeds: cuss theories as to the means by which afferent produces changes; for example, by what Robinson in work not yet published) calls pattern neurones dis ing pattern molecules into an extracellular space (cell?) nearby, where they form a storage unit which with time by formation of co-molecules. Retric obtained by release of pattern molecules from the act neurones and their recognition by the storage uni John recognizes that such hypotheses are so gene to be quite unsatisfactory and indeed a waste of t

the present state of knowledge.

The following sections return to the question of lo tion and the results of ablation experiments, and t again much exposition of Lashley and Hebb but wit critical discussion of more recent work. This is p the least original part of the book. Indeed, the au clearly not at home in the discussion of brain I He falls into the trap of using the word "function" out considering what it means. Lashley himself w immune from this, which indeed is difficult to a one starts to talk about tests for "the localization function". A major objective of experiment sho to try to discover what we do mean by "functions brain". What does John suggest instead? All he ca is "intuitive contradiction of the notion that the n of the response is localized in a discrete mediating re-

When Dr John proceeds to the relation of brain. tials to memory we feel that he returns to ground congenial to him. A review of the various el phenomena that can be observed in the brain is pa and will be useful for those not familiar with the himself has produced data on the distribution of el changes in the various phases of learning. Microel studies lead him back to the problem of whether discharge of a single neuron . . . might be suffice represent a particular item of information". cludes that it could not, in spite of Hubel and because of the lability of response of any given cell possibility of conditioning, as shown by Morell and His conclusion is that "characteristic modes of

tion, reflecting temporal patterns of average active are established during learning". To validate returns to experiments of the type he has studied s

rate to elicit 'labelled responses' with the same frequency in various parts of the brain". These sections contain much documentation to show that specific modes of activity emerge during conditioning throughout extensive parts of the brain and, furthermore, that an animal can learn to differentiate between two temporal patterns of activity applied to the cortex. There is undoubtedly much of interest here, but it is difficult to extract enough detail to be convinced of all that is claimed.

In short the author's wide review and interests make this an interesting book. His ideas for experiments on various aspects of the subject are stimulating, but many of the results have been only imperfectly reported in scattered and brief papers. The present work does not succeed in providing the missing data that will show us either the place of RNA or the significance of changes of patterns of electrical potential during learning. Nevertheless, we can be grateful to Dr John for his courage as one of the first to venture into many aspects of the subject.

J. Z. Young

ADVANCES IN TERATOLOGY

Advances in Teratology

Edited by D. H. M. Woollam. Vol. 2. Pp. 306. (London: Logos Press, Ltd., in association with Elek Books, Ltd., 1967. Distributed by Academic Press, New York and London.) 84s.

Until recently the history of the development of a scientific status for teratology, especially in man, has been a chequered one. Indeed, I can remember criticism of a distinguished physician who, in an examination with which he was concerned, set a question on the embryological origin and possible cause of a not uncommon foetal abnormality—coarctation of the aorta. Even the experimental evidence adduced by, among others, Giroud of Paris and the late Dr James Millen (in conjunction with the editor of the volume under review) and indicating the teratogenic influence of vitamin imbalance, received little general recognition. In the present decade, however, largely as a by-product of the tragic demonstration of the adverse effects on development of thalidomide. there has been an enormous increase in interest in the nature and causes of foetal abnormality. The subject of teratology is now accepted as one with a distinct status in biology and recognized to have considerable practical significance; the development of interest in it has been such that the publication of an annual volume on Advances in Teratology is now possible. Dr D. H. M. Woollam was asked to assume the responsibilities of the editorship of the series. As a colleague and friend of his, it is not really for me to assess his editorial work. I may, however, be permitted to write that, in my opinion, he has carried out his duties with discretion. In particular he has successfully persuaded a distinguished group of investigators to discuss specialized aspects of the current literature on teratology and teratogenesis in the light of their own studies in the fields concerned.

Of special interest in the volume under review is the contribution by the late Sir Denis Browne. As a paediatric surgeon at the Hospital for Sick Children, Great Ormond Street, who had to deal with many forms of bone and joint abnormality commonly found in human neonates and young infants, for many years he had stressed a mechanistic interpretation of the aetiology of certain of these malformations (for example, inverted feet, extended knees, dislocation of the hip, forms of scoliosis and torticollis). Browne was a skilled and very experienced photographer of clinical material, and the presentation of his views is accompanied by a large number of photographic illustrations of the conditions considered. There can be no doubt that his interpretations of a number of the

graphs. Nevertheless, most interested parties will probably continue to regard his specimens as falling within a group of deformations rather than malformations, but it was important that an authoritative expression of his views should have been made available. Browne took his personal, and often heterodox, views on malformations very seriously. He was fearless in presenting them, and his essentially clinical attitude to the problems discussed made him impatient of results of animal experiments. But he was a fair fighter; and it was characteristic of the man that he should have included in his presentation the sentence from Spinoza: "I have laboured carefully not to mock, lament or execrate, but to understand".

Other papers included in the volume are on rubella and antibiotics as teratogens, intra-uterine growth retardation, homocystinuria, chromosomal studies of spontaneous abortion, and teratogenesis in inbred strains of mice. Only special interest or personal taste can easily distinguish between their relative merits. All are authoritative and give good or excellent coverage to the literature concerned. There is also included a brief but useful account by Drs Cecilia Lutwak Mann and Mary F. Hay on the flat-mount blastocyst technique as applied to investigations of embryotrophic agents. Two of the contributions come from Britain, one from Italy; the remainder are products of American laboratories. second volume of Advances in Teratology is well up to the standard and level of interest of the first; it is very much to be hoped that the future will see the indefinite continuance of the series. The printing and illustrations are admirable. Unfortunately the price, though not excessive for these times, will make the volume for many a desideratum rather than a personal possession.

ALMOST ALL THE VIRUSES

The Molecular Biology of Viruses

(Proceedings of the Symposium held at the University of Alberta, June 27th to 30th, 1966, in conjunction with the Faculty of Medicine of the University of Alberta.) Edited by John S. Colter and William Paranchych. Pp. xvi+730. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 156s.

Nowadays the proceedings of almost every major international meeting are eventually published as a book. This is only as it should be, of course, because only a very small proportion of the world's scientists working in any particular field have the opportunity of actually going to meetings which interest them. Unfortunately, however, the inevitable delays in publication are usually so long that by the time the book appears everyone has heard what was said at second hand and, anyway, all the important new information has usually been published in the journals.

This book is no exception. It has taken the editors, who have a thankless task, and the publishers 15 long months to collect together and publish the thirty-six papers, and the discussions, given in June 1966 at the symposium on the molecular biology of viruses at Edmonton, Alberta. Almost all the material in this book is now available, less conveniently perhaps, elsewhere. None the less, the book will have its uses as a reference source and an archive. Collected together in one place are what boils down to a series of review articles of the ideas on various aspects of the molecular biology of bacterial and mammalian viruses that were current in 1966.

The symposium would have been better called North American rather than International. Only ten of the ninety-one authors work in Europe, and the rest of the world is unrepresented. But this does not matter all that much because most of the chief American groups, which so dominate the field, provided contributions. Perhaps the

most notable absentce was Professor Spiegelman who, although scheduled on the programme of the symposium to give a paper on the replication of Q3, was unwell at the time. Consequently, the section in the book on RNA bacteriophages gives few hints of the aerimonious dispute, on whether or not RNA bacteriophage replication involves a minus strand, which was raging between Spiegelman and almost everyone else, but Weissmann in particular. Those who went to the meeting were spared a repetition of the sparring match which took place at the Gordon Conference a week before the Edmonton Symposium and it is a great pity that the book contains none of this. For, after all, the main role of this book is as an archive and in the summer of 1966 the problem of RNA phage replication was one of the hottest topics in virology. Now, of course, Spiegelman has convinced himself that minus strands do exist while Weissmann, whose article in the book describes the role of replicative form and intermediate, has now gone on to question whether these structures really exist in vivo. All this goes to show just how out of date this section of the book is.

But it would be wrong to give the impression that the book is principally concerned with RNA bacteriophages. In fact, the symposium was ambitious enough to try to cover all the viruses with the exception of higher plant viruses. There are eight sections and only one of these is devoted to RNA bacteriophages. Three are on other bacteriophages, and there are sections on RNA and DNA mammalian viruses and two on oncogenic viruses. All these contain valuable reviews by leading workers. There is, just to take one example, a valuable paper by Brinton, who publishes less often than most, on his work on male specific F pili.

If I remember correctly, discussions of the papers at the symposium were quite extensive. They must have been severely edited or else the participants must have subsequently decided not to publish their remarks, because in the book there is never more than a page and a half of discussion after each section, which is a great pity.

A collection of review articles, such as this, is always valuable and this book is bound to find a place on library shelves if nowhere else. No doubt Academic Press had this in mind when they fixed the very high price which offectively excludes most individuals from the market.

JOHN TOOZE

LABORATORY ANIMALS

Husbandry of Laboratory Animals

(Proceedings of the Third International Symposium organized by the International Committee on Laboratory Animals.) Edited by M. L. Conalty. Pp. xvi+650. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 150s.; \$28.

This is a very interesting volume, the Proceedings of the Third Symposium of the International Committee on Laboratory Animals, held in Dublin in September 1965, and should be required reading by all those who use laboratory animals. Its importance is that this volume presents some of the known factors in the whole animal environment and the effects of these on tests using laboratory animals.

At present the use of laboratory animals is entering a new phase; the established pathogen and germ-free colonies has made it possible to pay more attention to the effects of environment on the animal, and, with more interest in animal behaviour, the influence of caging on the experimental animal is now becoming apparent.

This volume is the result of forty-six contributions from fifteen countries and a further thirty-two participants in discussion, and the individual chapters are of varied interest, but show the growth, scope and limitations of our present knowledge of these topics.

The text is divided into six sections. Seed handling, production and organization, include on handling scorpions and snakes, also technic skin grafting in rats and rabbits and a description experiments to assess the environmental in the mice.

Section 2 on nutrition is a series of papers which what is known about the vitamin, trace elements and calorie requirements of laboratory annuals, work on diets for SPF and gorm-free annuals.

Section 3 on ecology and disease includes aspects of stress in animals, the influence of age on the development of spontaneous neodes the successful limitation of mouse virus infections use of a filter fitting over each mouse cage.

Section 5 on physiology and psychophysiology in laboratory animals and of social stimulation and reproductive physiology.

Section 6 on pharmacology and pharmacology includes a paper on environmental influences of the responses in laboratory animals and pharmacology (a new term) and some nutritional paradoxes. The is used to describe the effects of environmental actual drug action. There is an author index to relieve text and a subject index.

The main impressions given by this book at although little is really known about many of the earlier attempts to stand of that many of the earlier attempts to stand of the response of laboratory animals will have to be seen that is now possible to detect the influence of the ment on experiments with laboratory animals of the ment on experiments with laboratory animals of the ment of the

we must know more about this important top.

This is a most stimulating book, not least to the need to study the total environment of the mental animal in the assessment of biological to the editor and the International Committee on the Animals are to be congratulated.

M. C. LA ANIMAL INTERNATIONAL TOP INTERNATIONAL

FLORA OF TURKEY

Flora of Turkey and the East Aegean Islands
Edited by P. H. Davis. Assisted by J. t. 1966
M. J. E. Coode. Vol. 2. Pp. xii+581. 1940
Edinburgh University Press, 1967.) 1898.

THE second volume of this invaluable work the heels of the first with a promptitude what e. congratulations to the editor and his col >: ! Volume 2 closely resembles its predecessor · · · format, being admirably printed, and admirable. what sparingly, illustrated. The do Cando' sequence of families has been modified to accord with modern opinions, with the apetalous Ille 19 Polygonaceae, Chenopodiaceae and Amarantae immediately after Caryophyllaceae, and for larger, and fairly obvious, alliance of Centralia Few will wish to argue with this departure, no v. re-unification of Hypericaceae and Guttiferac co much shaking of heads or shooting out of hip . sandwiching of Drosera between Frankenia and I' a little unexpected. For the rest, most of the control Malvaceae to a temporary stop at Celastraceae familiar to students of the Flora Orientalis, the wonders why the sequence has been altered to the Rhamnaceae and Celastraceae, and why Anacon has been interposed between these two sim 1. The permutations of phylogeny inscrutable, but the attitude of the Flora toward genera and species is on the whole so restreined to

mannered that an occasional demonstration of indi-

viduality is forgivable.

The decision to omit such internal geographical subdivisions as Mysia, Pisidia and Lycaonia will not be regretted, for this classical hangover was always something of a burden on our memories and a cause of confusion. It would, however, be useful to have some general indication of distribution within Turkey, apart from the grid and vilayet references, and it is to be hoped that some acceptable system will be devised in time for the next volume. R. D. MEIKLE

DESIGN IN FISHES

Functional Design in Fishes

By R. McN. Alexander. (Hutchinson University Library.) Pp. 160. (London: Hutchinson and Co. (Publishers), Ltd., 1967.) 10s. 6d. paper.

This book is about the structure of fishes. The functional design, as shown by the anatomical and physiological characteristics selected by the author, is related to evolu-tion by natural selection. Where appropriate the author supports his arguments with mathematics and physics, but, wisely, he warns the student that the subject must be approached in the manner of historians and critics. Mathematics and physics do not render the conclusions unassailable, because in any biological situation there are so many interacting structures and functions that there is always a danger of overlooking an important point.

The first chapter is about the coefficient of selection. The author examines the case of a genotype which increases the amount of food a fish obtains, or decreases energy consumption, thereby tending to increase the number of eggs laid by females, and being favoured by selection. He concludes that evolution by natural selection offers a plausible explanation for adaptations which result in small savings of energy.

The next five chapters deal with swimming, buoyancy, respiration, feeding and some sense organs. In each of these chapters analyses of the structures and functions of the organ systems are made, and related to the natural history of the fishes discussed.

A final chapter is concerned with the interactions between different functions or consequences of a single structure. This chapter is a most important one, because of the danger of oversimplification in a mathematical and physical analysis of a biological complex. It is so important that it should have been at the beginning rather than at the end of the book.

An appendix gives an outline classification of the fishes. It goes down to the level of orders, except in the case of the Cypriniformes and Perciformes, where some sub-orders are given. This brief systematic section is useful for students.

The reference list gives seven books on fishes, and there are eighty-eight other references, not representing an exhaustive bibliography, but serving as an entry into the literature of the subject.

There are sixteen clearly drawn figures. A highly commendable feature of the book is the complete relevance and full discussion of these figures in the text.

The book is a good introduction to the concept of functional design in fishes. It will be helpful to undergraduate students, for whom the series of books is intended. It will also be useful to workers in other fields of biology because it is a lucid and concise account.

One fault must be noted. Throughout the book the fish are called by their generic names only. This procedure is adopted even when it is clear that one species is meant and not all representatives of the genus. It is surprising that such nomenclatural inexactitude should occur, and it is to be hoped that this will be corrected in JAMES C. CHUBB subsequent editions.

SOIL BIODYNAMICS MISMANAGED

Soil Fertility

A Biodynamical Approach. By F. W. Pauli. Pp. xi+204. (London: Adam Hilger, Ltd., 1967.) 50s. net.

THE idea of the soil as a biologically active environment is well accepted. The relationships between plants and soil conditions continue to provide fascinating studies and we still have much to learn, In the preface to this book Professor Flaig draws our attention to the fact that recent emphasis on inorganic ions for plant nutrition has led to neglect of work on humus. Paradoxically, soil organic matter is most studied in countries using most mineral fertilizers. Dr Pauli aims to remedy the shortcomings of work with fertilizers by considering the soil ecosystem as a whole and relating the results of research on soil organic matter using modern analytical techniques to the problems of improving soil fertility measured by increased crop production. If this aim is achieved, then, as Dr Pauli hopes, some profitable new approaches to problems in soil fertility should develop. Unfortunately, he fails in his effort and what is worse sometimes confuses his interpretation of present knowledge. We shall have to wait longer for the inspired synthetical approach the subject demands.

The book is divided into seven chapters of text varying in length from three to seventy-three pages. Following four short introductory chapters, most of the text is in the fifth chapter on humus and soil fertility (59 pages), the sixth chapter on analytical techniques (73 pages) and the seventh chapter on man's exploitation of soil biodynamics (19 pages). There are two pages of conclusions and a glossary, bibliography and index.

The book is written without references inserted in the text so that the reader does not know either to whom to attribute the various statements or how much of the book represents published work and how much is interpretation by the author. The list of books, theses and papers provides no clue. The author states that his bibliography does not claim to be exhaustive and he does not indicate his method of selection. The bibliography does not contain several books one might expect to find, and references to published work by several established workers are missing from the list of papers.

Dr Pauli omits the role of fungi in the breakdown of plant material and the synthesis of humus. Normally, in aerobic soils the initial attack is by fungi, and subsequent decomposition by fungi and/or bacteria depending on the soil conditions. In anaerobic soils, plant remains are decomposed only by bacteria with the accumulation of lignified residues as peat. Terminology is bad throughout; for example, Dr Pauli writes of "low-molecular humus" when presumably he means "humus of low-molecular weight compounds".

On page 35, he describes clay minerals, "Many of the clay minerals consist of cations co-ordinated with water, OH-, O- and other anions, some of which are excellent bridging ligands and give rise to amorphous and crystalline solid particles. The crystalline particles show disorder and other imperfections, and may have amorphous coatings. The large surfaces contain many groups, among which are oxygen and different kinds of hydroxyl groups which may dissociate, yielding negatively and positively charged surfaces. In addition, electrical charges arise as a result of ionic substitution inside the lattice." Dr Pauli's basic description of the structure of a clay mineral differs so much from the accepted idea of layer structures of clay minerals derived basically from two units (a) of four oxygens at the corners of a tetrahedron with a silicon ion in the centre, and (b) of six oxygens or hydroxyls at the corners of an octahedron with typically an aluminium or magnesium ion at the centre, and with charges arising within the crystal from isomorphous replacement and at the edges of crystals by discontinuity, that one wonders at the source of his description and his interpretation.

Describing humus in hot and dry regions, Pauli says (page 65), "During a normal year, the atmosphere in hot and dry lands takes up much more water evaporating from the soil surface and transpired by the plants than the amount of water which reaches the soil as precipitation." The normal laws of physics demand that water balance should be achieved so that, starting and finishing with the same moisture content in the soil, water falling as rain must equal that lost by evapotranspiration and drainage. Presumably Dr Pauli means that in hot, arid regions, potential evapotranspiration is much greater than rainfall. Many other similar points occur throughout the book.

The book is well produced (although the copy received for review lacked plates 2 and 3). The price appears somewhat high for the length of the text.

The subject matter is one that should stimulate ideas in soil fertility and one hopes that another may soon succeed where Dr Pauli has failed. J. K. R. GASSER

STUDIES IN BIOLOGY

The Body Fluids and Their Functions By Garth Chapman. (The Institute of Biology's Studies in Biology, No. 8.) Pp. 68. (London: Edward Arnold (Publishers), Ltd., 1967.) 12s. 6d.

Guts

The Form and Function of the Digestive System. By John Morton. (The Institute of Biology's Studies in Biology, No. 7.) Pp. 58. (London: Edward Arnold (Publishers), Ltd., 1967.) 12s. 6d. net.

Microecology

By J. L. Cloudsley-Thompson. (The Institute of Biology's Studies in Biology, No. 6.) Pp. 48+2 plates. (London: Edward Arnold (Publishers), Ltd., 1967.) 12s. 6d.

THE Institute of Biology's series "Studies in Biology" is for sixth-formers, their teachers and first-year undergraduates. It has, as its aim, the presentation of concise reviews of "limited biological topics in which recent progress has been most rapid and important". In this it reflects an essential need. The problem is not so much that changes in research and ideas in biology are rapid but that they are extremely variable. Inevitably a major breakthrough in one field can be paralleled by virtual inertia in another. In such circumstances the maintenance of up to date editions of large texts becomes a difficult and uneconomical task and small, limited texts have to be employed.

It is the intention of the series to provide accounts not only of topics but also of "the methods that have been employed in elucidating the problems with which they deal" and to provide "suggestions for practical work for the student which should form a sound scientific basis for his understanding". Presumably implicit in the fact that none of the books has an index is the idea that they should be read, as it were, in one mental breath without the need for the resuscitation of cross-reference. How do these three books match up to these aims?

The Body Fluids and Their Functions is a comprehensive review. In parts it is so full of information and, in particular, taxonomic references that I suspect for the average student the physiological wood will get lost in the trees of specific names. It is chiefly at the first part of the book that this criticism can be levelled. It really is hard going with a first paragraph virtually a page long. What follows, however, is of great value. There are excellent sections on the dynamics of circulation, transport mechanisms and the homeostatic properties of the body fluids which are splendid examples of how the aims of the series can be achieved. Methods of investigations are integrated smoothly into descriptions of topics and the wider context of the subject is clearly demonstrated. The relation

between physiology and ecology and, most important, the role of physical science and mathematics in biological study are well illustrated.

Guts is written in a lively and, at times, humorous style; a virtue indeed in a textbook. At the same time it loses nothing in academic quality. It is a tribute to the author that he manages to make a potentially dull subject interesting and provides an insight into what laymen (and we can include students among them) see as the biologist's eccentric fascination in rather disagreeable subjects.

The framework for the text is a functional classification: herbivores and omnivores, deposit feeders, carnivores, filter feeders and fluid feeders. Unfortunately, within each category we find a review of species which although illustrating the variety of digestive systems, sometimes results in a rather barren catalogue of information. Possibly, it would have been better to use fewer examples and for more attention to be given to the gut as a selective barrier between the organism and the environment, emphasizing its ecological significance on the one hand and the physical processes involved in its functioning on the other. Methods of investigation are referred to, but mainly in a short appendix on practical work. There is little that illustrates how the methods and outcomes of investigations are related.

Microecology is not so much a review of the subject as a practical guide. The author is clearly more interested in getting students to find out about microecology than in telling them about the findings of microecologists; not a bad thing, but the result is that the book is a marked contrast to the other two. The depth and standard of presentation are much more elementary and clearly it is a book that would be used at the beginning of a course. Students would still need a review of the subject in its broader sense at the end.

The author argues the case for an autecological approach and through his review of measurement techniques and, particularly, his account of the ecology of cryptozoic animals which makes up virtually half the book, demonstrates the valuable part microecology can play in practical ecology classes. A chapter on microclimate and faunal distribution, however, is extremely shallow and the treatment of major ecological topics such as diurnal rhythm and food chains is appropriate to classes below the sixth form. Possibly the greatest disappointment is that no consideration is given to the place of microecology in applied biology. Pests of stored food products, and the role of soil organisms in agricultural practices, are only two examples that might have given the subject real meaning to the student.

Of the three books, I would select Body Fluids and Their Functions as being nearest to the aims of the series. But they all deserve a place in a school biology library. And a final comment: I am sure it would be very useful for students if indexes were provided.

P. J. Kelly

ROOTS OF MODERN MEDICINE

Medicine at the Paris Hospital

1794-1848. By Erwin H. Ackerknecht. Pp. xiv+242. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1967.) 72s. net.

THE present-day clinical approach to the patient originated with the Hippocratic physicians in fifth century Greece, and the interpretation of his disease on the basis of anatomy grew out of the Renaissance. The correlation, however, of these two, the findings in the living patient and those discovered in his body after death, did not receive widespread attention until the first decades of the nineteenth century. At this time the humoral pathology of the ancient Greeks was being replaced by morbid anatomy as an explanation of disease. Here then are the

exciting transition took place in the new French schools of health which were a product of the Revolution. That of Paris was by far the most significant, and in this excellent book Dr E. H. Ackerknecht, professor of the history of medicine in the University of Zürich, deals with its first 55 years, 1794 to 1848.

The period was dominated by brilliant men such as Bichat, the founder of histology, his teacher Pinel, systematizer of general medicine, great teacher and psychiatrist, Broussais, the creator of physiological medicine, Corvisart, the popularizer of percussion, Laennec, the inventor of the stethoscope, and many more. Ackerknecht discusses each of the principal contributors to this revolutionary era in clinical medicine, and his phenomenal knowledge of the subject, gained from almost twenty years experience, allows him to sketch with great skill their essential background; in so doing he captures the spirit that must have infused the hospitals of Paris at that time. Little wonder that students from all the world flocked to them to learn the new medicine from its distinguished practitioners. The main goal was to discover as much as possible about the patient in life and in death, and the new techniques that were introduced furthered this end. To this was added statistics which gave numerical order to the masses of data being accumulated. Just as important, therapeutics could now be objective, although on the whole treatment advanced the least; some would say it retrogressed owing to over-zealous therapists. Another feature of the period 1794 to 1848 was the closer association of medicine and surgery which was beneficial to both.

Professor Ackerknecht's book on the Paris school of the early nineteenth century is the first in English to describe this fundamentally important period. His ability allows of little criticism, and his book, engagingly illustrated by Daumier sketches and adequately documented and indexed, will be essential reading for anyone interested in the medicine, science or general history of the nineteenth century. EDWIN CLARKE

CHROMATOGRAPHY AGAIN

Advances in Chromatography

Vol. 4. Edited by J. Calvin Giddings and Roy A. Keller. Pp. xiv+380. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1967.) 130s. net. LIKE its predecessors, this volume contains interesting articles on a variety of topics; the greater part deals with gas chromatography, and the book is rather expensive.

The first article, by L. R. Snyder, attempts a systematic approach to the understanding and control of solute RF values on alumina and silica in thin layer chromatography. It is a praiseworthy clarification of a very complex subject. Steroid separation and analysis are next dealt with by R. Neher, who gives guidance on choice of chromatogram for particular steroid groups and useful advice for enhancing the efficiency of the methods discussed. Ion exchange cellulose is the subject of the next article, by C. S. Knight. Introduction of the microgranular type is a distinct advance for protein chromato-Its fundamental properties are discussed and compared with those of the more traditional microfibrillar type. Ion exchange cellulose sheets are also considered, and the article as a whole should provide a better understanding of the performance factors of all three types.

Adsorption gas chromatography, a comparatively neglected field, is covered admirably by A. V. Kisilev who, with others in the Soviet Union, has made important contributions to the subject. The standardized preparation of adsorbents with special properties has considerable potentiality. Applications and potentialities of the packed capillary column are also well brought out in the

main roots of modern clinical medicine. This vital and next article, by I. Halasz and E. Heine. This type of column has a diameter of 0.5 mm or less, and is prepared by drawing out a wider column already loosely filled with granular solid. The packing density of such columns is considerably smaller than that of conventionally packed capillary columns, and consequently they have higher permeability and higher separation power with only a moderate pressure drop. In the next article, by W. M. McFadden, the use of the mass spectrometer in the analysis of gas chromatographic eluants is discussed. The method is extremely sensitive, but interpretation of spectra of unknowns may sometimes be difficult. A good account of instrumentation is given and applications to various types of gas chromatogram are described. The object of the final article, by L. Rohrschneider, is to examine the relationship between polarity of stationary liquid phase as applied to gas chromatography and solvent polarity as deduced from physical chemical properties. possibilities for defining polarity and using this for predicting retention are examined. This is largely a theoretical article which points to important practical implications.

This volume maintains the high standard of the series and contains something of interest for all who use chromatographic methods. R. Consden

MORE HETEROCYCLIC CHEMISTRY

Pyrazoles, Pyrazolines, Pyrazolidines, Indazoles and Condensed Rings

By Lyell Behr, Rafaello Fusco and C. H. Jarboe. Edited by Richard H. Wiley. (The Chemistry of Heterocyclic Compounds: a Series of Monographs.) Pp. xvi+888. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1967.) 450s.

Specialists in the field of heterocyclic chemistry cannot do otherwise than welcome this new volume of collected information on pyrazoles and related types, painstakingly compiled, in very great detail, by the three authors. To quote from the editor's preface, the work covers "the transition from the classical period (1890-1920) through the development of the modern period (1905-1960)" and presents the research worker with necessary information on almost all known pyrazoles and their derivatives named in the title, in the form of preparative methods, properties, both physical and chemical, interspersed with good critical discussion. Physical methods for elucidation of structures are also well recorded. The reader is thereby saved much valuable time which would otherwise be spent in searching the literature on a subject which is very widely dispersed.

The book is in four parts. The first part, on pyrazoles themselves, is by Rafaello Fusco, whose name is well known in this field. The factual information is presented in a readable style with interesting discussion, and the section ends with a list of 1,161 references. The section is supplemented by the fourth part of the book, which comprises 110 tables, classifying pyrazoles into ten groups according to substituents, together with the main synthetic methods, physical characteristics, references, and some of the chief derivatives with their melting-points. One minor criticism could be directed at some of the formulae, which look a little odd, because of the author's determination to show which atom of a substituent is attached to the nucleus. It seems unnecessary to write H₅C₆- or H₅C₂OOC- just because the group is on the left.

The second part of the book, by C. H. Jarboe, covers the chemistry of pyrazolines and pyrazolidines equally exhaustively. The factual data are leavened by a certain amount of lively comment, and one may read "It was finally shown, amid much acrimony, that they were pyrazoline carboxylate esters" (page 195), or again, of a certain 1-pyrazoline, that the half-decomposition period is 34 minutes at 100°, and 9 years at 25° (page 207). An extensive list of references follows.

The third part, by L. C. Behr, dealing with indazoles and condensed systems, is also a good factual summary, providing an insight into the structural problems, and indicating the main lines of research which remain to be followed. References are again very numerous.

Regrettably, with such a wealth of material in one volume, the printing is close, and some of the structural formulae absurdly small. The older generation of chemists would do well to provide themselves with a good handlens!

W. J. BARRY

FIESER'S REAGENTS

Reagents for Organic Synthesis

By Louis F. Fieser and Mary Fieser. Pp. vii+1457. (New York and London: John Wiley and Sons, Inc., 1967.) 210s.

EVER since Louis Fieser's sixty-sixth birthday, two years ago, when his publishers announced the forthcoming publication of this book, we have been waiting eagerly for what promised to be a unique and extraordinarily useful book. The authors set out to list the reagents of organic chemistry, including under this heading catalysts, compounds like N-bromsuccinimide in which only part of the molecule is transferred, and compounds like tetraphenylcyclopentadienone in which the whole molecule can be present in the product. Because, when reagent is thus defined, every reaction of organic chemistry has a claim to inclusion, the book is necessarily selective. It is no surprise that in most cases the Fiesers have made the obvious choices of what to leave out.

The authors very largely achieve what they set out to do. In a check of a few reagents we wanted to use recently, we found all but three adequately covered (no purification for amyl nitrite [perhaps the American product needs no purifying], no entry for potassium or sodium cyanide, no entry for dimethylamine). But no one will find everything he wants in a book of this sort; it is a tribute to its success that we found so much and that our failures were so trivial. Indeed, because it is so inclusive that many people are going to become dependent on it, those relatively unusual reagents which have not been included are even likelier than before to get buried.

This book also has the fascination of a good dictionary: setting out to look up one reagent, one is continually waylaid by other absorbing entries, the more arcane the better. I not only came across all sorts of reactions I did not know about, but was continually entertained as I read, and almost found myself reading from cover to cover, no part of a reviewer's brief, I think, with this kind of book.

My one criticism (apart from the price, for which there are no doubt reasons) is that the organization into reagents occasionally leads to fragmentation of things that might be better treated as one topic. I can illustrate this with the example of enamines which appear under several headings: as a protecting group in a hydride reduction, under dibenzoyl peroxide (not indexed under enamines), under morpholinocyclohexene (not indexed), under ozonolysis, under sodium borohydride, under piperidine (not indexed), under pyrrolidnocyclohexene (not indexed), under 1,3-dichloro-2-butene (not indexed) and under toluene-sulphonic acid. Now the indexing failures are trivial; but in this kind of case an entry under the general heading enamines, rather than under several more specific headings, would do much for an understanding of the usefulness of the class and also afford an opportunity for a brief discussion of, in this example, the relative merits of the pyrrolidine and morpholine enamines. This would, of course, be no small task and I would

certainly hesitate to tackle it myself on the scale of the whole book,

This criticism is by way of saying that the book is so useful that one half expects to find everything in it.

IAN FLEMING

OBITUARIES

Professor Francis Bitter

On November 21, 1967, the National Magnet Laboratory at the Massachusetts Institute of Technology was rededicated as the Francis Bitter National Magnet Laboratory. Professor Bitter, long a leader in research on magnetism at MIT, died on July 26 in Centerville, Massachusetts, after a protracted illness.

Bitter, born in Weehawken, New Jersey, on July 22, 1902, was the son of Karl Bitter, a well known British sculptor. He attended the Taft School, and received the Bachelor of Science degree from Columbia University in 1924. After spending a year in Berlin, where he attended lectures by Einstein, Planck, and von Laue, young Bitter returned to Columbia, receiving the Ph.D. degree in 1928. In his thesis work he used a large electromagnet while studying the diamagnetic susceptibilities of organic gases, and during two years as a National Research Fellow at the California Institute of Technology, he worked further on magnetic problems under the direction of R. A. Millikan. In 1930 he joined the Westinghouse Research Laboratories in Pittsburgh, where he carried out applied research on ferromagnetism.

Bitter spent 1933-34 as a Guggenheim Fellow at the University of Cambridge, where he became interested in Kapitza's work on powerful pulsed magnetic fields. Returning to the United States as an associate professor in the Department of Metallurgy at MIT, he set out to develop magnets which would be capable of producing more intense, constant and uniform fields than had previously been available. He attacked this problem effectively by shifting the large masses of iron previously used in electromagnets from the magnets to the generators from which they obtained power. What came to be known as the Bitter magnet is a solenoid carefully designed to provide rapid heat removal by intimate contact with a large volume of water flowing through its turns. A magnet he designed for Zeeman effect studies in 1938 produced 100,000 Gauss, constant to 1/10 per cent and uniform within the same limits throughout a volume of 10 ml.

After serving for five years as a commander in the Naval Reserve, working on magnetic detection of submarines, counter-measures for magnetic mines and operations research, Bitter returned to MIT as a professor of physics, where he became active in research on nuclear magnetism and the optical effects of microwave resonance. He made important contributions to the theory of optical pumping.

Bitter's temperament was an interesting amalgam of the artistic and the scientific. The breadth of his scientific interests is indicated by the fact that he was successively professor of metallurgy, of physics and of geology and geophysics. Teaching was one of his favourite occupations, and he was an effective adviser to students, serving as master of Ashdown House at MIT from 1960 to 1965. He was active in the planning of courses and curricula, and was the author of several textbooks.

A large number of his colleagues, former students and friends gathered to honour his memory at a symposium on magnetism held in connexion with the re-dedication of the laboratory he helped to found.

GEORGE R. HARRISON

Appointments

MR HUBERT E. SAUTER, at present deputy director, has been appointed director of the US Clearinghouse for Federal Scientific and Technical Information.

Announcements

THE Agricultural Research Council has announced that Ditton Laboratory in Maidstone, Kent, will become a part of East Malling Research Institute, also in Maidstone, in April 1969. The new director who will take over from the two present directors—Dr R. G. Tomkins at Ditton and Dr R. F. Tubbs at East Malling—has not yet been named. The first move in the break-up of the laboratory at Ditton occurred about two years ago when research workers in the biochemistry department moved to the Food Research Institute in Norwich. The remaining staff will be split up between East Malling Institute and the Glasshouse Crops Research Institute in Sussex. Although East Malling and Ditton are essentially independent, some research work, on the conditions for the growth of fruit, has been shared for the past fifteen years.

DR A. G. FRASER, of the Department of Geology, University of Hull, has been awarded a Polar Medal in recognition of his survey work over a period of three years, two of which were spent in the Antarctic.

THE 87th annual general meeting of the Society of Chemical Industry is being held in Edinburgh during July 17-20, 1968. The chief feature of the meeting will be a two-day symposium on "The Future of the Petrochemical Industry". Further information can be obtained from Dr Magnus Pyke, Publicity Secretary, Scottish Grain Distillers, Ltd., Glenochil Research Station, Menstrie, Clackmannanshire, Scotland.

Meetings

Low Temperature Physics, August 21-28, 1968, St. Andrews (The Conference Secretary, School of Physical Sciences, University of St. Andrews, North Haugh, St. Andrews, Scotland).

INDUSTRIAL Measurement Techniques for On-Line Computers, June 10-14, 1968, Institution of Electrical Engineers (Conference Department, IEE, Savoy Place, London WC2).

CODATA Conference on the Critical Evaluation of Numerical Property Values in the Physical Sciences, June 30-July 5, 1968, Arnoldshain, near Frankfurt/Main (Dr Guy Waddington, Executive Director, Central Offices, CODATA, c/o National Academy of Sciences, 2101 Constitution Ave. NW, Washington, DC).

FEDERATION of European Biochemical Societies, July 15-20, 1968, Prague (The Secretariat of the Fifth FEBS Meeting, Nám Krasnoarméjců 1, Prague 1, Czechoslovakia).

PATTERN Recognition, July 29-31, 1968, National Physical Laboratory, Teddington, Middlesex (The Conference Department, The Institution of Electrical Engineers, Savoy Place, London WC2).

ERRATUM. In the second and third sentences of the article "Triads in Foetal Skeletal Muscle" by Sheppard M. Walker and G. Randolph Schrodt (Nature, 216, 985; 1967) "the Z level of the line" should read "the level of the Z line". In the second line of the legend to Fig. 6 "right" should read "left".

In the communication "Imidazole and Sequestration of Calcium Ions by Sarcoplasmic Reticulum" by B. P. Yu, E. J. Masoro and F. D. DeMartinis (Nature, 216, 822; 1967) the figures were wrongly numbered. Fig. 3 should be Fig. 5, Fig. 4 should be Fig. 3 and Fig. 5 should be Fig. 4. Text references to figures are correct for this revised numbering.

CORRESPONDENCE

Food Antioxidants in Tissue Culture

SIR,-Miss Milner has described some very interesting experiments on the effects of butylated hydroxytoluene (BHT) and related compounds on monkey kidney cells in tissue culture1, but it is a pity that she should have balked at the task of interpreting her findings in terms of the safety in use of BHT as a food antioxidant. Having spoken in three successive sentences of p.p.m. in individual items of food for human consumption, mg/kg body weight doses in animal experiments and p.p.m. in tissue culture media, without suggesting how these might be inter-related, she abdicates all responsibility by concluding that "...it is difficult to make a direct comparison with the in vivo experimental results because of the different effects measured".

Of course the selection of parameters to measure is at the discretion of the experimenter, but another source of difficulty in this instance is the absence of any data on the kidney concentrations of BHT in animals receiving BHT in the diet.

Because unchanged BHT is not excreted in the urine of rabbits receiving doses of up to 1 g/kg body wt2 it seems unlikely that the kidney concentrates this compound. We are, however, in the process of investigating this aspect of BHT metabolism.

In the meantime, reported results from liver analyses may be of interest. Daniel and Gage³ found that rats given 5,000 p.p.m. BHT in the diet averaged less than 2 p.p.m. BHT in the liver. Thus the amounts of BHT in the diet required to produce liver concentrations of 7.5-30 p.p.m. (those used by Miss Milner) would exceed the LD₅₀ of this compound in the rat⁴. Legal tolerances would allow an average BHT content of up to 4 p.p.m.5 in the human diet. In fact, the present dietary concentration in Great Britain is unlikely to exceed 1 p.p.m. If the intake and liver content of BHT have the same relationship in man as they do in the rat, the human liver would currently contain about 3×10^{-4} p.p.m.

We hope that these comments may help in the interpretation of the tissue culture data1 and in the design of further experiments to help us bridge the gap between animal studies and the human situation. But progress in this direction will only be possible if the results obtained with tissue cultures are considered against the background of toxicological data already in our possession.

Finally, one detail calls for correction. While it is true as stated that BHT is permitted for use in butter, this permission only applies to butter for manufacturing purposes6,7; this means that the addition of BHT to butter for retail sale in Great Britain is illegal.

Yours faithfully,

D. GILBERT A. D. MARTIN

The British Industrial Biological Research Association, Carshalton, Surrey.

- ¹ Milner, Susan M., Nature, 216, 557 (1967).
- ² Dacre, J. C., Biochem. J., 78, 758 (1961).
- Bantel, J. W., and Gage, J. C., Fd. Cosmet. Toxicol., 3, 405 (1965).
 Deichmann, W. P., Clemmer, J. J., Rakoczy, R., and Bianchine, J., A.M.A. Arch. Industr. Hith., 11, 93 (1955).
 Gilbert, D., and Golberg, L., Fd. Cosmet. Toxicol., 3, 417 (1965).
- ^e Hinton, C. L., in Fd. Addit. Control Ser. FAO, 2, 27 (1960).
- ⁷ The Antioxidant in Food Regulations 1966 (SI 1966, No. 1500) (HMSO, London, 1966).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Thursday, December 28

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 3 p.m.—Professor Richard L. Gregory "The Intelligent Eye". (The One Hundred and Thirty-Eighth Course of Six Lectures Adapted to a Juvenile Auditory. Further lectures on December 30, January 2, 4, 6 and 9).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

dates mentioned:
TEMPORARY LECTURER or ASSISTANT LECTURER (preferably qualified to teach philosophy of science) in the DEPARTMENT OF PHILOSOPHY—The Registrar, University College of North Wales, Bangor, North Wales (January 1).
ASSISTANT EXPERIMENTAL OFFICER (with a degree or equivalent in botany, horticulture, agriculture or a related subject) for work on the breeding of vegetable crops, particularly in Brassicae—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (January 5).
LECTURER IN ANATOMY—The Secretary, The University, Dundee, Scotland (January 6).

land (January 6).

RESPARCH FELLOW or ASSISTANT (graduate in mathematics, physics or electrical engineering) in ENGINEERING for studies in the synthesis of lumped linear electrical networks—The Registrar, University of Leicester, Leicester

(January 6).

CHAIR OF EDUCATION—The Registrar, University of Warwick, Coventry, CV4 7AL (January 8).

LECTURER (with previous research experience in the biochemical aspects of drug metabolism and distribution) in BIOPHARMACY—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, SW3

of drug metabolism and distribution) in Biopharmacy—the officerosty Chelsea College of Science and Technology, Manresa Road, London, SW3 (January 8).

Lecturer in Medicine in the University Department of Medicine, Western Infirmary—Secretary to the University Court, The University, Glasgow (January 13).

Lecturer (preferably with interests and substantial experience in low temperature physics, reactor physics or electronics) in the Department of Physics, and a Senior Research Associate in the Department of Physics, and a Senior Research Associate in the Department of Physics, and a Senior Research Associate in the Department of Physics, and a Senior Research Associate in the Computer Centre.—The Assistant Registrar (S), University of Birmingham, Box 363, Birmingham 15 (January 14).

Assistant Lecturers, Lecturers in the Computer Centre—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex, quoting Ref. MS/12 (January 15).

Lecturer of Assistant Lecturer in Botany—The Registrar, The University, Hull (January 15).

PROFESSOR OF CHEMISTRY at University Council, 33 Bedford Place, London, WC1 (January 16).

Senior Lecturer/Lecturer in the School of Physiology, University of Mew South Walcs—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Australia and London, January 15).

SENIOR LECTURER/LECTURER in the School of Physiology, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, January 15).

CHAR OF BOTANY at Fourah Bay College, University of Sierra Leone—The Inter-University Council, 33 Bedford Place, London, WC1 (January 18).

LECTURERS (2) (preferably with experience in teaching sociological methods and sociology of African societies) in Sociology at the University of East Africa, University College, Dar es Salaam—The Inter-University Council, 33 Bedford Place, London, WC1 (January 18).

SEMIOR LECTURER OF LECTURER IN BIOCHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, January 19).

DIRECTOR (with research experience in a field dealing with the production, processing and marketing of meat and meat products) of the M. C. Franklin Laboratory and William McIlraith Fellow in Animal Husbandry at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (London and Australia, January 26).

BAIRD AND TATLOCK RESEARCH FELLOW IN SCHENCE EDUCATION at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, WC1 (January 27).

LECTURER OF SENIOR LECTURER (preferably with research experience in blophysicai, or physico-chemical aspects of respiratory or circulatory physiology) in the Physiological Flow Studies University of Caro, Imperial College of Science and Technology, London, SW7 (January 31).

RESEARCH FELLOW (with a good knowledge of the techniques of protein chemistry, including amino acid analysis, sequence determinations, and the methods for studying active centres of enzymes) in Biochemistry in the John Curtin School of Medical Research, Institute of Advanced Studies, Australia and London, January 31).

CHAIR OF CELL BIOLOGY, HISTOLOGY

SENIOR LECTURER OF LECTURER IN PHYSICS at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, February 20).

BIOCHEMIST OF MICROBIOLOGIST in the BACTERIOLOGY DEPARTMENT for a group working on the natural defence mechanisms of the adder against infection by mastitis pathogens—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, Berkshire, quoting Ref. 67/25 (3).

JUNIOR TECHNICIAN/TECHNICIAN (male or female, preferably with experience of electron microscopy and histology) for a Research UNIT—The Establishment Officer, University College London, Gower Street, London, WCI, quoting Ref. Anat./11.

POSTDOCTORAL RESEARCH ASSISTANT IN GAS KINETICS for research on kinetics of Levis acid-base reactions in the gas phase—The Registrar, University College of Wales, Aberystwyth.

SENTION TECHNICIAN (preferably with histological training) in the BIOLOGY SECTION of the University Examination Laboratorics, for duties which will include work in connexion with University practical examinations at all levels, and in particular the making of permanent biological microscopical preparations—The Clerk to the Senate, University of London, Senate House (11/N), London, WCI.

TECHNICIAN (female, aged 18–23, preferably with experience of horticulture and laboratory work) in the Department of Horticulture—Professor O. V. S. Heath, FRS, University of Reading, Horticultural Research Laboratories, Shinfield Grange, Shinfield, Reading, Berkshire.

REPORTS and other PUBLICATIONS

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Psychology in Its Natural Habitat. By Professor P. L. Brondhurst. (An Inaugural Lecture delivered in the University of Birmingham on 16th February 1967.) Pp. 24. (Birmingham: The University, 1967.) 2s. 6d. [250 The National Council for the Unmarried Mother and her Child. Annual Report, April 1966-March 1967. Pp. 68. (London: The National Council for the Unmarried Mother and her Child. 1967.) [250 University of London. University College Calendar 1967-68. Pp. xcv + 256. (London: University College, 1967.) [250 Western Regional Hospital Board: Regional Physics Department. Collected Papers: 4—Work in Progress. (Glasgow: Western Regional Hospital Board, 1967.) [259 Ministry of Technology. Report of the Government Chemist 1966. Pv. i + 182+8 plates. (London: H.M. Stationery Office, 1967.) 15s. nct. [259 Pest Articles and News Summarles (PANS) Manual No. 2: Pest Control in Groundnuts. Pp. iv+188. (London: Topical Pesticides Research Headquarters and Information Unit, Ministry of Overseas Development, 1967.) 9s. [270]

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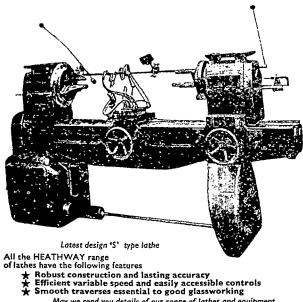
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Current research problems at Capenhurst—such as electrical machines; arcs and plasmas; distribution systems and equipment; human environment—present a range of control engineering problems and we wish to strengthen the team working in these areas by the appointment of a young ENGINEER (or MATHEMATICIAN) who will work on the design and analysis of control systems.

Ref. N/90.

NEW MATERIALS

We wish to extend the work on novel electrical motors by a thorough investigation of the design of new materials for such machines. These materials will probably have composite properties and an ideal background for this work would combine training and experience in METALLURGY, MATERIALS generally, and SEMI-CONDUCTOR work with particular reference to their mechanical and electrical properties.

Ref. N/91.

For all of the above posts inlitial salary, depending on ability, experience and age,

ror all of the above posts initial salary, depending on ability, experience and age, will be within one of the following salary bands: £980-£1.675; £1.605-£2.045; £1.825-£2.470; £2.430-£3.025.

Please apply, or write for further interests.

£2,470; £2,430-£3,025.
Please apply, or write for further information. to:
D. C. Pare. Head of Personnel Services,
THE ELECTRICITY COUNCIL RESEARCH CENTRE,
Capenhurth, near Chester,
quoting appropriate reference. (1047)

UNIVERSITY OF HONG KONG SENIOR LECTURESHIP/LECTURESHIP/ ASSISTANT LECTURESHIP IN APPLIED MATHEMATICS

MATHEMATICS

LECTURESHIP/ASSISTANT LECTURESHIP
IN PURE MATHEMATICS

Applications are invited for a post of Senior
Lecturer, Lecturer or Assistant Lecturer in Applied Mathematics, and a post of Lecturer or
Assistant Lecturer in Pure Mathematics. Candidates for the post in Applied Mathematics. Candidates for the post in Applied Mathematics shou'd
specialize in mathematical methods or theoretical
physics, and candidates for the post in Pure
Mathematics should preferably specialize in functional analysis, geometry or topology.

Annual salaries (superannuable) are: Senior

tional analysis, geometry or topology.

Annual salaries (superannuable) are: Senior Lecturer: \$HK47.360 by \$HK1.840 to \$HK56.560; Lecturer. \$HK30.880 by \$HK1.440 to \$HK49.600 (man) or \$HK26.720 by \$HK1,760 to \$HK32.000 to \$HK33.280 by \$HK2,000 to \$HK45.280 by \$HK1,440 to \$HK45.280 by \$HK1,440 to \$HK49.600 (woman); Assistant Lecturer: \$HK20.800 by \$HK1,440 to \$HK25.120 (man) or \$HK15.760 by \$HK1,520 to \$HK20,320 (woman), (\$HK14.60=£1.)

Appropriate increments may be allowed for approved experience after the age of 27, for appointment at Lecturer level.

Senior Lectureships and Lectureships are permanent appointments. Assistant Lectureships are for three years only, but Assistant Lecturers, it considered suitable, are promoted to Lecturer by the end of that period.

The equivalent of income tax in the Colony is comparatively low (at present from about \$HK656 to \$HK3,248 per annum for a married man with two children on a Lecturer's salary). There is a contributory Superannuation Scheme (5 per cent employee, 12½ per cent employer).

An appointee whose place of permanent home is agreed to be overseas is provided with accommodation at reasonable rental and economy-class air passages (or sea passage of the equivalent cost) on first appointment and on leaves. An appointee at Senior Lecturer level whose place of permanent home is not overseas is also provided with accommodation at reasonable rental.

Further information and application forms may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House; Pall Mall, London, S.W.1. Applications close in Hong Kong and London on January 22, 1968.

ZOOLOGY LABORATORY TECHNICIAN (male), Queen Mary College (University of London), Mile End Road, E.1. Previous experience in a physiological laboratory is essential. Salary according to experience and qualifications. Scale £653 to £938 per annum plus London Weightling £60 per annum and possible £30 or £80 special qualification award. Five-day week. Four/five weeks annual leave. Pension scheme.—Letters only to Registrar (ZT), stating full details of age, experience and present work. (2081)

UNIVERSITY OF THE WITWATERSRAND **JOHANNESBURG**

DEPARTMENT OF BOTANY

SENIOR LECTURER OR LECTURER IN PLANT PHYSIOLOGY

Applications are invited for appointment to a post of Senior Lecturer or Lecturer in Plant Physiology in the Department of Botany. The salary scales payable are: Senior Lecturer, R.4,200 by R.150 to R.4,800 by R.300 to R.5,700; Lecturer, R.3,150 by R.150 to R.4,800 (at the new rate of exchange R.1,716=£1 stg.) In addition an annual vacation savings bonus of up to R.200 is payable. Membership of the Associated Institutions Pension Fund is compulsory. Membership of the Staff Medical Aid Fund is also compulsory for an officer who is eligible for membership.

Intending applicants are advised to obtain a copy of the information sheet relating to the above vacancy from the Association of Commonwealth Universities (Branch Office), Marlbordugh House, Pall Mall, London, S.W.1. Applications close in South Africa and London on January 15, 1968. Duties are to be assumed on April 1, 1968, or as soon as possible thereafter.

THE DEPARTMENT OF PHYSIOLOGY and Pharmacology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, requires four faculty members to meet growing teaching and research needs, Persons interested in the following positions are invited to send curriculum vitae, grade transcripts, list of publications, and special qualifications and interests to Dr. H. G. Downie, Professor and Head of the Department.

- to Dr. H. G. Downie, Professor and Head of the Department.

 1. D.V.M. (or equivalent) with Ph.D. in Pharmacology. Duties include participation in the team approach to teaching undergraduate and graduate Pharmacology, and research in areas of own interest. Rank; Associate Professor.

 2. D.V.M. (or equivalent) with Ph.D. in Pharmacology, Toxicology, or Biochemistry. Duties include participation in the team approach by teaching undergraduate and graduate Toxicology and Pharmacology, administrative supervision of a veterinary toxicology testing laboratory, and research and development in forensic analysis. Rank: Associate Professor.

 3. D.V.M. (or equivalent) with Master's degree in any related science. Duties include participation in the team approach to teaching undergraduate Pharmacology. The candidate would be expected to pursue graduate work at the Ph.D. level in Pharmacology. Rank: Lecturer.

 4. PHYSIOLOGIST with Ph.D. qualification. Duties include instruction in Physiology to Physical Education undergraduates and participation in a laboratory course on Human Physiology. Consideration will be given to those individuals having a special research interest in respiratory or cardiovascular physiology. Rank: Assistant Professor.

 Moving expenses up to \$1,000 will be provided for each position. All salaries are commetitive

Moving expenses up to \$1,000 will be provided in each position. All salaries are competitive ith industry. (2074) with industry.

UNIVERSITY COLLEGE OF **NORTH WALES**

THE MARINE SCIENCE LABORATORIES MENAI BRIDGE

Applications are invited for a Post Doctoral Fellowship or Post Graduate Assistantship to study the mechanism of cold hardiness in marine invertebrates. The post will be a full-time appointment, initially for a period of three years.

The salary in the case of a Post Doctoral Fellow would be in the range, £1,105 to £1,340 plus F.S.S.U. benefits or for a Post Graduate Assistant, £760 to £865 plus F.S.S.U. benefits, If a Post Graduate Assistant is appointed, he will be allowed to read for a higher degree, subject to the terms and conditions of an S.R.C. studentship.

ship.
Applicants should have a first class or upper second class Honours degree or have had research experience in the field of electronmicroscopy or biochemistry.
Applications, giving details of age, qualifications and experience, together with the names and addresses of two referees, should be sent before January 8, 1968, to the Registrar, from whom further particulars about the post may be obtained. (2085)

PHARMACOLOGIST

Expanding Pharmaceutical Research Laboratory located near Montreal, Canada, is seeking a Ph.D. with 0 to 5 years experience and a good general background in pharmacology.

Opportunity for basic research.

Send résumé to PERSONNEL MANAGER

BRISTOL LABORATORIES OF CANADA

100 INDUSTRIAL BLVD. CANDIAC, P.Q., CANADA

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE, NORWICH

FOOD RESEARCH INSTITUTE, NORWICH Assistant Experimental Officer/Experimental Officer required within the Microbiology Division for each of three posts concerned respectively with work on: (a) Microbial products—Reference 67/5; (b) Biochemistry of micro-organisms—Reference 67/21; (c) Microbiology of food spotlage—Reference 67/26, Minimum qualifications—Pass degree, H.N.C., F.I.M.L.T., or equivalent in appropriate subjects. Experience in biochemistry of microbiology an advantage in posts (a) and (b). Experience in microbiology an advantage in post (c).

Salary according to age and experience in Assistant Experimental Officer or Experimental Officer grade. A.E.O. on a scale £803 (age 22), £1,017 (age 26 or over) rising by annual increments to £1,243. E.O. £1,365 to £1,734 by annual increments. Optional contributory superannuation scheme.

annuation scheme.

Application forms
from Secretary, Low Temperature Research
Cambridge, quoting reference number(s) of

LAURENTIAN UNIVERSITY SUDBURY, ONTARIO, CANADA

DEPARTMENT OF GEOLOGY AND GEOGRAPHY

Applications are invited for a new staff position in Mineralogy, appointment effective from September 1, 1968. Duties will include teaching mineralogy and X ray crystallography, some assistance in the development of the first year laboratory, and responsibility for initiating and conducting research in mineralogy and allied fields. Interests and experience in X-Ray analytical procedures are desirable. New Philips X-ray diffraction and spectrographic research equipment are available in the Department. Unique geological setting of Sudbury provides excellent research opportunities, Department to move in the Spring of 1969 to new building presently under construction.

Applicants must have Ph.D. degree, and experience with research programmes as well as teaching ability at the undergraduate level. Salary and rank dependent on qualifications and experience. Please submit complete personal research interests and experience, and the names of at least three referees who are in a position to assess the applicant's character, scholarly ability and aptitude for teaching to: D. H. Williamson, Professor of Geology, Geology Department, Laurentian University, Sudbury, Ontario, Canada. (2052) Applications are invited for a new staff position

GENERAL HOSPITAL SOUTHEND-ON-SEA

SOUTHEND-ON-SEA

Medical Physics Technician (male or female), grade V or IV, according to experience, required in the Physics Department of the above hospital, the duties including work in the radio-active isotope unit. Applicants must have passed the Ordinary National Certificate in Applied Physics or its equivalent. Salary scale for grade V, £711 to £1,004 per annum, or grade IV. £850 to £1,050 per annum. Possibility of residential accommodation for single woman. If required. Further details may be obtained from the Principal Physicist at the Hospital.

Applications, stating age, qualifications and previous experience, together with the names of two referees, to be sent to the Secretary, General Hospital, Southend-on-Sea, Essex, as soon as possible.

UNIVERSITY OF LEEDS DEPARTMENT OF GENETICS

The Department requires an assistant to help with a project involving amino acid sequence determination in a protein. The successful applicant could either be a young graduate in Chemistry or Biochemistry. graduate in Chemistry or Biochemistry, who would have the opportunity for some independent work towards a higher degree, or a technician with appropriate experience. In the former case the appointment would be made as a Research Assistant within the salary range £785 to £975 per annum, in the latter on the Technician scale £683 to £968, with the possibility of promotion to the Senior Technician grade. The appointment will be for three years in the first instance.

Applications, stating age, qualifications

Applications, stating age, qualifications and experience and naming two referees, should be sent to Professor J. R. S. Fincham, the University, Leeds, 2 before January 5, 1968. (2066)

THE CITY UNIVERSITY

The DEPARTMENT OF CHEMISTRY has a vacancy for a

TECHNICIAN

to assist a forward-looking, S.R.C.-sponsored research in which a combination of advanced techniques is used to study reactions on single crystals. The work is varied and experience with high vacuum equipment, the preparation and polishing of metal specimens, or electronics would be useful.

Salary in the range £800 to £850 per

Write to Professor R. C. Pitkethly, The City University, St. John Street, London, E.C.1. (2064)

UNIVERSITY OF WARWICK SCHOOL OF PHYSICS

SCHOOL OF PHYSICS

Applications are invited for LECTURESHIPS in both THEORETICAL AND EXPERIMENTAL PHYSICS available from October 1, 1968. The School of Physics has special interests in solid state physics and the physics of materials, and preference will be given to candidates with experience in this field of research. Salary in the scale £1.470 to £2.630, together with F.S.S.U. benefits. Removal allowance payable.

Further particulars and application forms may be obtained from the Registrar, University of Warwick, Coventry, CV4 7AL; closing date January 15, 1968. (2084)

UNIVERSITY OF WARWICK SCHOOL OF MOLECULAR SCIENCES POST IN BIOCHEMISTRY

POST IN BIOCHEMISTRY

Applications are invited from Biochemists for a Lectureship or Assistant Lectureship in the School of Molecular Sciences. The successful applicant will be expected to assist both in the teaching of undergraduates and in the running of an M.Sc. course in Molecular Enzymology. Salary in the scale of £1.470 by £90 to £2.010 by £85 to £2.180 by £90 to £2.270 (bar) by £90 to £2.630 (Lecturer), or £1.105 by £75 to £1.180 by £80 to £1.340 (Assistant Lecturer), together with F.S.S.U. benefits. Removal allowance payable.

Application forms and further details may be obtained from the Registrar, University of Warwick, Coventry CV4 7AL. Applications should, if possible, be received by January 15, 1968. (2083)

PLANT BREEDER FOR STUDIES IN VEGE-table breeding and their application to develop-ment of improved varieties. A good honours degree in plant science and postgraduate experi-ence in plant breeding or genetics expected. Ap-pointment in grade Scientific Officer (£926 to £1,574) or Senior Scientific Officer (£926 to £2,155), depending on age, qualifications and ex-perience. F.S.S.U.—Applications, quoting three referees, to Secretary, National Vegetable Re-search Station, Wellesbourne, Warwick (from whom further particulars may be obtained), by January 12, 1968. (2082)

UNIVERSITY OF SHEFFIELD CHAIR OF GEOGRAPHY

CHAIR OF GEOGRAPHY

Applications are invited for a CHAIR of GEOGRAPHY which will become vacant on the retirement of Professor Alice Garnett in September, 1968. Candidates should have a special interest and experience within the general field of human geography and, preferably, some research experience in the application of quantitative methods in their chosen field. Salary in the range approved for professorial appointments. F.S.S.U. provision.

Further particulars from the Registrar, to whom applications (fourteen copies), should be sent by February 19, 1968. (2078)

UNIVERSITY OF BIRMINGHAM CHAIR OF CANCER STUDIES

CHAIR OF CANCER STUDIES

Applications are invited for the newly-established Chair of Cancer Studies and the Directorship of the Laboratories of the Birmingham Branch of the British Empire Cancer Campaign. The joint appointment will be held in association with the Division of Pathological Studies in the Faculty of Medicine and Dentistry. Applicants should preferably but not necessarily, have interests in the field of cell biology and/or immunology. Salary in the range: £3,570 to £4,990 (Non-medical and Pre-clinical) or £3,465 to £4,885 (Clinical). F.S.S.U.

Further particulars may be obtained from the Registrar (R.S.). University of Birmingham. P.O. Box 363, Birmingham 15, to whom applications (twelve copies), naming three referees, should be sent by February 12, 1968. (2076)

UNIVERSITY OF VICTORIA VICTORIA, B.C.

DEPARTMENT OF PHYSICS

Applications are invited for faculty positions from July 1, 1968. A Ph.D. is required. Duties will include teaching and research. Preference will be given to applicants with research interests in one of the following fields: Astronomy, Geophysics. Fluid Mechanics, Nuclear Physics. Appointment may be made at any rank depending upon background and experience. Salary floors in 1967-68 are \$14,500 for Professor. \$11,500 for Associate Professor, and \$9,200 for Assistant Professor. Professor.

Letters of application should be sent to Pro-fessor J. L. Climenhaga, Head, Department of Physics, and should include a complete curricu-lum vitae and the names of three or four feet (2075)

QUEEN'S UNIVERSITY KINGSTON, ONTARIO, CANADA DEPARTMENT OF CHEMISTRY

Applications are invited for admission to the graduate programme leading to the M.Sc. and Ph.D. degrees in Theoretical, Physical, Inorganic and Organic Chemistry. Teaching assistantships of \$3,700 involving a maximum of three periods per week laboratory supervision are available. Applications should be made to: Head, Department of Chemistry, Queen's University, Kingston, Ontario, Canada. (2073)

CHRIST'S COLLEGE CAMBRIDGE

CAMBRIDGE

The Master and Fellows of Christ's College propose to elect two schoolmasters as Fellow-Commoners of the College, one for the Michaelmas Term 1968 and another for the Lent Term 1969, in order to provide them with an opportunity to pursue their own intellectual interests and to follow recent developments in their subjects. A schoolmaster Fellow-Commoner will have no duties but he will be expected to reside in Cambridge for the period of Full Term; he will be provided with accommodation, free meals including dinner at High Table, and an emolument of £50. Preference will be given to candidates who have taught for at least seven years, who are below the age of 45, who are not graduates of Oxford or Cambridge, and who are teaching in a direct grant school or a maintained grammar school. It is hoped on this occasion to appoint at least one schoolmaster who teaches a subject or subjects in Natural Sciences.

Applications should be made before March 1, 1968, on the standard application form which should be obtained from, and returned to, the Master, Christ's College, Cambridge, (2070)

PRE- AND POST-DOCTORAL RESEARCH APPOINTMENTS. Biochemistry and Biophysics, competitive stipends, wide selection of research areas.—Inquire to Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma, 74074. (X1728)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BACTERIOLOGY

Applications are invited from medical candidates for a post of Lecturer in the Department of Bacteriology. The salary will be within the range £1,470 to £3,110 per annum, according to qualifications and experience.

experience.

Applications, stating age, qualifications and experience, together with the names of three referees, should be sent not later than January 17, 1968, to the Registrar, the University, Liverpool 3, from whom further particulars may be obtained.

Please quote Ref.: RV/292/N. (2056)

PROJECT ENGINEERS-MEDICAL ENGINEERING

MEDICAL ENGINEERING

Applications are invited from graduates in aplied or pure science for posts in the medical engineering team of Vickers Limited Research Establishment. The appointees will be required to assist in the development of equipment for both laboratory and clinical use. A main line of work at present in tissue and organ storage for spare part surgery.

The basic qualifications necessary are a good degree, inventive ability and experience in project engineering. Experience in bioengineering could be an advantage.

Applications, accompanied by a brief curriculum vitae, should be addressed to the Controller of Research, Vickers Limited, Vickers Research Establishment, Sunning-hill, Ascot, Berkshire. (2072)

UNIVERSITY OF DUNDEE DEPARTMENT OF PHYSICS

DEPARTMENT OF PHYSICS

Applications are invited from honours graduates for a post of Research Assistant in the Department of Physics to work on X-ray diffraction investigations of virus structure. The post will be supported by a Medical Research Council grant for three years at a salary of £700 per annum. The Research Assistant will be encouraged to read for a higher degree.

Applications, with the names of two referees, should be submitted not later than January 13, 1968, to Professor K. J. Standley, Department of Physics, The University, Dundee, from whom further information may be obtained. (2090)

LIBRARIAN/SECRETARY CANADIAN RED CROSS MEMORIAL HOSPITAL

TAPLOW, NR. MAIDENHEAD, BERKS

Required to take full charge of medical library and assist in preparation of manuscripts, etc. at M.R.C. Rheumatism Research Unit. Post vacant January 1. Salary in accordance with age and qualifications.

Applications to Hon. Director.

(2091)

UNIVERSITY OF LIVERPOOL DERBY CHAIR OF ZOOLOGY

Applications are invited for the Derby Chair of Zoology. The salary will be within the range approved for full-time professorial appointments.

Applications (twelve copies), together with the names of three referees, should be received not later than January 31, 1968, by the undersigned, from whom further particulars may be obtained. (Candidates overseas may send one copy only, by airmail.) Please quote Ref.: RV/295/N.

H. BIRCHNALL.

H. H. BURCHNALL, Registrar.

(2071)

ICI POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for ICI Research Fellowships tenable under a new scheme commencing October 1st, 1968. Fellowships may be held at the following universities:

University of Aberdeen
University of Aston in Birmingham
Bath University of Technology
The Queen's University of Belfast
University of Birmingham
University of Bradford
University of Bristol
Brunel University
University of Cambridge
The City University
University of Dublin, Trinity College
University of Duhdee
University of Durham
University of East Anglia
University of Edinburgh
University of Essex
University of Exeter
University of Glasgow
Heriot-Watt University
University of Hull
The National University of Ireland
University of Keele
University of Kent at Canterbury

University of Lancaster

University of Leeds
University of Leicester
University of Liverpool
University of London
Loughborough University of Technology
University of Manchester
(including University of Manchester Institute
of Science and Technology)
University of Newcastle upon Tyne
University of Nottingham
University of Oxford
University of Reading
University of St. Andrews
University of Salford
University of Sheffield
University of Southampton
University of Stratholyde
University of Surrey
University of Sussex
University of Wales
(including University of Wales Institute
of Science and Technology Designate)
University of Warwick
University of York

The Fellowships, normally tenable for two years, may be held in any field that falls within ICI's general sphere of interest: this includes many branches of chemistry, physics, the biological sciences, applied mathematics, engineering, and technology. Candidates should not be more than 28 years of age at the date proposed for taking up their Fellowship and must hold the Ph.D. degree or have equivalent research experience.

The initial stipend will depend upon qualifications and experience but will generally be within the range £1,155-£1,365 per annum, together with FSSU benefits. Awards of up to £1,680 may be made to engineers and technologists where industrial experience or other special circumstances warrant it. Up to one year of the tenure of an award may be spent at an approved European research centre. In such cases a small grant may be made towards travel expenses.

Application should be made, not later than February 16th, 1968, to the Registrar/Secretary of the university at which the candidate wishes to pursue his research. Candidates may apply to not more than three universities. In the case of such multiple applications each university must be informed of the other applications and the candidate must clearly state his own order of choice. Strong preference will be given to candidates proposing to carry out their research at institutions other than that from which they make application. Each application must be accompanied by a brief curriculum vitae, a clear indication of the field of research proposed, and the names of two referees. The final choice of candidates will be made by a National Selection Committee, comprising representatives of the universities and of ICI.

HUNTINGDON RESEARCH CENTRE RESEARCH OFFICER

is required to organise a large research project associated with comparative anatomical studies of the respiratory tract. The person appointed will be required to work mainly unsupervised and will be encouraged to publish results. The post carries an attractive salary.

Applications should be made to the Personnel Officer, Huntingdon Research Centre, Huntingdon (Tel. 2522), quoting LEM-T/10/G. (2100)

UNIVERSITY OF CALGARY

DEPARTMENT OF BIOLOGY

invites applications for positions at the level of Assistant or Associate Professor for posts in Invertebrate Physiology, Immuno-logy (or Immuno-temistry), Bacterial Physiology, Environmental Vertebrate Physiology, Developmental Plant Physiology, Anticipated minimum salaries: \$9,500 (Assistant level) and \$13,000 (Associate level).

Applications with curriculum vitae, including names and addresses of three referees, should be sent to:

Dr. J. B. Cragg, Dept. of Biology, The University of Calgary, Calgary, Alberta, Canada. (2099)

WEST OF SCOTLAND AGRICULTURAL COLLEGE ASSISTANT IN CROP HUSBANDRY

ASSISTANT IN CROP HUSBANDRY
Applications are invited for a post of Assistant in the Crop Husbandry Department (headquarters at Auchineruive) from candidates with an appropriate degree (preferably honours) and particular interest in practical and theoretical aspects of experimental work in Crop Husbandry.

The salary, according to age, experience and qualifications, will be on one or other of the following grades: Grade III, £1,396 rising to £2,193; Grade IV, £899 (Honours £926) rising to £1,277.

Conditions of appointment and application forms, obtainable from the Secretary, 6 Blythswood Square, Glasgow, C.2, should be lodged not later than January 12, 1968. (296)

UNIVERSITY OF ABERDEEN CHAIR OF PHARMACOLOGY

Applications are invited for a newly instituted CHAIR OF PHARMACOLOGY, with the Department of Therapeutics and Pharmacology. Further particulars should be obtained from the Secretary, the University, Aberdeen, with whom applications (12 copies) should be lodged by January 20, 1968. (X1898)

RESEARCH ASSISTANT REQUIRED FOR RESEARCH ASSISTANT REQUIRED FOR X-ray crystallography group working on the structure of organic compounds. Candidates should have a degree or comparable qualification in physical sciences or engineering and relevant experience would be useful but not essential. Salary in the range £900 to £1,400.—Apply in writing to: The Superintendent, University Chemical Laboratory, Cambridge. (2077)

HOSPITAL BIOCHEMIST (BASIC GRADE) required. Opportunities for research.—Apply to Dr. D. M. Matthews, Department of Chemical Pathology, Vincent Square Laboratories, 124 Vauxhall Bridge Road, London, S.W.1.

TECHNICIANS SENIOR AND JUNIOR with experience in Pharmacology, required for modern Research Laboratories.—Apply, in writing, with fullest particulars of education, previous experience, etc., to The Research Director. Biorex Laboratories Limited, Apsley House, 198 City Road, London, E.C.1. (2097)

FOR SALE AND WANTED

SCIENTIFIC ALMOST ANY learned periodicals, proceedings, transactions, etc., wanted. Also most books in print supplied.—H. Pordes, 529B Finchley Road, London, N.W.3., Tel.: 01-435 9878. (2065) WM. DAWSON & SONS LTD.
Back Issues Department,

16 West Street, Farnham, Surrey, England.

Tel.: Farnham 4664.

Offer top prices for: 'BACK RUNS OF JOURNALS IN SCIENCE AND THE HUMANITIES

FELLOWSHIPS AND **STUDENTSHIPS**

CHRIST'S COLLEGE CAMBRIDGE

CENTRAL ELECTRICITY GENERATING BOARD FELLOWSHIP

BOARD FELLOWSHIP

The College, in consultation with the C.E.G.B. and the University Department of Metallurgy offers a Senior Fellowship in Metallurgy offers a Senior Fellowship in Metallurgy for a period of five years in the first instance at a stipend of £1,400 per annum by £50 to £1,600 maximum and usual Fellowship privileges and allowances. The Fellow appointed will be required to become a member of the F.S.S.U.

The Fellow will be expected to work in one of the following areas of Materials Science: ceramics, intermetallic compounds, materials for electrical and magnetic applications.

Candidates should send their names, together with a brief curriculum vitae and the subject of their research to the Master before February 1, 1968. It is hoped to proceed to an election in the Lent Term and the date of commencement will then be fixed by arrangement with the successful candidate. (2069)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

DEPARTMENT OF CHEMISTRY

Several graduate studentships in chemistry are available, value \$2,500 to \$3,100.

Interested applicants who have, or expect to receive, an honours degree in chemistry or graduateship of the R.I.C., or equivalent qualification, should write to: The Head, Department of Chemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, (1841)

Date-1968

11. March 26.

12. April 2.

CHRIST'S COLLEGE CAMBRIDGE

RESEARCH FELLOWSHIP IN PLANT BIOCHEMISTRY

The College proposes to proceed, in consultation with the University Department of Biochemistry and Botany, in the Lent Term 1968, to the election of a Fellow for research in Plant Biochemistry if a candidate of sufficient merit presents himself.

Applicants for this Fellowship should, by the beginning of the Lent Term 1968, have completed three but not more than ten terms of research; but candidates falling outside these limits of standing may be admitted at the discretion of the Master.

The tenure of the Fellowship is for four years with a stipend of £750 a year if the successful candidate is the holder of a Ph.D. degree or £600 if he does not hold such a degree, together with a marriage allowance of £250 a year and a children's allowance of £50 per child a year in appropriate cases. In addition a Research Fellow is entitled to receive rent-free accommodation in College, dinners, and a commons allow-

Candidates should send their names, together with a brief curriculum vitae and the subject of their research, and the names and addresses of two referces, to the Master, before February 1, (2086)

LECTURES AND COURSES

UNIVERSITY OF LEEDS

ELECTRONIC COMPUTING LABORATORY

The Laboratory offers a ONE-YEAR POST-GRADUATE COURSE in ELECTRONIC COMPUTATION leading to the degree of M.Sc.

Please write for further particulars to Professor G. B. Cook, Computing Labora-tory, The University, Leeds, 2. (2067)

WALTHAM FOREST TECHNICAL COLLEGE AND SCHOOL OF ART Forest Road, London, E.17

SCHOOL OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

RADIOACTIVE TRACER TECHNIQUES

Tuesday evenings-6.30-9.30 p.m. JANUARY-APRIL, 1968

This course is designed to give an introduction to the major uses of radioisotopes in inorganic chemistry, organic chemistry, biochemistry and clinical chemistry. The emphasis will be on practical techniques sultable for adaptation to a wide variety of purposes. The course is recognized by the Department of Education and Science as supplying the additional requirements for school teachers as laid down in Administrative Memorandum 1/65.

Introduction. Fundamentals of Radioactivity Physics. Properties of Ionising Radiations. Ionisation Chambers, Proportional counters, Geiger-Müller counters, Scintillation counters. 1. January 16. Properties of Radio-isotopes. Properties of Iionising Radiations. Characteristics of Geiger-Müller counters. Characteristics of Scintillation January 23.
 January 30. 4. February 6. counters.
Double Radioactive Tracer tech-5. February 13. Statistics of Radioactive Assay. niques.
Protection against Ionising Radia-6. February 20. Safe handling of Radioactive Iso-Radioactive Assay. 7. February 27. Principles of Radioactive Tracer Trechniques.
Isotope Dilution Analysis.
Autoradiography.
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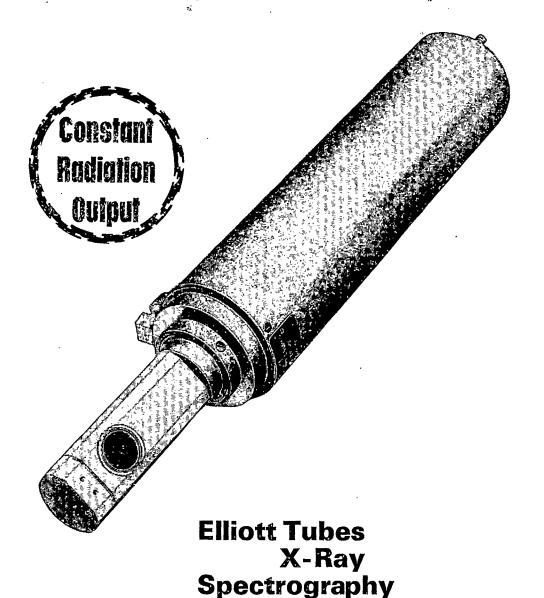
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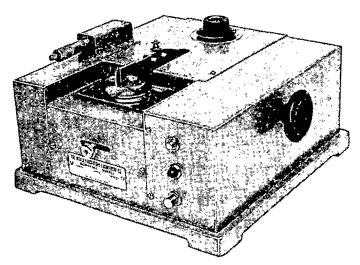
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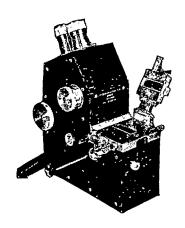
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NATURE Volume 216 DECEMBER 30, 1967

Does every Apple have a Worm?

THE Reith Lectures are a splendid device by means of which the British Broadcasting Corporation provides each year a public platform from which some individual can launch a strictly personal view of the condition of society. Although the lecturers are usually distinguished scholars-often scientists-they and their listeners are usually aware that the best performances are almost by definition the most provocative. Reith lecturer can be counted to have done his job if his listeners are robbed of some natural complacency. It follows that Reith lecturers have a good deal in common with old-fashioned preachers, which implies that people are prepared to tolerate from them a degree of directness and even of piety that would not usually be seemly. There is very little doubt that this year's lecturer, Dr Edmund Leach, the social anthropologist, now also Provost of King's College, Cambridge, has succeeded admirably in conveying to what seems to have been an attentive audience a clear and even controversial view of the problems of modern society. In the process, he has been amusing enough for his assertiveness not to seem too smug, which is more than a little to be thankful for.

Much of what Dr Leach has had to say is concerned with the social consequences of science and technology. Briefly, his theme is that science and technology have now enabled people to change the natural world, not just to understand it. But people—this is the lamenthave not risen to the occasion. As Dr Leach would put it, circumstances require that people should behave like gods, and they persist in behaving like people. One of Dr Leach's examples is the problem of population growth. It is plain, he says, that the trend of population has been "up and up" and that "if the human population goes on increasing continuously at anything like its present rate, then social life as we now know it will rapidly cease to be possible". It does not follow, of course, that a more crowded world would seem intolerable to those who lived in it, for people are remarkably adaptable. But a more crowded world would certainly be different. So does it not follow that people should seek somehow to influence the course of events, first of all perhaps by trying to work out criteria for deciding what kinds of social attributes must be counted desirable? And even if it should prove hard to influence events, is it not more fun-Dr Leach's word—to try to do so and fail than to stand passively aloof from all problems except those which are known in advance to be soluble?

So far, so good. Few would dissent from this opinion. Indeed, it has something in common with the old Edwardian view that the opportunities afforded by

science and technology will enable people to establish a far greater degree of control over circumstances than could have been possible only a few centuries ago. A part of the trouble with Dr Leach's argument is, indeed, that it pays too little attention to the reasons why this cheerfully optimistic view has been modified by the sometimes painful experiences of the past few decades. In the first of his six lectures, he spent a good deal of energy complaining about the "cult of objectivity" among scientists which, the argument goes, is assumed to be a good excuse for not seeking to anticipate the consequences of innovation.

Why do so many of us talk as if the advancing sweep of technology were a natural catastrophe beyond all human control? If you ask a professional scientist that question, he will probably simply reinforce your alarm by insisting that genuine human control is impossible. . . . That being so, the wise man must avoid all involvement in practical affairs; only by detachment can he hope to gair true understanding. That last sentence is a Buddhist precept, but it summarizes the basic philosophy of our science-laden society: all true science must aim at objective truth, and that means that the human observer must never allow himself to get emotionally mixed up with this subject matter. His concern is to understand the universe, not to improve it. Detachment is obligatory. It would be wrong as well as foolish for any scientist to accept responsibility for the practical consequences of his investigations. It is not the scientists' fault that we are threatened by the bomb.

To be fair, Dr Leach went on to admit that this argument may not "seem quite right" even to scientists. The difficulty is that this argument is not simply an exaggeration but a travesty.

The truth is not so much the opposite as something quite different. One of the most important of the developments in science in the past decades has been the spread of the positivist doctrine that objective truth is often an illusion. Particularly in the physical sciences, people are well aware that theories can be no better than the experiments which they suggest. The notion that radically different descriptions of the same system can be equally valid has been commonplace at least since the mid-twenties. This, of course, is why it is unfair of Dr Leach, later in his series of lectures, to tease the high-energy physicists for their attempts to define the properties of particles of matter too unstable to be observed directly. "So what?" is the answer. Logically, these particles are not so much physical realities as testable hypotheses, and the people most of all concerned would probably be as delighted as Dr Leach—though for a different reason—if the concepts were shown by experiment to be invalid. And certainly there is nothing in high-energy physics to justify Dr

Leach's fear that too great a preoccupation with the bits and pieces of which physical systems are made will preclude attempts to deal with systems as a whole.

Much more important, however, is the recognition which has grown up in recent decades of the limitations of the speed with which technical innovations can influence the conditions of society. This is at once a frustration and a safeguard. People still die of tuberculosis, for example. Agricultural productivity is lower than it could be, particularly where the pressure of the population is greatest. Lack of capital, not lack of technical knowledge, restricts the use at present made in developing countries of irrigation schemes, nuclear power stations and even schools and hospitals. Everybody knows that telecommunications are a boon, yet they are grossly under-used in most parts of the world, usually for reasons which have nothing to do with science and technology.

To many scientists, the apparent inertia of society in the face of technical innovation has been a chastening discovery, but it has usually been followed quickly by an avowal that the social sciences contain the means by which science and technology can be more tightly geared to the needs of society as a whole. And who can deny that the handling of the population problem would be much simpler if there were a better understanding of the reasons for the continuing increase of the population as well as better means of conveying simple information about agriculture and physiology to large numbers of badly educated people? In the same way, modern industrial techniques which could theoretically bring prosperity to impoverished people are often inadequately applied because those most in need of them lack quite simple skills which may have no direct connexion with science as such. The frustrations which these circumstances create have helped to spur on what now seems to be a growing appreciation of the social sciences, and even if what may be called the hard scientists are not universally enthusiastic about this prospect, it is a great pity to postulate a gulf which does not exist between science and the social sciences.

All this suggests that Dr Leach may have overlooked some of the difficulties of the problems with which he dealt and some of the tentative solutions for them which are being developed. Nevertheless it is valuable that he has in several ways provided a reminder that in trying to estimate the directions in which society should be encouraged to make fuller use of technology, people should consider quite radical possibilities. In this sense it is useful to be reminded that conventional patterns of family life may be as much of a strain as a source of stability. Certainly the English seem to be as much attached to boarding schools as the Israelis are to kibbutzim, and both of these are devices for diminishing the interaction between parents and children. Whether it is as valuable for him to suggest that everybody should be compulsorily retired at 55 is quite a different matter—there are surely more subtle and less wasteful ways of making sure that young people are not kept perpetually in chains by their elders, and there is plenty of American experience to bear this out. But perhaps Dr Leach has been more concerned to stir things up than to suggest realistic solutions for the problems which concern him. As a gadfly he has succeeded well enough, but it is unfortunate that in the process he has helped to encourage the view that there is somehow an inherent conflict between the interests of scientists and those of society.

Does the Past predict the Future?

THE conventional exercise of trying to decide what features of the past year have been most memorable is complicated, as far as science is concerned, by the fact that a year is altogether too short a time scale. In politics, momentous events can be contained entirely within a year. But discoveries in science are hardly ever as unexpected and unpredictable as are external events. A meaningful summary of past events would ideally be spread over a period more like three years than one. But, that said, there is some virtue in trying to make a list of what has happened in the year now nearly finished, if only for the sake of being more able to anticipate what is likely to happen next.

In this connexion, it is plainly important that a great deal has happened in the past few months to suggest that problems of cosmology are coming to the boil. The discovery in the past two years of what seems to be a cosmic microwave background has, of course, been a great spur to speculation about the origin and evolution of the universe. It is no doubt significant that this has now led to a renewal of interest in the construction of cosmological models (see this issue, page 1297) and a concern for the correctness or

otherwise of the general theory of relativity. Nearer home, events have shown the value of geophysical methods of studying the continuing movement of portions of the Earth's crust (see, for example, this issue, page 1276). Magnetic anomalies have turned out to be valuable means of following the spreading of material outward from the oceanic ridges on to the floor of the deep oceans. It is easy to predict that oceanographers will now be more concerned than ever to be given opportunities for studying deep ocean sediments, but it is, of course, impossible to know in advance what they will learn from them. It is also worth noticing that the success of the particle accelerators in high-energy physics has given people a familiarity with the unstable particles of matter which could not have been guessed at even a year ago.

In the biological sciences, there is plainly a great future for the studies which have in the past year or so helped to define the molecular structure of the substances which are effective in immunology, particularly the immunoglobins. It remains, of course, to be seen how quickly this will lead to a description of the tertiary structure of these materials and thus to a detailed understanding in molecular terms of the interaction between antigens and antibodies. If anything, the experience of the past year, during which there has been some cheerful progress with the X-ray diffraction analysis of tertiary protein structure, suggests that there is an awkward step between the description of a tertiary structure and an understanding of how this influences its function. That said, there is no doubt that strictly practical considerations will spur on attempts to understand in greater detail the mechanism of the immune response.

The year past has seen a further refinement of the genetic code, and, although it is now probably fair to expect that powerful uses will be made of the theoretical structure which has been established in the past few years, only a rash man would predict just what these will be. The experiment in which Professor A. Kornberg was able to replicate in the laboratory an infectious viral DNA is as much a proof of the rudimentary state of DNA chemistry at present as of the more awesome possibilities to which his sponsors at the National Institutes of Health gave some publicity. But if molecular biologists have a hard road ahead, so too do those who now believe that neurophysiology will bring important benefits.

Technologists will not be the only ones to look forward with pleasure to the use of increasingly sophisticated computing machinery. Artificial intelligence may smack too much of science fiction, but the phrase does also accurately suggest the wealth of exciting possibilities now opening up. To begin with, at least, the interest of computing machines which are more than mere automatic abacuses is that they will sharpen the faculties not merely of those who design machines and programmes for them, but of all those with an interest in problems of an essentially analytical character. Who knows, for example, what will be the intellectual consequences of the interest of the machine designers in methods of pattern recognition? comparison, the development of computing machinery as such may be at present less spectacular, but that is only a relative judgment. The consequences of the continuing trend towards faster and smaller electronic components will also be considerable. By contrast, the excitement which there has often been in recent years about the promise of superconducting materials seems not to have been too vigorously sustained by the experience of the past year, and it will probably be a long time before they are used to any great extent in heavy electrical engineering in the commercial world as distinct from the high-energy physics laboratories. In much the same way, the interest of transport engineers in new devices for moving people from one place to another is likely to be most fruitful in suggesting ways in which transport networks may be constructed. On the experience of the past year, the design of new vehicles as such is likely to be much less productive.

If it is hard to predict particular developments in science or even accurately to assess the significance of events in the recent past, it is, however, possible and even instructive to pick out trends in what seems to

be the temper of science. In this sense, it is important that in the past year there has been a marked slackening of the enthusiasm of governments for the lavish support of scientific research and industrial development. In the United States, for example, it seems as if special historical factors such as the commitment in Vietnam have tightened purse-strings, but the chances are high that, even if it were not for historical circumstances like these, the rapid increase of the science budget in recent decades would by now have slackened. Much the same is true of the way that things have gone in Britain. Although it is at first sight tempting to link the slackening of the growth of the research budget to the continuing economic crisis, there is also a possibility that expenditure would have tailed off even if there had been no external shortage of funds. What seems to be happening is that governments and the scientific institutions responsible for planning strategies of research are more than ever anxious to find criteria for relating the cost of research to the potential benefits. In the long run this is a good development, but it is a great misfortune that the first result has been to reduce the sums being spent on educationally desirable objectives.

Curriculum Development

THE Nuffield Foundation Science Teaching Projects. which became administratively under Chelsea College-University of London in August, have now moved to the temporary quarters of the Centre for Science Education established by the college at Pulton Place, SW6 The director of the centre is to be Professor K. W. Keohane, recently appointed to the chair of science education in the university, who is also co-ordinator of the Nuffield Science Teaching Projects.

A grant of £5,000 per annum for two years has also been received from Charter Consolidation Ltd. for use with the project to enable a study to be made of the ways in which applications of science might be introduced into the Nuffield teaching materials at a level. Dr Mansell has been seconded from Hatfield College of Technology to work with Dr Van Praagh on this work during 1967 when the main emphasis will be on chemistry. Later, similar studies will be undertaken in physics.

Less to Spend

THERE seems very little danger that the first round of restrictions on the scale of public expenditure in Britain will seriously hamper the prosecution of important projects in research and development. Ever since the devaluation of sterling on November 14, it has been clear that the British Government would have to make considerable economies, and the new Chancellor of the Exchequer announced a few days before Christmas that a total of £71 million would be cut from the expenditure of the nationalized industries in the coming financial year. In practice, however, the collective expenditure of the nationalized industries is so great that £71 million is not an important sum. For many of the industries concerned, the reduction will be most simply obtained by better management—

the General Post Office, for example, hopes to save £11 million in the coming year by running down its stocks of supplies and by postponing the renewal of various kinds of ancillary equipment-motor vehicles, for example. The postponement of various items of capital expenditure is, however, the most favoured expedientthe railways, for example, will put off the start of the electrification of the line between London and Glasgow for a year, while the British Airports Authority will postpone its scheme for a helicopter terminal for a similar period. The Central Electricity Generating Board will probably take the opportunity afforded by the Government's request to reduce expenditure by £17 million to put off the scheme for building a power station at Seaton Carew in north-east England. In the past few months, there has been some dispute about the fuel to be used for this power station—coal, oil or nuclear power. Coal interests have been urging that it would be unseemly to burn anything but coal in a power station built on the edge of a coalfield, and there seemed at one time to be a possibility that the Prime Minister had given an undertaking that this point of view would not be overlooked. It would be understandable if the Central Electricity Generating Board welcomed the opportunity with which it has now been

Research is likely to be more directly affected by the request for a reduction of expenditure of £3 million in the budget of the Atomic Energy Authority than by any of the other measures, larger though they may have been, which were announced by the Chancellor of the Exchequer. It seems to have been decided that the chief reactor projects will not be affected, which is entirely sensible. It is, however, significant that the Atomic Energy Authority now considers that the Dragon reactor is somewhere near the top of this list, along with the advanced gas-cooled reactor and the fast reactor. Evidently there are now high hopes that the technology of the Dragon will help enormously with future development of the advanced gas-cooled reactor. But if most reactor projects are sacrosanct, it probably follows that the authority will have to look to its research establishments for economies. The trouble here is that most of what could be done to economize at Aldermaston would not count in the authority's favour because it is paid for out of the defence vote, while the fusion laboratory at Culham is already faced with the prospect of trimming its sails by 50 per cent over the next five years.

In the circumstances, it is perhaps inevitable that the main burden of the economies ahead will fall on the laboratory at Harwell. It may even be that the Chancellor of the Exchequer is hoping that the dilemma which he has created will persuade the Minister of Technology finally to decide what should be done about Harwell. His colleagues in the British Government—and certainly those outside who reckon to keep a watch on developments like these—will be hoping that the necessarily stop-gap reductions of expenditure now announced will quickly be followed by policy decisions which promise a more permanent reduction of the scale of government expenditure.

London Intelligences

THE Research and Intelligence Unit of the Greater London Council has now been in action for nearly a year, the director of the unit, Dr Bernard Benjamin, having been appointed in 1966. Two divisions of the unit—GLC Statistics, and Research, are well established and a third which will serve the London boroughs will begin work early in 1968. The work of the unit, with particular reference to the field of information and intelligence, was described in a paper by R. G Dunsmore and R. E. Fry at a recent meeting of the Institute of Information Scientists.

The Research and Intelligence Unit does not have complete responsibility for statistics and research in the GLC, for separate departments such as housing took them over between the setting up of the GLC in 1965 and the formation of the unit. The unit's job, among other things, is to co-ordinate such activities. Mr Fry quoted three other aspects of the original terms of reference of the unit that are particularly important, and discussed the actions taken by the unit in these directions. The purposes mentioned were the setting up of an information system, which would contain statistics for forecasting material trends, and the publishing of a regular series of London statistics.

With the wide range of activities of the GLC involving an annual budget of over £400 million, the unit had to decide what statistics would be most useful. The conclusion was reached that information is required to support the needs of strategic planning in the widest sense. The unit would be the only body within the GLC with a comprehensive view of information kept in the different departments, and because of this could advise on gaps, unnecessary overlaps, coding and classification. One working group that has been set up is seeking agreement between certain GLC departments and the London boroughs on standardization of units for statistical data on transport and land use.

As the clerk to the council, in whose department the unit is located, has recently been up-ranked to director-general, to be the GLC's principal adviser on policy, the unit will be in effect his co-ordinator.

Schools Council and A-Level

THE Schools Council has at last published the document which embodies its proposals for a new pattern for the sixth form curriculum (Some Further Proposals for Sixth Form Work, HMSO, 3s. 6d.). This document has already been circulated to universities and was indeed the subject of the discussion among representatives of the universities at a meeting of the Senate House of the University of London on November 17. The proposals have been circulated to universities by the Standing Conference on University Entrance. The first reaction of the universities at the meeting in November was one of hostility to the Schools Council's proposals, chiefly on the grounds that they do not cater adequately for the need for a broader curriculum in the sixth form. The matter is to be discussed more formally at another meeting in the new year, and the universities are hoping to give the Schools Council a formal reply at Easter or thereabouts. The chances are that the universities will suggest, through the standing conference, an alternative to the sixth form pattern suggested by the Schools Council which takes the form of a broader pattern of studies (see Nature, November 25, 1967).

The essence of the new proposals by the Schools Council, which are said to have evolved from the

previous proposals for a mixture of "major" and subjects in the sixth form, is that externally examined A-level subjects should be restricted to two, even for intending candidates for university entrance. In addition, the Schools Council proposes that there should be a number of "elective" courses designed by schools to reflect the interests of staff and students and able to support and sometimes complement the remainder of students' work in the sixth form. It is intended that elective subjects should be examined or assessed internally by the schools and not externally by the existing examinations boards. One feature of the proposals is that the Schools Council has outlined ways in which elective studies could form a part of the sixth form curriculum for students not bent on university entrance but who continued to follow courses at Ordinary level instead of at Advanced level. The Schools Council also emphasizes the need somehow to diminish the pressure of competition for university places and much of its concern is with the conditions on which universities may be prepared to give students suitable assurances.

The argument is sustained by statistics which illustrate the changing pattern of the sixth form in British secondary schools. For one thing, for example, the population in the sixth form is growing rapidly, at least as far as can be seen from the increase of the proportion of seventeen year olds staying on at school from 8·1 per cent in 1955 to 11·1 per cent in 1960 and 13·8 per cent in 1965. At the same time, the proportions of sixth formers not following A-level courses seem to be increasing—the Schools Council has been given access to figures collected by the Inner London Education Authority which show that non-A-level pupils in the first year of the sixth form accounted for 7 per cent of the age group in 1963 and 10·5 per cent in 1967, only four years later.

Mathematics for the Less Talented

MUCH of the current interest in the reform of the school curricula has been concerned with the more talented pupils. In mathematics, the range of experimentation in the United Kingdom is unprecedented, with four large projects in progress, and a number of smaller ones, but again there is a danger that the less talented pupil will be overlooked. The Schools Council has now produced a pamphlet-Mathematics for the Majority, by Mr Philip Floyd—which sets out to remedy the deficiency. (In the introduction he does point out that the Schools Mathematics Project is undertaking a revision of its texts to cater for the less talented, and that the Midlands Mathematical Experiment could be used for below average pupils by extending the time scale.)

The report begins by establishing the greatest limitation to progress—the dismal lack of mathematics teachers in the schools. Because this deficiency does not seem likely to be made good within the next few years, any scheme which is devised has to be appropriate to the teaching talent available—talent which does not always correlate closely with the actual mathematical ability of the teacher. The report suggests a variety of ways in which the interest of children can be sustained by relating the teaching of mathematics to things which they understand, and by the use of equipment—calculating machines, conic

sections, equipment for simple surveying—which can help to make mathematics seem less dry and theoretical. Collaboration between mathematics departments and departments of English, history, geography and science can also help to relate arithmetic to real life. The Stock Exchange, opinion polls, wages and systems of payment, old people's budgets, defence costs—all these can be incorporated into a general scheme which brings mathematics to life. "It might be said," Mr Floyd writes, "that this is, overall, very ordinary mathematics; but these are very ordinary children, and this is the world of mathematics they see around them." But schemes such as this, he emphasizes, do not close the door to the more imaginative mathematical world which the children do not see around them.

Fortunately, the adoption by a number of schools of the Mode II and Mode III forms of examination for the Certificate of Secondary Education enables much more freedom with syllabuses than would otherwise be possible. (In Mode II, the syllabus is determined within the school, but examined outside; in Mode III. the syllabus and the examination are both the responsibility of the school, subject to an external moderator.) This offers a real opportunity for variety, and Mr Floyd makes some suggestions—the mechanics of flight, a study of the computer, the construction of logical circuits, the study of gears, the mathematics of betting and gaming, more respectably called actuarial science. He also provides a series of charts which provide a basis for planning syllabuses. But the final word in the report goes to A. W. Whitehead—"The whole of mathematics consists in the organization of a series of aids to the imagination in the process of reasoning." Teachers, as well as pupils, need these aids to the imagination, Mr Floyd concludes.

Architects

TALKS are in progress between the Architectural Association and the Imperial College of Science and Technology over the proposed link-up, whereby the AA will become the fourth constituent college of Imperial College. To some, this statement might seem as old as the Albert Memorial, but at last it seems that light is beginning to dawn, and it is hoped that agreement will be reached early in the new year. The University Grants Committee, the third party in the discussions, has agreed to its part of the bargain, which is to provide a recurrent grant, as part of its allocation to Imperial College, for the running of the architectural school. A letter from Sir John Wolfenden to this effect, sent to the AA in August, broke through the deadlock that had been reached. Up to that point, the UGC had promised the grant only if the AA had enough money for the necessary new building in South Kensington, while the AA in its turn felt unable to raise the building money unless the recurrent grant was assured. Now that this part of the problem has been solved it remains to the AA and Imperial College to agree over the building funds. Once the merger has gone through, the AA will cease to be a private institution and will instead be financed by government money, In this way it will be the second private institution of any size that has joined the UGC fold, the other being the Institute of Estate and Farm Management which has just been incorporated into Reading University.

There may be other private institutes and research centres in Britain that are reflecting on the possibility of becoming part of a university, with the accompanying measurable benefit of government grants, but the UGC seems not anxious to encourage such unions at present, when funds are so limited. There are several architectural schools, for example, at present attached to local authority art colleges, which would dearly love to become university departments. The Leeds school is particularly keen on the idea, but has not so far had much joy from the UGC. When there is not enough money in the kitty to pay for expansion in existing university departments, it is natural that there is reluctance on the part of the UGC to take on extra establishments. When the choice lies between paying fees that would have come from other sources for existing students, and providing money for additional students, the UGC's policy is understandable.

Canadian Research Flourishes

The National Research Council of Canada has spent five times more on scholarships and research in the universities in the past ten years than in the previous forty years of its existence. According to the council's annual report, the sum spent on Canadian universities has risen even in this past decade from \$10.5 million in 1962 to more than \$35.5 million last year. Nearly half of this sum is spent on grants and equipment, and the report estimates that, on the average, each professor received \$6,400 for equipment last year, a thousand dollars more than two years ago. Expenditure on university research is expected to continue to increase.

In 1966, the council established post-industrial experience research fellowships of \$6,000, to allow engineers and scientists in industry a year for research in government or university laboratories. The scheme is to run for a trial period of two years, five fellows being elected in each year. This year, to celebrate its own golden jubilee and the centenary of Canadian confederation, the council has decided to set up a new group of scholarships. These will be awarded to graduates for research leading to doctorates in universities other than those from which they obtained their first degrees. It is hoped by this means to foster cultural exchange between the different regions of Canada, in the spirit of the centennial year,

Homeless Doctors

SINCE the University of St Andrews became independent of Dundee University in October this year, it has been impossible for St Andrews medical students to do their clinical training. Most have continued to

go to Dundee for this purpose.

On December 19, however, the university announced that a link-up had been established between St Andrews and the University of Manchester. From 1973 onwards, preclinical students at St Andrews will go to do their clinical training in hospitals in Manchester, at the United Manchester Hospitals, and also at Withington Hospital, which is being developed into a full teaching hospital. Students at St Andrews will study for three years in the faculty of science on an extended preclinical course leading to a BSc degree, before going to Manchester.

A number of medical schools were approached in the hope of finding accommodation and several were willing to take a few students. The agreement with Manchester, on the other hand, will take the whole output of premedical students. According to Professor A. E. Ritchie at St Andrews, the liaison committee which will operate the scheme will probably be made up of Professor A. C. P. Campbell, Dr F. B. Beswick and Mr A. R. Anscombe representing Manchester, and Professor G. R. Tristram, Professor A. E. Ritchie and Professor R. Walmsley representing St Andrews.

Training Manpower

Last week the Ministry of Labour announced a new scheme for the training of craft apprentices in the development areas. The scheme extends the range of training grants already made under the levy and grants system introduced by the Industrial Training Act of 1964. The new grants are of two distinct types; one will provide capital grants towards the cost of providing places for training off the job, and the other will reward-employers who take on additional trainees for on-the-job training.

The capital grants, the ministry explains, are intended to encourage the provision of places for training off the job. The ministry will agree with the training boards a standard capital cost for providing the premises and equipment for the training. The employers who provide the new premises will get a grant worth 60 per cent of the cost of providing the places. If the employer can increase the number of training places without building new premises, the grant will be 60 per cent of the costs incurred. Grants of similar scale will be given in industries where there is as yet no recognized form of training. The per capita grant is intended for industries where training is normally done on, rather than off, the job. Employers who make more places available for trainees will be entitled to grants equal to £100 for each additional trainee.

So far, the industrial training boards cover industries which employ over 10 million people, a figure which will soon be increased to 15 million when new boards, already announced, come into operation. Next year the ministry estimates that some £120 million will be distributed to individual firms as training grants. The ministry is keen to make the schemes known to more firms, and has produced a pamphlet—Assistance with Industrial Training in the Development Areas (HMSO)—which explains them.

British Aircraft

THE British Government is now the dominant force in British aviation. There is nothing new in this, of course, but the point has been hammered home with particular emphasis in recent months. First there was the decision to build London's third airport at Stansted, then the apparent dithering over the Concorde, finally the announcement that British Aircraft Corporation would not be allowed to build the BAC 2-11. All these are important commercial decisions, but only in the last is it easy to see why the Government acted as it did. Although BAC wanted to build the 2-11, and British European Airways wanted to fly it, nobody wanted to pay for it. According to Mr Crosland, the President of the Board of Trade, it would have cost £120 million

to develop, against a mere £15 million for the Trident 3b, the alternative aircraft. In the circumstances, it was obvious what the outcome would be:

Despite the cancellation, BAC seems to have plenty of work; for one thing, it will be responsible for the British end of the Concorde production. The BAC 1-11, the company hopes, will continue to sell for a good time yet, and there may still be a few orders for the VC 10 and the Lightning. And the news that the Trident 3b is to be developed is bound to be an encouragement to Hawker Siddeley, disappointed by the Government's refusal to sell Buccaneers and Nimrods to South Africa. BEA is expected to want about 40 Tridents, and there may be a few orders from elsewhere now the decision to go ahead has been taken. But BEA will only accept the aeroplane on sufferance, and presumably as collateral to a Government agreement to provide the corporation with a financial reconstruction along the same lines as that enjoyed by BOAC. This is because BEA believes that the Trident will be more expensive to fly than the alternatives, one of which is the Boeing 727-200.

The alarming feature of the situation is not that the Government now pays the piper—that is probably inescapable—but that it is calling such a peculiar medley of tunes. While the Board of Trade has been mulling over the 2-11, the Ministry of Technology has been lavishing its formidable enthusiasm on the European airbus, a collaborative project in which France, West Germany and Britain are involved. To judge by the published reactions of some of the airlines, the airbus is no more immediately attractive than the What the Government should now be asking itself is whether the 2-11 would have been more attractive clad in the guise of European collaboration. Looking at it another way, would the airbus have seemed so desirable if BAC had proposed to build it alone? 'And why should two projects which clearly influence each other be the responsibilities of separate ministries? There is a real danger that the worthwhile aim of European collaboration is being used to justify projects which cannot justify themselves. That kind of muddled thinking will serve nobody's purposes but those of the United States aircraft companies.

Fall-out over Britain

Three Chinese nuclear explosions have occurred since the last recorded measurements were made in the United Kingdom of fission products in rainwater and airborne dust. In addition, France tested several nuclear devices in the South Pacific between June 1966 and July 1967. But in spite of this the latest Atomic Energy Authority research group report, Radioactive Fallout in Air and Rain: Results to the Middle of 1967 (UKAEA, 7s.), shows that the concentration of long-lived fission products over the United Kingdom has continued to decrease.

Prepared by the Health Physics and Medical Division of the Atomic Energy Research Establishment in Harwell, Berkshire, the report describes methods of sampling and analysis which were used over the period January 1966—July 1967: the data have been tabulated and, where appropriate, have been presented in graphical form. Airborne dust was sampled at one metre above ground by passing appropriate quantities of air through cylindrical filters of esparto grass paper

at a number of stations in Britain, Gibraltar, Hong Kong, and Pretoria and Aspendale in Australia. Samples of rainwater were collected in polythene bottles, containing carrier solution to reduce loss by adsorption, at eight stations in Britain and twenty stations elsewhere. Individual activities were determined either by radiochemical methods or by gamma-ray spectrometry.

The mean deposition of Sr-90 in rainwater at Milford Haven for the first half of 1967 was found to be 50 per cent of that for the first half of 1966. About onetenth of the long-lived fall-out in early 1967 was attributed to the Chinese explosion of December 28. 1966: this increase was sustained for several months because debris had been injected into the stratosphere. No short-lived fission products, such as iodine-131 from the Chinese explosion of June 17, 1967, were detected in Britain although there is evidence of debris from this explosion in the autumn of 1967. It is interesting to note that plutonium-238 was released over the southern Indian Ocean in April 1964 when an American satellite containing a SNAP-9A power source of 17 KCi of Pu-238 failed to orbit. During the last few months of 1966, cumulative deposition of long-lived fission products was reduced for the first time as radioactive decay exceeded new deposition. In early 1967, however, deposition again slightly exceeded radioactive decay.

Partially-hearing Children

A SURVEY of the special arrangements made to help partially-hearing children has been published this week by the Department of Education and Science. In 1966 there were 162 units for such children in England and Wales, of which the survey, conducted by HM inspectors of schools and medical officers, has covered 74.

The units are attached to schools and their purpose is to enable partially-hearing children to join the ordinary classes as soon as they are capable of doing so. Great care must be exercised in selecting children for the units, because severely deaf or otherwise retarded children will hold back the progress of the others. The survey recommended that more careful attention should be paid to the criteria for selecting children for the units. Children should be seen to have the prospect of learning to speak in a natural way more by listening than by lip-reading, and the unit's teacher should be consulted before selection so as to ensure that the child does not present too heavy a load of additional handicaps.

The survey suggested that units functioned better when they were large enough to include more than one class and more than one teacher. Though most of the units inspected are well supplied with audio-visual equipment and other aids, it is surprising that there has so far been little serious development in the use of programmed instruction or film loops.

programmed instruction or film loops.

All teachers of partially-hearing children are suitably qualified as teachers of the deaf. The survey found little to criticize in the standard of instruction within the units and indeed commended the progress made, but it pointed out that teachers who devote all their time to the units are often unaware of, and tend to underestimate, the standards reached by normal children.

The encouraging tone of the survey is marred only by the widespread lack of understanding found to exist in parents' attitudes towards their children's social and educational handicap. The survey stresses that parents need guidance and support in helping them to accept that their child may not make normal progress without special assistance.

Water Development

In its annual report, whose principal conclusion is that Britain has too much water—not too little—the Water Resources Board gives details of some interesting research being carried out under its aegis. There has, for example, been a programme of research into the regulation of the river Dee. Work on the generation of synthetic river flow data has been in progress at the Water Research Association and Lancaster University, and mathematical models representing catchment response are being studied at Imperial College. At the same time, attention is being paid to the problems of fisheries in the river, and it is hoped to build a fish counting station with an experimental electric fish barrier.

Less progress has been made in investigations of artificial recharge as a means of augmenting resources, largely owing to lack of staff. The report suggests that, although desalination plants similar to those built and projected in the United States will not be profitable in Britain in the immediate future, there is sense in the use of flash distillation plants in conjunction with conventional surface reservoirs to meet intermittent excess demands. It is important, therefore, that operating experience of a multi-stage flash distillation plant should be obtained in Britain. The pilot electrodialysis plant in Essex for desalting brackish ground water from the chalk is now in operation, however, and there is a possibility that such a system could be used for reclamation of water from industrial and sewage effluents.

A most important part of the Water Resources Board's programme is the development of automatic hydrometric instrumentation. It is hoped that by mid-1968 equipment will be available for recording climatological and river water quality data on magnetic tape in such a way that the information can easily be fed into a computer. The board has negotiated with instrument manufacturers as a group, rather than individually, and feels sure that the advantages of this particular procedure have been significant. An ICT 1901(S) computer has very recently been acquired, and a team of staff to handle it recruited. The new computer section will serve all the divisions of the board. At present river flow data have been processed for river authorities, and the new computer will allow the service to be extended to cover climatic and other data.

The total expenditure of the board on research and on contributions for the support of university and river authority work was £212,500 last year.

Production in Japan

Japan is expected to overtake West Germany in the production of motor vehicles this year, and so become the second largest motor manufacturer in the world. More than three million cars will have been built, an increase of no less than 40 per cent since 1966. Nor will this rate of growth fall, for production in 1968 should easily exceed four million vehicles.

Japanese exports used to be thought of as imitative and voluminous. Japan's achievements in shipbuilding and light engineering have convinced Westerners that the first of these epithets is unjustified, but it would surprise many to know that Japan's exports in 1966 were little more than half those of Britain—\$8,450 m against \$14,000 m, at the old rate of exchange. But if those in the West have exaggerated Japan's export performance, they have paid little attention to her importance as a producer of goods, as the table shows.

		W. Ger	_		
	Japan	many	Britain	Franc	e Italy
Sulphuric acid	5-66	3.06	3.3	2.9	4.75 million tons
Synthetic	0-00	9-00	0.0	2.9	4.19 Hillion sons
fibres	1,098	1,025	846	475	650 million lb.
Steel	41	36	27	19	12.6 million tons
Motor					
vehicles	1.88	$2 \cdot 9$	$2 \cdot 2$	1.5	11.4 million
Electricity	180	172	148	101	82 thousand million units
TV sets	4.2	$2 \cdot 8$	1.6	1.25	1.2 million
Ships	5.4	1.02	1.07	0.48	0.44 million tons launched

Statistics of Industrial Production, 1965.

The foregoing table demonstrates the somewhat surprising fact that even in 1965, Japan was producing more steel, electricity, television sets, and, of course, ships than France and Italy combined, and that her output of synthetic fibres and motor vehicles was even then comparable with the combined totals of those two countries. It is interesting to reflect on the difference between the economic and political weights of the first four countries listed here. Japan's reliance on the United States as a trading partner precludes extravagant political gestures. Perhaps the next few years will see Japan rise to a diplomatic importance commensurate with her strength.

Pooling Ocean Resources

TWENTY-EIGHT nations have joined in a proposal to set up a United Nations committee to study the peaceful international exploitation of the ocean floors of the world: the United States is a sponsor of the proposal.

According to a draft resolution presented to the General Assembly's main political committee, the proposed committee will be required to conduct a survey of the past and present activities of the United Nations and its various agencies regarding the ocean floor and of existing international agreements concerning them; to summarize the scientific, economic, technical, legal and other problems which are involved in international use of underwater resources; and, finally, to suggest the practical means by which international co-operation could be promoted in the exploration, conservation and use of the ocean floor and its sub-soil.

The recommendation grew out of a debate on the subject in the political committee, and it was Malta which suggested that resources beneath the sea should be exploited for the benefit of all mankind. In deference to national claims of territorial waters, the draft resolution restricts its recommendations to the "sea bed and the ocean floor, and the sub-soil thereof, underlying the high seas beyond the limits of present national jurisdiction". Before the new committee can be set up, however, the draft resolution must

first be endorsed by the political committee and then by the General Assembly as a whole.

Marketing is King

THE British engineering industry must give greater attention to marketing. This was the message delivered by the managing director of Massey Ferguson (UK), Mr J. W. Beith, in his lecture last week to the Institute of Mechanical Engineers.

Of the 14,992 engineering establishments in Britain, six hundred, or 4 per cent, account for two-thirds of the industry's total exports. An NEDC report in 1965 indicated that the probable rate of return on new investments in the engineering industry has been between 6 and 8 per cent during a period when the acceptable break-even rate was regarded as 15 per cent. Figures such as these, and the high import levels of foreign machine tools, indicate that some manufacturers fail to exploit in the market place the undoubted technical expertise which they possess.

Marketing, Mr Beith declared, should be the paramount activity in a company's operations, and must antedate and determine even the discovery in the research laboratories. If a product is to be tailored to its market, the whole strategy of a firm must be oriented towards the complex of research and planning activities which constitute marketing. Market research includes studies of such factors as the political and economic influences on a market, the demographic trends, the distribution of incomes and expenditures, and the likely appeal of alternative products. These findings form the basis for market planning, the function of which is to specify the product which will most profitably meet the situation described by market research. Market planning defines the physical characteristics of the product, its price, utility and styling; only then can design and manufacture begin, and any afterthoughts will be increasingly expensive. The goal of market planning is to strike the best balance between minimizing unit production costs and maximizing market appeal.

"The future of large sectors of engineering production," Mr Beith said, "will be influenced by the impact of marketing on engineering firms, and by the changes which it will bring about within those firms." The increasing importance of marketing is likely to favour the large firm against the small and to lead to fewer and larger firms. But perhaps the greatest change as firms become more market oriented, Mr Beith suggests, is that engineers will be expected to work within the parameters of the marketing approach and to think in terms not of the product itself but of its marketability.

Parliament in Britain

Nuclear Power

ASKED what effect the proposed reductions in capital spending by the nationalized industries would have on the construction of nuclear power stations, Mr Richard Marsh, the Minister of Power, replied that the start of one power station would probably be deferred. (Written answer, December 18.) At question time on the following day, Mr Wedgwood Benn, the Minister of Technology, defended his decision to close the Culham Establishment, set up for research into the generation of electricity by means of fusion. He said that that

programme was well over the horizon, perhaps as much as 25 years ahead. As for the proposal, put to him by Mr David Price, that he transfer Culham from the Atomic Energy Authority to the Science Research Council, he said that work on astrophysics at Culham was to be undertaken by the SRC, and that he was taking Mr Price's proposals seriously.

Sonic Booms

THE subject of sonic booms came up in both written and oral questions on December 19. The Minister of State for the Ministry of Technology, Mr Stonehouse, said that no decision had been made about restrictions to be placed on the speed of the Concorde airliner over inhabited areas. He estimated that the total market for the aircraft would be reduced to 65 per cent if a ban were to be imposed on the making of supersonic booms overland. Mr Stonehouse went on to say that he did not think it would be wise to start international discussions on supersonic flight over land. Britain, he said, was in touch with France and the United States and would co-ordinate with them in any decision. Nobody could be certain about the characteristics of the bang from Concorde until the aircraft was flying, but there was a great deal that could be learned, and was being learned, from the intensive tests carried out in the United States. In written replies to questions put to him by members, Mr Stonehouse said that the Ministry of Technology would shortly issue a statement about the July sonic boom tests. Thirteen local authorities had commented on various aspects of these tests.

American Aircraft Imports

In a written answer, Mr Stonehouse gave figures for the trade in aircraft and space products between Britain and the United States in the first ten months of this year. Britain imported £64,541,000 worth of aircraft, exporting £37,645,000 worth to the US. Mr Stonehouse thought that the export prospects of the aircraft industry would be enhanced as a result of devaluation. A Procurement Committee had been established under the British National Export Council and the Confederation of British Industries with the object of securing orders within the aerospace and avionics fields. (Written answer, December 20.)

Civil Defence Warning System

LORD STONHAM, Minister of State, Home Office, in a debate in the House of Lords, gave details of the warning system which would be used to alert the civil population in the event of war. He claimed that the warning system was virtually instantaneous and nation-wide. There were two types of warning signal. The first was a siren similar to that used in the last war. The difference was that the system would now be operated centrally, and warning would be communicated within 30 seconds to 22,000 different points. Each warning point was equipped with a radiac survey meter and, in the event of a breakdown in the communication system, the warning point operator would sound a warning when fall-out registered on the survey meter reached 0.3 röntgens per hour. the actual fall-out would be sounded by maroon. Distribution of maroons would begin early next year. In addition, fall-out warnings would be given by the BBC's wartime broadcasting system, picked up by transistor radios. (Debate, civil defence warnings, December 19.)

NEWS AND VIEWS

New Hearts for Old

THE publicity which surrounded the Washkansky heart transplant operation in Cape Town has caused the blurring of some details and the exaggerated prominence of others. And the picture has not been clarified by the dispute between those who say the operation was premature and those who claim that Professor C. Barnard anticipated other similar operations only through the fortunate concurrence of a suitable donor and recipient.

The morality of the operation has several times been questioned, but more by way of hypothesis than accusation. It is hard to see what special ethical conundrums are raised by a heart transplant. The doctor who decides that a patient is sufficiently dead for him to remove the heart while it is still functional takes a difficult decision, but this problem is not new to medicine. There is no sharp dividing line between life and death, and assessment of the transition has always rested on a complex of factors, not all of which may be objective. The first heart transplant does not raise a new problem, it exacerbates an old one. By doing so, it may help to draw attention to what seems to be the best of all safeguards—that the recipients of an organ transplant and the potential donors should be cared for by different doctors.

The interests of the recipient also matter, but Washkansky was undoubtedly made aware of the experimental nature of the operation, and his immediate assent for it precludes any reasonable doubt of his willingness to take the chance. In general, however, there is a case for asking that transplant operations should be undertaken only when there is a reasonable prospect that they can materially improve the chances that a patient can expect to enjoy a substantial period of reasonable health. This kind of decision has long been familiar in the treatment of cancer. It does not mean that treatments cannot be given for the first time, but merely that the pace of innovation is contained.

Not only surgeons will want to move cautiously, however. It has often been supposed that once heart transplanting becomes a viable operation, hospitals will be overwhelmed with applicants and that whatever selection method is devised will be, at the least, invidious. But a heart transplant is unlikely ever to become a straightforward and routine operation, and for many years yet it will require a team of highly experienced doctors. Ordinary people are unlikely to be overjoyed at the prospect of having to live with somebody else's heart, at least for several years to come.

If the moral and social implications of the operation have been exaggerated, the medical ones have remained uncertain, largely because the problems of tissue-typing

are as yet unsolved. Professor Barnard and his team were confident that enough was known to justify the risks, and Washkansky's survival beyond the usual period of tissue rejection must to some extent support their assessment. Nonetheless, for all the splendid surmounting of technical difficulties, the operation was not in the event successful. The crucial test lay not in the transplant of the heart itself but in the treating of the patient's immune defences. The failure of the immune defences to combat the final pneumonia signified the failure of the operation. The moral, if there is one, is that there would be great profit in spending more effort and money on research in tissue typing than is at present being spent. This, in the long run, is how successful transplants will be made.

Isolation of Permeases

from a Correspondent

The permeases—carriers or transporters—were until recently merely hypothetical entities, invented by biochemists to account for the puzzling phenomenon of membrane transport. Yet one by one permeases are being isolated, characterized and even, in one case, crystallized (Science, 156, 1627; 1967). A permease, defined by its ability to catalyse transport across an intact cell membrane, cannot be directly assayed when the membrane is no longer present. All procedures for monitoring the isolation of permeases therefore make use of ancillary features of the transport process—in most cases the ability of the isolated molecule to bind its substrate. Thus treatment of Escherichia coli with osmotic shock liberates a galactose-binding protein (J. Biol. Chem., 242, 793; 1967) and addition of the purified protein to cells depleted of binder restores their ability to accumulate galactose.

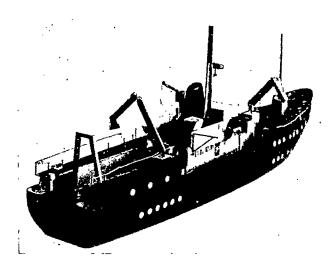
An experiment as striking as this has not yet been reported for the classic lactose permease of E. coli, but genetic evidence has now been obtained (Proc. US Nat. Acad. Sci., 57, 698; 1967) that the M-protein, a component of the lactose permease system, is indeed coded for by the permease gene. This gene maps between the β -galactosidase and galactoside transacetylase genes of the lactose operon. Binding of substrate to the permease is again the basis of the assay for the isolated M-protein, but the substrate is now used to protect an active centre against inactivation by N-ethyl maleimide, a reagent attacking thiol residues. The protected thiol is identified by its ability to react with additional, isotopically labelled, N-ethyl maleimide after the substrate is washed away. The M-protein is firmly bound to the spheroplast membrane, and released only by treatment with detergent. Permease negative mutants of E. coli do not synthesize the *M*-protein while in a temperature-sensitive revertant. Conditions which lead to the loss of permease activity in the intact cell lead also to a reduced yield of M-

protein. Mutants with deletion of the transacetylase gene are perfectly capable of forming M-protein. That the natural substrate, lactose, and also the excellent synthetic substrate thiomethylgalactoside do not block the inhibitory action of N-ethyl maleimide on the permease, and hence cannot be used in the labelling procedure, might reflect some property of the active centre of the protein rather than cast doubt on the correctness of its identification as the permease.

On the assumption that each molecule of M-protein possesses a single reactive thiol, a lower estimate of 9,000 permease sites per bacterium can be obtained. This compares well with the 3,000 sites per cell membrane shown by Kolber and Stein (Currents in Modern Biology, 1, 244; 1967) as being specifically labelled when the lactose operon is induced using thiomethylgalactoside. Extraction of cell membranes with sodium iodide solution liberates a labelled protein of molecular weight about 60,000. An earlier report of the labelling of cytoplasmic fraction apparently coded by the permease gene may represent some transient (nascent or degraded) form of the M-protein.

More Oceanography

The Natural Environment Research Council has not only approved the construction of the new oceanographic research vessel mentioned in its last annual report (see Nature, 216, 954; 1967), but it has also issued details of the specifications. The ship has been designed, by Yarrow-Admiralty Research Development, for operations in the regions of the Continental shelf and North Atlantic. The new ship will therefore be smaller than RRS Discovery, with a displacement of 1,000 tons and an overall length of 165 ft. She will be operated by the Scottish Marine Biology Association, and will be stationed at Oban, Argyll, where the S.M.B.A.'s new laboratories are at present being built, but she will be available to university workers.



Perhaps the most important innovation in the design of the vessel is the unorthodox stern, which allows the trawl to be brought in aft, instead of over the side as in most trawlers. Drag on towropes is a great inconvenience when delicate electronic equipment is being towed, partly because it is difficult to tow deep objects

close to the boat, and partly because of the induced vibration. The towing cables will therefore be faired to reduce turbulence, and this in turn has required the provision of a specially designed helical capstan. Steam turbines, not usually fitted in so small a ship, are also there to provide vibration-free movement.

Nine scientists will be accommodated on board, and they are apportioned a thousand square feet of laboratory space—a considerable area for so small a vessel. Some of this is to the side of the working deck at the stern, but all the laboratories are convenient of access to this deck, where the specimens are landed. Certain navigational features will doubtless further the scientific purposes of the ship: the tall sides at the stern, which will serve to head the bows into the wind and so reduce drifting, and the provision for bow thrusters to move the ship sideways.

Although it is expected that most of the ship's work will be biological, facilities have been provided for geological research. It will be possible, for example, to operate a deep sea geological corer, pneumatically powered, to take samples of rock at down to 1.500 metres. Trawling will be possible at a depth of 1.000 metres.

With this new hydrographic vessel about to be built -contracts will shortly be put out to tender, and the ship should be operating early in 1970—NERC will now be thinking about some of the other ships mentioned in its annual report. The largest of these, for the combined use of the universities and the Institute of Geological Sciences, would be among other things a mother ship for submersibles. The heavy lifting equipment needed for this task might also be useful for the operation of the deep sea geological sonar at present being developed by the National Institute of Oceanography. This piece of equipment, which goes by the alluring acronym of GLORIA (for Geological Long Range Inclined Asdic), uses a fan shaped beam 2.5° wide in the horizontal plane and 15° in the vertical plane to provide a picture of submarine geological strata over a range of ten miles. A model has been tested, and it is hoped to use the device itself from Discovery next April.

Autotropic Metabolism

from our Microbiology Correspondent

SOME micro-organisms are autotrophic: that is, they synthesize their cell components from simple chemical substances. Obligate autotrophs are limited to this way of life while facultative autotrophs are adaptable. Obligate autotrophs always require carbon dioxide as a source of carbon and either light or reduced inorganic compounds as a source of energy. Recent use of isotopic tracers has shown that, contrary to the pioneering studies of Winogradsky, certain organic compounds can be assimilated by obligate autotrophs: consequently the notion that these cells are impermeable to organic substances no longer provides a tenable hypothesis to account for obligate autotrophism.

Smith, London and Stanier (J. Bact., 94, 972; 1967) have examined an alternative explanation of this phenomenon, namely that obligate autotrophs are characterized by having defects in their intermediary metabolism which preclude the use of organic compounds as principal sources of carbon and energy. This

hypothesis was tested in obligate blue-green algae of species *Thiobacillus*, and two facultative autotrophs, *Hydrogenomonas eutropha* and *Thiobacillus intermedius*.

Acetate was most effectively assimilated by the bluegreen algae, but although it contributed up to 10 per cent of cellular carbon in these algae, the latter retained an overwhelming dependence on carbon dioxide for their carbon. In contrast, the facultative autotroph T. intermedius synthesized 40 to 88 per cent of its cell carbon from exogenous glucose, acetate, succinate or Incorporation of [14C]-acetate by the obligate autotrophs was almost entirely restricted to leucine and the glutamate group of amino-acids. This indicates an inability to convert acetate to oxaloacetate through a-ketoglutarate. In T. intermedius, however, carbon from acetate and pyruvate was incorporated into all of its amino-acids. The conclusions drawn from these incorporation experiments were confirmed by monitoring a number of enzymes in the TCA cycle and electron transport chain. Iso-citrate dehydrogenase was highly active in both obligate and facultative autotrophs, while malic and succinic dehydrogenases, although having similar distributions, had much lower activities in the obligate species. Significantly a-ketoglutarate dehydrogenase was not found in obligate species; thus, the accompanying low levels of malic and succinic enzymes indicate their purely biosynthetic role in an impaired TCA The obligate species also lacked reduced nicotinamide-adenine dinucleotide (NADH₂) oxidase activity.

The Berkeley group argue that the principal obstacle to a heterotrophic habit in obligate autotrophs is their incapacity to couple the catabolism of organic substrates to ATP production; a consequence of the lack of NADH₂ oxidase. The blue-green algae can maintain a balanced production of NADH₂ and ATP by appropriate regulation of the cyclic and non-cyclic photophosphorylations. In thiobacilli, the coupling of NAD reduction with the oxidation of sulphide, thiosulphate, tetra- and tri-thionate has not been observed, but such oxidations could instead be coupled to the reduction of a c-type cytochrome. The authors propose that generation of reduced pyridine nucleotide by an ATP-mediated reversal of electron transport may be a property of all chemoautotrophs with the exception of hydrogen bacteria. The NADH2 oxidase is probably essential for the metabolism of Hydrogenomonas, because the low potential of the $H_2: H^+$ couple requires a carrier at the potential level of pyridine nucleotides. This provides a cogent reason why hydrogen bacteria are the only facultative chemoautotrophs. The loss of NADH₂ oxidase by other chemoautotrophs would conserve the reducing power generated by reversed electron transport and, as biosynthetic demands can be met without the necessity of a complete TCA cycle, a-ketoglutarate dehydrogenase could have been the second enzyme to be eliminated in the evolution of obligate chemoautotrophs from heterotrophic ancestors.

Irradiating Cells

from our Cytogenetics Correspondent

RADIATION and many drugs which block DNA synthesis are used in cancer therapy, because they are able to

kill actively proliferating cells, although the way in which they kill cells is not at all clear. Some cells may be more sensitive than others because they spend more time in a particularly sensitive phase of the cell cycle. If this is so, which are the sensitive stages in the Tolmach and colleagues have recently reported experiments on this problem with human HeLa cells subjected to either X- or ultraviolet irradiation. They devised a technique (Pfeiffer and Tolmach, Nature, 213, 139; 1967) for selecting cells which are all in the same phase of the cell cycle. These cells then progress more or less synchronously through the subsequent phase of the cycle and so can be irradiated at definite phases and their sensitivity in these phases estimated. Djordjevic and Tolmach (Radiation Res., 32, 327; 1967) collected HeLa cells in the G_1 phase (before DNA synthesis, or the S phase) and irradiated them with X-rays. They found that fewer cells survived irradiation at the G_1 phase than if irradiated at either the S or G_2 (post $\overline{\text{DNA}}$ synthesis) phases. By contrast, sensitivity to ultraviolet irradiation increased towards the end of G_1 and reached a peak at mid-S. Cells irradiated with ultraviolet can enter S but the rate of DNA synthesis then slows down; previous work had shown that cells irradiated with a nearly comparable dose of X-rays at G_1 showed little reduction in the rate of DNA synthesis. The basis for these differences in sensitivity is unknown, but presumably biochemical events in cells at different phases have different responses to ultraviolet and X-rays, and damage to these events leads to cell death.

Ultraviolet radiation was found to stimulate a small amount of DNA synthesis in both G_1 and G_2 . Djordjevic and Tolmach suggest that this may represent the DNA synthesis needed to repair damage done by the ultraviolet. They found, however, that this "repair" synthesis was insensitive to inhibitors of DNA synthesis such as fluorodeoxyuridine (FUdR) and hydroxyurea (HU).

Weiss and Tolmach (Biophys. J., 7, 779; 1967) reported further experiments on the effects of X-rays on synchronized HeLa cells. They found that various inhibitors of DNA synthesis, including FUdR and HU, enhanced the killing effect of X-rays. example, many more cells were killed by X-irradiation at G_1 . HU was present at a concentration which prevents cell proliferation but is not toxic. Even if cells are not given HU immediately after irradiation at G_1 but a few hours later as the cells proceed into S, many more cells are killed than if given X-rays alone. But there is a lag period before the effect of HU is realized and this is roughly proportional to the time between irradiation and addition of HU. Again the biochemical basis for the enhancement of cell killing is unknown, but it is quite clear that HU increases the sensitivity of cells to X-rays and the cells remain susceptible to the influence of HU up to 12 hours after irradiation. Can cells and their contents be protected from radiation damage? When chromosomes replicate in the presence of bromodeoxyuridine (BUdR), this is taken into the chromosomes in place of thymidine. Subsequent ultraviolet irradiation causes many more breaks in the chromosomes containing BUdR than in those without it. Trosko and Brewen (Radiation Res., 32, 200; 1967) found that if cysteamine is present at the time of irradiation the number of breaks induced in chromosomes containing BUdR is very much reduced

but there is no effect on the number of breaks induced in chromosomes free of BUdR.

Repairing DNA

from our Molecular Genetics Correspondent

DURING the past six months an exciting addition to our knowledge of DNA synthesis has appeared, most of it from the work of four groups in the United States. These groups have discovered enzymes able to join a break in one strand of a DNA helix. This enzyme activity has been detected and the enzyme purified from E. coli and E. coli infected with T4 or T7 bacteriophages. The detection of this enzyme, which has been named a DNA sealase or DNA ligase, requires a rather sophisticated substrate. Basically two types of substrates have been used. Phage λ DNA molecules which are either hydrogen bonded to one another or hydrogen bonded to themselves into a circular DNA molecule can be covalently sealed giving covalent dimer DNA helices or covalently intact circular DNA helices. Alternatively, double-stranded DNA from a variety of sources can be "nicked" with an endonuclease, thereby producing a few single breaks in the helix. These breaks can be labelled specifically with radioactive phosphate using the enzyme polynucleotide kinase and y-labelled adenosine triphosphate, ATP. The breaks can then be rejoined by the ligase. Once the ligase has joined the labelled phosphate into the chain, the phosphate can no longer be removed from the DNA by the enzyme alkaline phosphatase.

The ligase isolated from *E. coli* has the surprising requirement for the co-factor diphosphopyridine nucleotide, DPN. The DPN has been shown to combine with the DNA ligase in the absence of DNA, forming an enzyme-adenylate complex and free nicotinamide nucleotide. This ligase-adenylate complex will now join a single-stranded break in a DNA helix, concomitantly releasing adenylic acid. The enzyme isolated from phage-infected cells requires ATP as a co-factor, and again forms an enzyme-adenylate complex in the absence of DNA, releasing pyrophosphate.

These enzymes, from whichever source they are isolated, apparently repair breaks in a DNA double helix only when the break has a 5' phosphate and a free 3' hydroxyl group in the break. It is likely that these enzymes play an important part in genetic recombination, the repair of radiation-induced damage, and DNA synthesis, but exactly how is unknown. (References: Gellert, M., Proc. US Nat. Acad Sci., 57, 148 (1967); Weiss, B., and Richardson, C. C., Proc. US Nat. Acad. Sci., 57, 148 (1967); Olivera, B. M., and Lehmann, I. R., Proc. US Nat. Acad. Sci., 57, 1426 (1967); Gefter, M. L., Becker, A., and Hurwitz, J., Proc. US Nat. Acad. Sci., 58, 240 (1967); Becker, A., et al., Proc. US Nat. Acad. Sci., 58, 1996 (1967); Little, J. W., et al., Proc. US Nat. Acad. Sci., 58, 2004 (1967).)

Neurone Geometry

from our Neurophysiology Correspondent

FIVE papers in the current issue of the Journal of Neurophysiology deal with the location of excitatory

and inhibitory synapses on motoneurones. (Smith, Wuerker and Frank, J. Neurophysiol., 30, 1072-1096. 1967; Nelson and Frank, *ibid.*, 1097-1113; Burkem. ibid., 1114-1137; Rall, ibid., 1138-1168; Rall, Smith, Frank, Burke and Nelson, ibid., 1169-1194.) Rall has proposed a model for the contribution of dendritic potentials to postsynaptic excitation and inhibition measured at the motoneurone soma which is in several respects at variance with Eccles's model of the postsynaptic membrane. Eccles's model implies that to be effective in changing neuronal excitability a synapse must be located close to the neurone soma. Using this model Fatt showed how relatively ineffective synapses located on distant dendrites would be (Eccles, The Physiology of Synapses, Springer. Berlin, 1964). Several predictions can be made from the model: the maximum amplitude of a postsynaptic potential (PSP) should be linearly related to the postsynaptic membrane potential; there should be an equilibrium potential at which no postsynaptic currents would flow and no postsynaptic potential therefore be generated; and it should be possible to reverse the postsynaptic potential when the equilibrium potential is surpassed. These predictions are satisfied by the neuromuscular junction, which has become the "model synapse", but there are several discrepancies in motoneuronal behaviour. While inhibitory postsynaptic potentials (IPSPs) fit the Eccles model well. excitatory potentials (EPSPs) do not.

On the basis of their experiments, Rall and his colleagues contend that, while inhibitory synapses are located on, or close to, the motoneurone soma, excitatory synapses may be largely located on the dendrites. They measured changes of membrane impedance during PSPs and found that, while the majority of IPSPs were accompanied by impedance changes of the somatic membrane, EPSPs were accompanied by a variable degree of impedance change, less than half. in fact, producing a measurable change at the soma. If Eccles's model were correct, all PSPs should produce measurable impedance changes at the somatic membrane; however, if some excitatory synapses were dendritically located, then the impedance changes they produced would be extremely small or even too small to measure. Similarly, when the somatic membrane was hyperpolarized, the effect on EPSPs was extremely variable and was quite consistent with the suggestion that excitatory synapses are located on remote dendrites, making their EPSPs relatively immune to the effects of current passed through the somatic membrane. Furthermore, it seemed that EPSPs, unlike IPSPs. could have a quite complicated composition, reaching maxima and decaying over a wide range of times. This would be entirely consistent with the remote location of some dendritic synapses which would affect the somatic membrane electrotonically, and whose potentials would therefore be "smoothed" to a varying extent dependent on the area of neuronal membrane between the synapses and the soma.

All these experimental results fit Rall's theoretical calculations well, and are good evidence for his hypothesis that remotely located synapses contribute significantly to potentials measured at the motoneurone soma. This hypothesis has been developed for several years and is quite comprehensively discussed in one of Rall's earlier papers (Rall, Biophys. J., 2. 146-147; 1962).

Prejudices of Science Students

What does research mean to science undergraduates in universities? Are their attitudes towards industry based on prejudice, or on a rational assessment of where their best chances lie? A study by Mr Donald Hutchings of the University of Oxford Department of Education may help to provide the answers.

A survey carried out by the Oxford University Department of Education in 1963 showed that among scientific sixth formers, pure science has a prestige far greater than that of technology. Pure scientists, the schoolboys felt, led glamorous and exciting lives. They spent their time doing research work which was barely work at all, with the chance of making discoveries which would make them famous. Technologists, on the other hand, were drudges, less intelligent and less well paid than scientists, and often obliged to do boring work. Mr Donald Hutchings, who carried out the original survey, has now followed it up by examining the attitudes of science students in universities. How far are the original attitudes maintained? How do undergraduates make decisions, and on what are they based?

The results of the survey have now been published by the department as a book, The Science Undergraduate, a study of science students at five English universities. The evidence was gathered by interviews with more than 2,000 students of science and technology, at the Universities of Oxford, Cambridge, Leeds and Bradford, and at Imperial College, London. What emerges is a picture now becoming familiar in British education-of an undergraduate population only too eager to stay at university for a higher degree. A common remark, Mr Hutchings reports, was "If I get a good enough degree, I hope to stay on and do research". Almost three-quarters of the total sample felt this way, as Table 1 shows. But there were marked differences both between different subjects and between different universities. At Bradford, for example, a smaller percentage said that they wanted to study for doctorates, and only at Leeds did a significant number contemplate teaching. As Mr Hutchings points out, "It is disconcerting to note how pitifully few students intended to go for teaching: this may be understandable at Bradford, with its industrial emphasis, but if no more than the 4 or 5 per cent of scientists, including mathematicians, at Imperial College and at Oxford and Cambridge intend to teach, the serious shortage of science teachers is hardly likely to be alleviated".

Among pure scientists, by far the largest group was planning to stay on for a doctorate. But there were variations; comparatively few mathematicians, for example, chose a PhD rather than a master's degree; while 63 per cent of physicists were thinking in terms of a PhD, only 35 per cent of mathematicians had the same idea. And although the rather idealized view of research which Mr Hutchings had discovered among sixth formers had been modified, he reports that "it was heartening to see that the idealized picture of higher studies, and of research in particular, had not entirely disappeared". But third year students were at least beginning to show a more realistic view of their chances of being accepted; 46 per cent of first year undergraduates were thinking of

Table 1. POSTGRADUATE INTENTIONS, BY UNIVERSITY I.C. Leeds Bradford Ox/Cam 68 (13) 92 (15) 64 (16) 126 (24) 350 (17) None

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 859 (42) 211 (10) 123 (6) 504 (25) 142 (7) 2189 572 675 411 531 Total (Percentages in parentheses)

Table 2. POSTGRADUATE INTENTIONS BY STUDENTS' CHOICE OF PLACE TO TAKE HIGHER QUALIFICATION

	Same univ.	Another Brit. univ.	British industry	Abroad
PhD	444 (65)	153 (23)	46 (7)	33 (5)
Master's degree	113 (61)	43 (23)	19 (10)	11 (6)
Dip. Ed.	49 (47)	51 (49)	2(2)	3 (3)
Prof. Inst.	107 (22)	37 (8)	319 (66)	22 (4)
Other	65 (54)	36 (20)	12 (10)	7 (6)
Total	778 (50)	320 (20)	398 (25)	76 (5)
	(Percentag	ges in parenth	eses)	

a PhD, but by the third year the proportion had fallen to 40 per cent.

Among the undergraduates interviewed there was a general unwillingness to go into industry, even to do research. A very large proportion said that they would prefer to stay on at university—only engineers viewed academic research with anything approaching suspicion. One engineer, Mr Hutchings reports, said that research "delays earning power", others thought that it led to pettiness and stuffiness, even triviality. The reluctance to leave university seems to be matched by a reluctance to consider even leaving the university of their first degree; Table 2 shows that 65 per cent of those contemplating PhD degrees wanted to stay at their own university. Only 5 per cent wanted to go abroad, a pattern repeated at all the British universities studied.

The effect of the undergraduate course itself was hard to detect, although an increasing number of undergraduate courses provide opportunities for students to do some research—at Oxford, in particular, the Chemistry and Metallurgy courses provide for a fourth year and a short research project. Mr Hutchings reports that this fourth year gave students an excellent opportunity of finding out whether they were really suited to research work.

The survey also enabled the variations in the background of the students to be examined. At Oxford and Cambridge—almost by definition—there were many more students from public schools, and no students at all from technical schools; at the other extreme, Bradford had as many as 28 per cent of students from technical schools in departments of applied science. But the remarkable feature (see Table 3) was that students from public schools showed a slight preference for the applied sciences (except at Bradford)

The greater glamour of pure science is shown in another way, by the proportion of the best pupils—judged admittedly by the crude criterion of A level marks—who choose

Table 3. PERCENTAGE OF STUDENTS FROM DIFFERENT TYPES OF SCHOOLS AT EACH INSTITUTION

		Gram- mar	Inde- pendent	Tech- nical	Other
I.C.	Pure	78	14	5	3
	Applied	57	35	7 .	1
Leeds	Pure	· 81	12	5	2
	Applied	77	19	2	2
Bradford	Pure	69	8	16	7
	Applied	62	3	28	7
Ox/Cam	Pure	55	45		
,	Applied	43	57	_	
Total	Pure	70	22	6	2
	Applied	62	27	9	2
Average %		67	24	7	2

pure science in preference to applied. Of pure scientists, 53·1 per cent had achieved a best A level mark of 75 per cent or over, while among applied scientists only 32·1 per cent had reached this standard. But in the range of marks from 71 to 75 per cent, candidates were divided almost equally between pure and applied science. The figures are interesting in another respect, Mr Hutchings says; they indicate that A levels have now become the necessary qualification for university entrance. Even at a technological university like Bradford, only 46 out of the 390 who were interviewed were admitted on Ordinary National Certificate or Diploma qualifications. And the figures suggest that HNC and ONC are now in full retreat as a means of entry; at Bradford, only 9 per cent of first year students had come in by this route, compared with 18 per cent of final year students.

Most of the students at Oxford and Cambridge were there by choice, Mr Hutchings reports. Among engineers at Cambridge, no less than 96 per cent had made it their first choice, and 82.6 per cent of pure scientists at Oxford had made it their first choice. Despite this, Oxford does not seem a particularly popular choice for science—only 3 per cent of those who did not get their first choice would have preferred Oxford, compared with 24 per cent who would have preferred Cambridge. Of the 128 students who were at Imperial College by second choice, Mr Hutchings says, 64 per cent would have preferred Cambridge. But Leeds shows a completely different result—only 4 per cent of those at Leeds by second choice would have preferred Cambridge, but 70 per cent preferred other principal universities as their first choice. The students were also asked why they chose the universities they did. At Oxford and Cambridge, the reputation of the university and recommendations from school seem to have been the important criteria, with the reputation of the particular department coming in down the list. In contrast, students at Leeds said that the reputation of the department was the most important criterion, and students at Bradford named particular types of course—normally a sandwich course with an industrial bias. Nearness to home seems not to have been important.

Some doubts intrude into the estimates given by students for hours of private study. At Oxford and Cambridge, where private study is expected of students, 45 per cent said that they did more than 20 hours a week. Elsewhere, in universities where lectures and formal instruction tend to take up more of the working day, estimates were lower, with the largest group—about a third of the students interviewed—saying that they spent between 11 and 15 hours a week working privately. As for vacations, Oxford and Cambridge students claimed to work harder than others. A hard core of 4 per cent denied doing any academic work at all in the vacation, but 40 per cent said they worked for about half the vacation, and 10 per cent stretched credibility by claiming to work for nearly all the vacation. In contrast, at Bradford 50 per cent said that they did very little, and 21 per cent that they did none at all. Oxford and Cambridge students spent less time in lectures than others-Bradford was notable in that 60 per cent of the students spent between 10 and 15 hours a week in lectures. And only 1 per cent of the students at Bradford were spending no time at all in the laboratory, against 32 per cent at Oxford and Cambridge. Practical work, in fact, was not highly regarded by the sample—only about 10 per cent thought it was the most helpful form of instruction (see Table 4). As for the form of instruction found least helpful (Table 5), Oxford and Cambridge students were particularly sceptical of the value of lectures. Apart from this, most students found little difference between different forms of instruc-This, Mr Hutchings comments, indicates a high degree of complaisance on the part of present university science students.

A similar attitude is hinted at in the answers given about research interests. "The most striking single fact

Table 4. FORMAL INSTRUCTION FOUND MOST HELPFUL

1-		,	
Lectures	Practicals	Tutorials	No difference
46	10	22	22
42	9	18	31
41	11	16	32
16	3	60	21
	46 42 41	Lectures Practicals 46 10 42 9 41 11	46 10 22 42 9 18 41 11 16

Table 5. FORMAL INSTRUCTION FOUND LEAST HELPFUL (PERCENTAGES)

	Lectures	Practicals	Tutorials	No difference
I.C.	5	16	26	46
Leeds	5	16	12	65
Bradford	5	19	11	60
Ox/Cam	36	19	8	37

to emerge from the student's interviews was the almost complete lack of ideas about future postgraduate research," Mr Hutchings writes. Only 2.5 per cent of the students who firmly intended to do research had any idea what the topic would be; 43 per cent had no idea at all. Few students had considered the possibility of working in a field other than their main university subject, although a large proportion of pure science students were expecting to do applied research. If this is so, why did so many of the students want to do a further degree? The answers, Mr Hutchings says, divide into two categories, "idealized" and "mercenary". Table 6 shows the proportions falling into these two groups. Even among the mercenary answers, Mr Hutchings reports that few seemed very hard headed about salaries, although one engineer put it nicely by saying "Let's face it, a PhD is a good meal ticket". Although students were vague about the salaries they could expect, with a few taking a very pessimistic view of their chances, most said that they were more concerned with responsibility than with money.

Table 6. REASONS FOR TAKING HIGHER QUALIFICATIONS (By type of study)

(- y - y <u>-</u>		,	
	Pure Science	Applied Science	Total
"Mercenary"			
Improve job prospects	188 (19)	180 (27)	368 (22)
Higher degree necessary	(,		0
(i.e. BSc inadequate)	224 (22)	84 (12)	308 (18)
Essential prof. qualification	` '	(- ,	
for engineers	32 (3)	242 (35)	274 (16)
for teachers	111 (11)	11 (2)	122 (7)
Benefit financially	95 (9)	81 (12)	176 (11)
Stay on at university	77 (8)		116 (7)
Status	27 (3)	34 (5)	61 (4)
	754 (75)	671 (99)	1,425 (85)
"Idealized"			
Enlarge own knowledge	218 (22)	114 (17)	332 (20)
Interest in, enjoyment of			(,
subject	193 (19)	68 (10)	261 (16)
Research enjoyable and	` '	, ,	, ,
rewarding	187 (19)	67 (10)	254 (15)
Continue present (academic)			
activity	42 (4)		68 (4)
Satisfy personal ideal	39 (4)	25 (4)	64 (4)
	AMO (AO)	000 (45)	000 (50)
Other	679 (68)		979 (59)
Other	72 (7)	52 (8)	124 (7)
Total	1,505	1,023	2,528
N (i.e. those intending som	ıe		
kind of higher qualification		676	1,674

Three-quarters of the students questioned said that they intended to work abroad at some time in their career. The majority thought that work overseas was a vital part of their training, as well as being interesting and desirable.

The Change to Metric Units

The British Government seems to be making rapid progress in its attempts to convert British institutions to the SI version of the metric system.

THE Government of France may say that the United Kingdom is not yet ready for membership of the European Economic Community, and there may be some truth in that. But there now seems every prospect that most British institutions will have changed over to a particularly pure form of the metric system by the mid-seventies. For better or worse, the days of the rod, pole and perch are numbered. What has happened may be counted as a cheerful victory for the campaign which some departments of the British Government, particularly the Ministry of Technology and the Board of Trade, have been conducting in the past few years.

The starting point for most of what has happened recently seems to have been the recognition by British industry and by the British Government, more or less at the time of the first negotiations for membership of the EEC in 1962, that a full-blooded adoption of a thoroughgoing metric system would be a slow process. By 1964, the Federation of British Industry (now the Confederation of British Industry) formally urged the Government to make preparations for what has since become known as metrication. On May 24, 1965, the British Government gave the project its blessing, and the President of the Board of Trade allowed himself to hope that the change would have been completed by 1975 or thereabouts. Since 1966, the Ministry of Technology has been chiefly responsible for encouraging the adoption of the change, chiefly through the Standing Joint Committee on Metrication under Mr A. H. A. Wynn, formerly chief scientist at the National Coal Board.

The British Government's success so far is quite considerable. Manufacturers are impelled along by the recognition that roughly half of the goods exported from Britain now go to countries in which metric systems of measurement are in use. The British construction industry announced its intention of adopting metric measurements at the beginning of 1967, and there are good reasons to expect that other sectors of industry will quickly follow suit. There have also been some modest successes in the field of education. The Council of Engineering Institutions has, for example, already decided that examinations held under its auspices shall rely exclusively on metric units in 1971 and thereafter. The Oxford and Cambridge Schools Examination Board has come to a similar decision about examination papers in physics at advanced level. Then, as reported in this journal two weeks ago, a group of editors of scientific journals brought together by the Royal Society has settled for the uniform and rapid adoption of metric units in the years immediately ahead.

Whether the trend towards metrication will go as far and as quickly as the Ministry of Technology would like to see remains, of course, to be determined. It may well be significant that the Confederation of British Industry has awakened, rather late in the day, to the fact that the distributive trades seem to be lagging behind the manufacturers in their readiness to adopt a metric system. Plainly it will be a great inconvenience if retailers insist on selling sugar by the pound and cloth by the yard when manufacturers have already been converted to kilograms and metres, but the distributive trades will probably be quick to point out that there is still a sense in which the customer must be considered to be right, and, that if people want to go on buying sugar by the pound, shop-

keepers have a duty to supply them with suitable packages. Because there are at present no plans for legislation to enforce a metric system, the Ministry of Technology must obviously prepare itself for a great deal of vigorous propaganda in a wider circle than that which is occupied by the manufacturers and the scientific journals.

In this connexion, the details of the metric system on which the Ministry of Technology has set its heart call out for more detailed attention. Metrication is more of a slogan than a specification of which of several versions of a metric system is to be introduced. The British Government is working for acceptance of the set of metric rules known as SI (which is an abbreviation of système internationale) promulgated by the meeting in 1954 of the Conference Générale des Poids et Mesures. This in turn is an elaboration of the MKS system, or of the MKSA (Giorgi) system. Altogether there are six basic quantities for length (m), mass (kg), time (s), electric current (A), thermodynamic temperature (°K) and light intensity (cd). To the metricians, the great virtue of SI units is that the system which they form is coherent. In other words, units of derived quantities follow directly from units of the fundamental quantities. Thus surface tension (expressed as force per unit length) is properly measured in units of newtons per metre. (The use of the newton as the unit of force is, of course, also consistent with the SI system as with the MKS system which is a sufficient specification of dynamical properties, so that one newton is equivalent to 100,000 dynes or to rather less than a quarter of a pound-weight.)

The SI system has also codified the rules for forming multiples and submultiples of the basic units. Briefly, multiple or fractional units which are not related to the basic units by factors of a power of a thousand are discouraged. This means that the centimetre is less well favoured than the millimetre as a measure of length, although it seems to be quite widely recognized that several years will pass before the centimetre is altogether banished. What will happen to the angstrom is less clear—the affection of X-ray crystallographers and chemists may be too strong for the SI system to prevail. Engineers are also putting up a strong fight against the Newton per square metre as a unit of pressure. (It is in any case agreed that the bar should remain for the time being.)

The system is summarized conveniently by the document prepared by the Royal Society's working party on metrication under Professor James Lighthill. This will be published early in 1968. A draft of that text follows below.

THE Royal Society Conference of Editors, after considering the part that scientific journals can play in connexion with the Government's policy of promoting the general adoption of the metric system in Britain, makes two main recommendations:

1. That the system of units known as SI should be adopted in all scientific and technical journals.

2. That, in order to keep to a minimum the difficulties which will inevitably arise during the period of transition, the change-over should be effected as quickly as possible.

SI (which is the abbreviation in many languages for Système International d'Unités) is an extension and

refinement of the traditional metric system. It embodies features which make it logically superior to any other system as well as practically more convenient: it is rational, coherent and comprehensive.

The metric system, which had spread to several countries in the aftermath of the French Revolution, began to be adopted in scientific work in Britain in the last quarter of the nineteenth century. Its use extended more and more widely, although a few branches of science remain where Imperial units have continued to predominate. It is fortunate that now that the time has come to discard completely the time-honoured native units (which are not without their advantages), there is to hand a fully developed International System to take their place. Over the years much thought has been given to extending and improving the metric system until finally in 1960 the Conference Générale des Poids et Mesures, the body responsible for maintaining standards of measurements (of which the U.K. is an active participant), formally approved SI. Already nearly thirty countries have decided to make it the only legally accepted system and it is clearly destined to become the universal currency of science and commerce. In many spheres in the U.K. (schools, universities, industry) the adoption of SI is being actively encouraged. The Conference of Editors is anxious that the journals devoted to science and engineering should seize the opportunity of playing a crucial part in helping to end the confusion and wastefulness (both mental and material) resulting from the present multiplicity of units.

The main features of SI are as follows:

1. There are six basic units (see below), the metre and kilogramme taking the place of the centimetre and gramme of the old metric system.

2. The unit of force, the newton (kg m s⁻²), is independent of the Earth's gravitation, and the often confusing introduction, in some branches of science and technology, of g into equations is no longer necessary.

3. The unit of energy in all forms is the joule (newton \times metre), and of power the joule per second (watt); thus the variously defined calories, together with the kilowatt hour, the B.t.u. and the horsepower, are all superseded.
4. "Electrostatic" and "electromagnetic" units are

replaced by SI electrical units.

5. Multiples of units are normally to be restricted to steps of a thousand and similarly fractions to steps of a thou-

Lists of the basic SI units follow, as well as lists of some derived SI units, of compatible units, and also examples of units which run counter to SI the use of which is accordingly to be actively discouraged. Also listed are the names and symbols of the prefixes representing numerical factors: these are both convenient in obviating the need to write large numbers of zeros or in some instances high powers of 10, and also helpful in establishing familiarity with the numerical framework of modern science. It will be noted that the recommended prefixes are limited to $10\pm 3n$.

The rate of the change-over towards complete metrication will vary from journal to journal, depending on the subjects covered and the extent to which the metric system already holds sway. In certain branches of science and engineering editors may decide to proceed to their target along the following route (with equivalent values given in parentheses):

$$\begin{array}{ccc} \text{non-metric (SI)} {\rightarrow} \text{SI (non-metric)} {\rightarrow} \text{SI} \\ \text{(stage II)} & \text{(stage III)} \end{array}$$

In some branches full metrication will have to wait upon the installation of metric machinery and equipment. (Where measurements are expressed in the form of instrument readings they should be so recorded and a conversion factor quoted.) In many journals, on the other hand, change-over to SI units can be achieved in one step, and the experience of some editors and authors where changes have already been introduced is that such changes are

more readily accepted than would have been supposed before their introduction.

Basic SI Units

physical quantity	name of unit	symbol for unit
length	\mathbf{metre}	m
mass	kilogramme	kg
time	second	S
electric current	ampere	A
thermodynamic temperature	degree Kelvin	$^{\circ}\mathrm{K}$
luminous intensity	candela	cd

Symbols for units do not take a plural form.

Supplementary Units

	•	symbol for
physical quantity	name of unit	unit
plane angle	radian	rad
solid angle	steradian	sr

These units are dimensionless.

Derived SI Units with Special Names

sym	bol	for
-----	-----	-----

physical quantity nam	e of unit	unit	definition of unit
energy	joule	J	kg m ² s ⁻²
force	newton	\mathbf{N}	$kg m s^{-2} = J m^{-1}$
power	watt	W	kg m ² s ⁻³ =J s ⁻¹
electric charge	coulomb	C	As
electric potential difference	volt	\mathbf{v}	kg m ² s ⁻³ A ⁻¹ = J a ⁻¹ s ¹
electric resistance	ohm	Ω	kg m ² s ⁻³ A ⁻² =V A ⁻¹
electric capacitance	farad	\mathbf{F}	A^2 s ⁴ kg ⁻¹ m ⁻² =A s V ¹
magnetic flux	weber	Wb	$kg m^2 s^{-2} A^{-1} = V s$
inductance	henry	\mathbf{H}	kg m ² s ⁻² A ⁻² =V s V ⁻¹
magnetic flux density	tesla	${f T}$	kg s ⁻² A ⁻¹ =V s m ⁻²
luminous flux	lumen	lm	ed sr
illumination	lux	lx	cd sr m ⁻²
frequency	hertz	Hz	cycle per second
customary	degree	$^{\circ}\mathrm{C}$	$t/^{\circ}C = T/^{\circ}K - 273.15$
temperature, t	Celsius		

Examples of Other Derived SI Units

physical quantity	SI unit	symbol for unit
area	square metre	$\mathbf{m}^{\mathbf{s}}$
volume	cubic metre	n_1^3
density	kilogramme per cubic metre	kg m ⁻³
· velocity	metre per second	m s-1
angular velocity	radian per second	rad s-1
acceleration	metre per second squared	m s-2
pressure	newton per square metre	$N m^{-2}$
kinematic viscosity, diffusion coefficient	square metre per second	m² s-1
dynamic viscosity	newton second per square metre	$N \text{ s m}^{-2}$
electric field strength	volt per metre	$ m V~m^{-1}$
magnetic field strength	ampère per metre	A m-1
luminance	candela per square metre	· cd m-2

Units to be Allowed in Conjunction with SI

omis to be intowed in conjunction with Si				
	symbol for			
physical quantity	name of unit	unit	definition of unit	
length	parsec	pc	30·87 × 1015 m	
area	barn	pc b	10^{-28} m^2	
	hectare	ha	$10^4 \mathrm{\ m^2}$	
volume	litre	1	$10^{-8} \text{ m}^8 = \text{dm}^3$	
pressure	bar	bar	$10^5 \ { m N \ m^{-2}}$	
mass	tonne	t	$10^{3} \text{ kg} = \text{Mg}$	
kinematic viscosity, diffusion coefficient	stokes	St	10-4 m ² s-1	
dynamic viscosity	poise	\mathbf{P}	10-1 kg m ⁻¹ s ⁻¹ 10-4 T	
magnetic flux density (magnetic induction)	gauss	G	10-4 T	
radioactivity	curie	Ci	$37 \times 10^9 \text{ s}^{-1}$	
energy	electronvolt	eV	$1.6021 \times 10^{-19} \text{ J}$	

The common units of time (e.g. hour, year) will persist. and also, in appropriate contexts, the angular degree.

6894.76 N m⁻²

Fractions and Multiples		Examples of Units Contrary to SI,			
fraction	prefix	symbol	n hyeleni	with their Equi	valents
10^{-1} 10^{-2}	deci	d c	physical quantity	unit	equivalent
. 10-3	milli	' m	length	angström .	10 ⁻¹⁰ m
10-6	micro	μ		inch	0·0254 m 0·3048 m
10-9	nano	n		foot yard	0.3048 m 0.9144 m
10 ⁻¹² 10 ⁻¹⁵	pico femto	$\mathbf{p}\\ \mathbf{f}$		mile	1.609 34 km
10-18	atto	a	area	nautical mile square inch	1·853·18 km 645·16 mm²
multiple	prefix	symbol		square foot	0.092 903 m ²
10	deka	da*		square yard square mile	0.836 127 m ² 2.589 99 km ²
$\frac{10^2}{10^3}$	hecto kilo	h* k	volume	cubic inch	$1.638 \ 71 \times 10^{-5} \ \text{m}^3$
106	. mega	M G		cubic foot U.K. gallon	0.028 316 8 m ³ 0.004 546 092 m ³
10^{6} 10^{12}	giga tera	G T	mass	pound	0.453 592 37 kg
*To be restricted as mu	ch as possible.		density	slug pound/cubic inch	14.593 9 kg 2.767 99×104 kg m ⁻³
		ised. Thus 10-8 metre	<u>.</u>	pound/cubic foot	16·0185 kg m ⁻³
is represented by 1 prefix to a unit in ef		. The attaching of a	force	dyne poundal	10−5 N 0·138·255 N
	$1^2 = 1 \text{ (km)}^2 = 1$			pound-force	.4·448 22 N
2 200	$not 1 k(m^2) = 1$		pressure	kilogramme-force atmosphere	9·806 65 N 101·325 N m ⁻²
Where possible any r	numerical prefix	should appear in the	F- Sandar	torr	133:322 N m ⁻²

Organochlorine Pesticides in Seals and Porpoises

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numerator of an expression.

Seals and porpoises in Scotland and Canada, far from the sites of application of pesticides, can accumulate high concentrations of residues in their blubber. These chemicals are spreading through the long food chain which ends with seals and porpoises and obviously cannot be confined to their place of discharge.

pound (f)/sq.in.

THE presence of organochlorine residues in marine life in many areas of the world, including the Antarctic, has been reported recently^{1,2}. In British waters the accumulation of residue in the eggs of sea birds³ and the process of concentration of pesticides through certain food chains in the marine environment⁴ have been studied. Analyses of various species of marine fish and mamnals from Scottish waters during the past 5 years^{5,6} have shown that organochlorine residues are now a normal occurrence, the highest concentrations being found in organs or tissues with the highest lipid content. Thus the muscle tissue of salmonids, the livers of cod and the blubber of seals act as storage sites for these residues.

The aquatic mammals, such as whales, seals and porpoises, have a relatively high proportion of the body weight in the form of subcutaneous fat (blubber). Seals feed chiefly on gadoids and salmonids, and porpoises chiefly on small gadoids and clupeoids. The process of concentration of pesticides through their food chain might be expected to result in high pesticide levels in the fat of these mammals, and samples analysed at this laboratory confirm this expectation. In recent years, specimens of the grey seal (Halichoerus grypus), common seal (Phoca vitulina) and porpoise (*Phocaena phocaena*) have been sampled from the coasts of Scotland. Samples of blubber were available for all the specimens examined, and in a few cases other organs were also available. As well as recognized pesticide residues, other electron-capturing substances have been detected by gas-liquid chromatography and, in order to establish whether such substances are as widely distributed as the pesticides, blubber samples from grey seals and harp seals (*Phoca groenlandica*) on the Canadian Atlantic coast have also been examined and the results of these analyses are reported here. Examination of samples of seal blubber from other areas is continuing in order to assess the variation between different regions of the marine environment.

All tissue samples from Scottish waters were frozen at -18° C until required for analysis. The Canadian samples were preserved in 10 per cent formalin, which showed no evidence of any influence on the analytical procedure, and in this and other cases involving fish it has not affected the efficiency of recovery of pesticide residues. Aliquots of the samples (5 g) were ground to a powder with anhydrous crystalline sodium sulphate (AR grade) and extracted in a Soxhlet apparatus with redistilled AR grade n-hexane (previously tested and found to be free of electron-capturing materials by gas chromatography). The hexane extracts were made up to 100 ml. and 25 ml. aliquots were subjected to clean-up by the hexane-dimethylformamide partition method of de Faubert Maunder et al., followed by column clean-up using alumina containing 5 per cent water. The loss of pesticide residues from fat extracts so treated is up to 20 per cent, but, as is customary in pesticide analyses, no correction has been made for such losses in the results presented here.

The cleaned-up hexane extracts were analysed by gasliquid chromatography on a Varian 'Aerograph 205-2B' instrument employing two glass columns 5 ft. long $\times \frac{1}{8}$ in. outer diameter. One column was packed with 'Chromosorb WAW/DMCS 80–100' mesh coated with 10 per cent

DC-200 silicone oil, and the other with the same support coated with 5 per cent DC-200 + 7·5 per cent QF-1 fluorosilicone. The oven temperature was 200° C, the nitrogen gas flow 50 ml./min and the column resolution for dieldrin equivalent to 1,600 theoretical plates. There was no significant degradation of DDT on the columns. While complete separation of the common pesticide residues is possible on one or other of these columns, interference by polychlorinated biphenyl (PCB) residues8,9 with p,p'-TDE and p,p'-DDT peaks necessitated a further analytical stage. Aliquots (5 ml.) of some of the extracts were evaporated to dryness in a stream of cold filtered air, and the residues refluxed with 2 ml. of 2.5 per cent alcoholic potassium hydroxide for 15 min on a warm water-bath. After cooling, 5 ml. of n-hexane was added, with mixing, followed by 40 ml. of 2 per cent sodium sulphate solution. The supernatant hexane layer was then analysed by gas-liquid chromatography. By this technique, quantitative conversion of p,p'-DDT to p,p'-DDE, and of p,p'-TDE to p,p'-MDE (=DDMU), is obtained, and the conversion products confirm the identity of the original residues. The residual peaks in the p,p'-DDT and p,p'-TDE positions, where present, can be subtracted from the values of the pesticides originally determined in these positions. Hydrolysis also destroys α and γ-BHC, where present, but polychlorinated biphenyls are un-A cyanosilicone (XE-60) column¹⁰ has also been used to separate p,p'-DDT and p,p'-TDE from PCB interference, confirming the latter.

The principal pesticide residues found in all samples examined were those of the DDT group, with dieldrin Canadian seals contained in much smaller quantities. lower concentrations of dieldrin than Scottish seals, while Scottish porpoises contained remarkably high concentrations of all pesticide residues (see Table 1). Interference on the DC-200 silicone column in the p,p'-TDE position caused by a non-hydrolysable residue was responsible for about 25 per cent of the total estimated p,p'-TDE in the Canadian and Scottish samples examined. In the p,p'-DDT position, the non-hydrolysable residue constituted about 10 per cent in the Canadian samples and about 30 per cent in the Scottish samples examined for this interference, the total amount of non-hydrolysable material being smaller in the Canadian samples. values in Table 1 have not been corrected for this interference, for only a proportion of the samples were hydrolysed. Results from the various species of seals were not noticeably different in a given area, and have been combined in Table 1.

Table 1. CONCENTRATION RANGES OF ORGANOCHLORINE RESIDUES IN FAT OF SEALS AND PORPOISES (p.p.m.)
(Means in parentheses)

	lo.ii mpl	ı e Dieldrin	$p,p' ext{-DDE}$	p,p'-TDE	p,p'-DDT
E. Scotland, adult	18	0.15-2.1	1.0-11.1	0.27-3.0	1.5-23.3
seals (1965–66)		(0.79)	(5·5)	(1-2)	(7.8)
N. and W. Scotland,	6	0.08-0.39	1.2-7.0	0.17 - 0.83	1.7-7.7
adult seals (1965-66)	-	(0.20)	(3.4)	(0.41)	(3.8)
N. and W. Scotland,	9	0.06-0.44	1.0-4.0	0.13-0.59	1.1-3.7
seal pups (1966)		(0.18)	(2.5)	(0.32)	(2.3)
Canada, Magdalen Is	2	0.02-0.04	0.67-1.8	0.08-0.16	0.08-0.63
juy, and adult seals	_	(0.03)	(1.2)	(0.12)	(0.36)
(1967)					
Canada, Cabot Straits,	6	0.03-0.10	$1 \cdot 2 - 17 \cdot 3$	0-35-2-1	2-1-15-6
juvimm. and adult		(0.07)	(5.9)	(0.78)	(5.5)
seals (1967)				, ,	, ,
Scotland, Orkney,	1	0.59	1.1	0.58	2.2
adult porpoise (1967)					
E. Scotland, adult	3	4.9-18.0	9-6-15-3	5.2-14.3	13-1-25-7
porpoises (1965, 1967)	(9.9)	(12.8)	(8.9)	(21.1)
*	•	/	/	,	\

Contamination by pesticides is greater in the seals on the east coast of Scotland (taken in an area roughly from Aberdeen to the Tay estuary) than in specimens from the west and north coasts, including the Orkney Islands. Seal pups from the Orkney and Harris (west coast) breeding grounds contained only slightly lower residues than adults from the same areas. By comparison, the Canadian samples from the Magdalen Islands (Gulf of St. Lawrence) contained very low levels of both dieldrin and the DDT group, while seals from the Cabot Straits (Gulf of St. Lawrence) contained little dieldrin but higher DDT group residues, similar to those of Scottish seals. Of the few porpoises examined, that from Orkney was the least contaminated, but the three taken on the east coast of Scotland had markedly higher contents of all the residues measured. One specimen contained a total residue concentration (dieldrin and DDT group) of 73·3 p.p.m. Analyses of a few tissues other than the blubber have been made, but concentrations in the blubber have always been found to be the highest (Table 2). Concentrations in other tissues seem likely to be consistent with their lower lipid contents.

Table 2.	TOTAL DDT	CONCENTR.	ATIONS (p.	.p.m.) IN V	ARIOUS T	ISSUES
Sample	Blubber	Liver	Brain	Kidney	Spleen	Muscle
Grey seal	5.8	0.77	0.26		*****	
Grey seal	9-2	0.36	0.15	*****		
Grey seal	14.8	0-97	0.24		0.53	
Grey seal	8.4	0.46	0-17		0.13	
Grey seal	2.4	0.11	0.07		0.06	-
Porpoise	3.8	0.58	0.02	0.04	0.10	0.56

The proportion of blubber extractable by hexane was determined for some samples. Values ranged from 72 to 86 per cent (mean 80 per cent) for the Canadian seals, and from 88 to 98 per cent (mean 93 per cent) for Scottish seals samples on the breeding grounds off the west coast. (Values for other seal samples were not estimated.) Concentrations of pesticides in terms of extractable fat for these samples would thus be up to 40 per cent greater than the values in Table 1, but the general comparison is not significantly affected. The lower extractable fat values for the Canadian samples may possibly be a result of being preserved in formalin.

Concentrations of pesticide residues in the extractable fat of various organs and tissues of one porpoise show an important relationship (Table 3). While the concentrations of the DDT group in the original tissues vary widely, those expressed in terms of extractable fat are in much closer agreement, with the sole exception of the brain. A similar anomaly for tissues of trout was described by Holden¹¹. It seems likely that a large proportion of the lipid fraction of the brain may not contain pesticides, and the brain lipid composition is known to differ considerably from that of depot fat.

Table 3. CONCENTRAT	ions of t	OTAL DD	r (p.p.m.)	IN TISSU	ES OF A P	DRPOISE
Tissue or organ	Blubber	Muscle	Liver	Spleen	Kidney	Brain
Concentration in tissue Percentage extract-	3·8 67 · 0	0·56 6·1	0·58 13·2	0·12 5·1	1·4 1·4	0·02 8·3
able fat Concentration in extractable fat	5-6	9-2	4.8	2.4	2.9	0-27

The unidentified peaks on the chromatograms seem to be similar to those produced by polychlorinated biphenyls and, to enable comparison to be made, the ratios (Rx) of the retention times of all regularly occurring peaks (relative to dieldrin=100) have been calculated for the operating column temperature of 200°C, these values being temperature-dependent. The Rx values of PCB compounds from commercial PCB formulations were also determined for both types of column (Table 4). At least seven PCB-type peaks were found, including those which interfere with p,p'-TDE and p,p'-DDT. Heptachlor epoxide and p,p'-MDE could not be confirmed on both columns and are not therefore recorded.

The most significant aspect of the results is that, although seals and porpoises inhabit an environment far removed from the site of application or discharge of pesticides, they can contain much greater concentrations of dieldrin and DDT group residues than those recorded for most other species, including man. Robinson and Hunter¹² found a mean concentration of dieldrin of 0·22 p.p.m. (range 0·10–0·73 p.p.m.) and a mean total con-

centration of DDT of 4.0 p.p.m. (range 1.0-11.1 p.p.m.) in samples of human depot fat in England in 1964. No evidence of any significant change was found during 1962-65.

Table 4. RELATIVE RETENTION VALUES (Rx) OF RESIDUES (DIELDRIN = 100) DC-200 column DC-200/QF-1 column

recount		Dampie	residue	Sample
PCB	= 68.5	68	PCB = 68.5	70
p,p'-DDE Dieldrin	-100	100	p, p'-DDE = 84	83.5
PCB	, = 121	125	$\begin{array}{ll} \text{Dieldrin} &= 100 \\ p, p'\text{-TDE} &= 121 \end{array}$	100
p.p'-TDE	= 129	128	PCB = 121 PCB = 123	121 122
p,p'-TDE PCB	= 148	149	PCB = 146.5	
p,p'-DDT PCB	= 170	171	p.p'-DDT = 147	147
PCB	≈ 174 207	175	PCB = 234	231
PCB	=205 $=282$	205 287	PCB = 282	278
PCB	= 339	338		

The seals and porpoises from the east coast of Scotland were usually more seriously contaminated than those from the north and west coasts. This, at least in respect of seals, may be because, as is believed, the populations in the two areas come from different breeding sites. The seals south of Aberdeen breed principally on the Farne Islands, off the north-east coast of England, while the seals of north and west Scotland are primarily from the breeding grounds of the Orkney Islands in the north, and other islands off the west coast of Scotland. The sea off eastern Scotland is also likely to receive more contamination from estuarine discharges than that off north and west Scotland. Similarly, the two separate seal populations sampled off the east coast of Canada may inhabit areas with different levels of contamination. Pups contain pesticide residues in concentrations only slightly lower than in the parent population. Common seals in Holland have been found with concentrations of residue similar to those of seals on the Scottish east coast13.

While the residue concentrations in subcutaneous fat are high, those of other tissues and organs are found to be much lower. This suggests that there is no risk of physiological effects, unless the metabolism of much of the fat in times of stress leads to a considerable increase in the concentration of the residue in circulating lipids, and thus in residue concentrations in other organs or tissues.

Grey seals on the coast of the Scottish mainland feed primarily on salmon (Salmo salar) and cod (Gadus morrhua)14. The highest concentrations of pesticide in salmonids in the marine environment have so far been found in sea trout (S. trutta) on the east coast with up to 0.2 p.p.m. (dieldrin + total DDT) in muscle tissue15, and salmon from the same area would probably have similar contents. Cod examined from the east coast have contained up to 0.35 p.p.m. (dieldrin + total DDT) in muscle tissue (mean 0.12 p.p.m.) and from the west coast up to 0.56 p.p.m. (mean 0.22 p.p.m.), while cod livers contained up to 4.0 p.p.m. and 12.0 p.p.m., respectively. Grey seals are estimated to eat about 15 lb. of food daily, and thus an adult seal could consume its own weight in food in 20-30 days. Assuming a high proportion of the pesticide intake to be stored in body fat rather than to be excreted or metabolized, an increase of two orders of magnitude in pesticide concentration from food to body fat within a few years is feasible. This degree of accumulation of residue in an environment not deliberately contaminated, and in species ecologically far distant from the target organisms of persistent pesticides, underlines the impossibility of confining such chemicals to the areas of application.

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The North Pacific: an Example of Tectonics on a Sphere

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Individual aseismic areas move as rigid plates on the surface of a sphere. Application of the Mercator projection to slip vectors shows that the paving stone theory of world tectonics is correct and applies to about a quarter of the Earth's surface.

THE linear magnetic anomalies1,2 which parallel all active ridges can only be produced by reversals of the Earth's magnetic field if the oceanic crust is formed close to the ridge axis³. Models⁴ have shown that the anomalies cannot be observed in the North Atlantic unless most dyke intrusion, and hence crustal production, occurs within 5 km of the ridge axis. The spreading sea floor³ then carries these anomalies for great horizontal distances with little if any deformation. The epicentres of earthquakes also accurately follow the axis and are offset with it by transform faults^{5,6}. The structure of island arcs is less clear, though the narrow band of shallow earthquakes suggests that crust is consumed along a linear feature.

These observations are explained if the sea floor spreads as a rigid plate, and interacts with other plates in seismically active regions which also show recent tectonic activity. For the purposes of this article, ridges and trenches are respectively defined as lines along which crust is produced and destroyed. They need not also be topographic features. Transform faults conserve crust and are lines of pure slip. They are always parallel, therefore, to the relative velocity vector between two plates—a most useful property. We have tested this paving stone theory of world tectonics in the North Pacific, where it works well. Less detailed studies of other regions also support the theory.

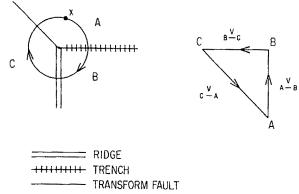


Fig. 1. The circuit and its vector diagram show how a ridge and a trench can meet to form a transform fault.

The movement of blocks on the surface of a sphere is easiest to understand in terms of rotations. Any plate can clearly be moved to a given position and orientation on a sphere by two successive rotations, one of which carries one point to its final position. a second about an axis through this point then produces the required orientation. These two rotations are equivalent to a single rotation about a different axis, and therefore any relative motion of two plates on the surface of a sphere is a rotation about some axis. This is Euler's theorem, and has been used to fit together the continents surrounding the Atlantic. If one of two plates is taken to be fixed, the movement of the other corresponds to a rotation about

some pole, and all relative velocity vectors between the two plates must lie along small circles or latitudes with respect to that pole. If these small circles cross the line of contact between the two plates, the line must be either a ridge or a trench depending on the sense of rotation. Neither of these structures conserves crust. If the line of contact is itself a small circle, then it is a transform fault. This property of transform faults is very useful in finding the pole position and is a consequence of the conservation of crust across them. There is no geometric reason why ridges or trenches should lie along longitudes with respect to the rotational pole and in general they do not do so. The pole position itself has no significance, it is merely a construction point. These remarks extend Wilson's concept of transform faults to motions on a sphere, the essential additional hypothesis being that individual aseismic areas move as rigid plates on the surface of a sphere.

There are several points on the surface of the Earth where three plates meet. At such points the relative motion of the plates is not completely arbitrary, because, given any two velocity vectors, the third can be determined. The method is easier to understand on a plane than on a sphere, and can be derived from the plane circuit in Fig. 1. Starting from a point x on A and moving clockwise, the relative velocity of B, $_{\lambda}v_{s}$ is in the direction AB in the vector diagram. Similarly the relative velocities $_{x}v_{o}$ and $_{v}v_{s}$ are represented by BC and CA. The vector diagram must close because the circuit returns to x. Thus:

$$_{\mathbf{A}}v_{\mathbf{B}} + _{\mathbf{B}}v_{\mathbf{c}} + _{\mathbf{c}}v_{\mathbf{A}} = 0 \qquad (1)$$

The usual rules for the construction of such triangles require three parameters to be known, of which at least one must be the length of a side, or spreading rate. Transform faults on both ridges and trenches are easy to recognize, and they determine the direction, but not the magnitude, of the relative velocities. The magnetic lineations are one method of obtaining $_{\rm B}v_{\rm c}$, though this value must be corrected for orientation unless the spreading is at right angles to the ridge. Then the triangle in Fig. 1 determines both $_{\rm a}v_{\rm a}$ and $_{\rm c}v_{\rm a}$. This method is probably most useful to determine the rate of crustul consumption by trenches. Equation (1) must be used with care, because it only applies rigorously to an infinitesimal circuit round a point where three (or more) plates meet. If the circuit is finite, the rotation of the plates also contributes to their relative velocity, and therefore these simple rules no longer apply.

Equation (1) is easily extended to the corresponding problem on a spherical surface because angular velocities behave like vectors.

$$_{A}\omega_{B} + _{B}\omega_{\sigma} + _{\sigma}\omega_{A} = 0 \tag{2}$$

The sign convention takes a rotation which is clockwisewhen looked at from the centre of the sphere to be a positive vector which is pointing outward along the rotation axis. By adding more terms, equation (2) can be extended to circuits crossing more than three plates and applies to all possible circuits on the surface. ω diagrams for three plates are no more difficult to construct than those for v, because the third vector must lie in the plane containing the other two. This result does not apply

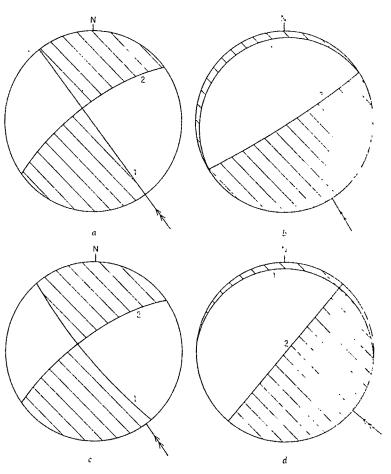


Fig. 2. Mechanism diagrams for four circum-pacific earthquakes. The lower half of the focal sphere is projected stereographically on to a horizontal surface, and the rarefraction quadrants are shaded. The horizontal projection of the slip vector in plane 1 is marked with a double arrow. (a) June 28, 1966, Parkfield¹⁵, strike slip. (b) September 4, 1964, Alaska¹⁶, overthrust. (c) June 14, 1962, Near and Aleutian Islands¹⁴, strike slip. (d) October 20, 1963, Kurile Islands¹⁷, overthrust.

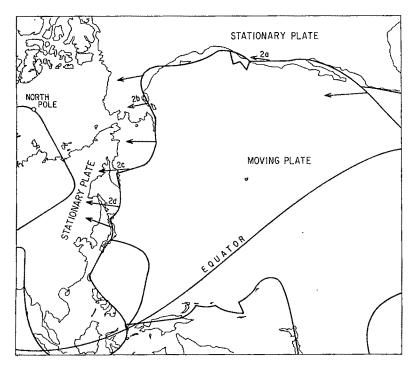


Fig. 3. A Mercator projection of the Pacific with a pole at 50° N., 85° W. The arrows show the direction of motion of the Pacific plate relative to that containing North America and Kamchatka. If both plates are rigid all slip vectors must be parallel with each other and with the upper and lower boundaries of the figure. Possible boundaries of other plates are sketched.

to diagrams for four or more plates, which are three dimensional and therefore less easy to draw.

These geometrical ideas can now be applied to the North Pacific. There are many fault plane solutions for earthquakes in the area, and these are used in a new way in order to determine the direction of the horizontal projection of the slip vector. Unlike the projection of the principal stress axes, that of the slip vector varies in a systematic manner over the entire region. This is clearly a consequence of spreading of the sea floor, which determines the relative motion, not the stress field.

The North Pacific was chosen for several reasons. The spreading rate from the East Pacific rise is the most rapid yet measured², and should therefore dominate any slight movements within the plate containing North America and Kamchatka¹⁰. The belt of earthquake epicentres which extends from the Gulf of California to Central Japan without any major branches¹⁰ suggests that the area contains only two principal plates. Also, the belt of seismic activity between them is one of the most active in the world and many fault plane solutions are available^{6,11-17}. It is an advantage that the trend of the belt which joins the two plates varies rapidly over short distances, because this illustrates the large variety of earthquake mechanisms which can result from a simple rotation (Fig. 2). It is also helpful that the outlines of the geology and topography of the sea floor are known.

Fault plane solutions which were obtained from the records of the world-wide network of standardized stations now give excellent and consistent results^{6,18}. The directions of principal stress axes, however, which were determined from first motions, vary widely over short distances (Fig. 2) and are therefore difficult to use directly. The concept of spreading of the sea floor suggests that the horizontal projection of the slip vector is more important than that of any of the stress axes, and Fig. 2 shows that this is indeed the case. The examples which are illustrated are stereographic projections of the radiation field in the lower hemisphere on to a horizontal plane¹⁶. The direction of the projection of the slip vector in plane one is obtained by adding or subtracting 90° from the strike of plane two

if the planes one and two are orthogonal. The slip directions which are shown give the motion of the oceanic plate relative to the plate containing North America and Kamchatka. For each case in Fig. 2 there are two possible slip directions, but, whereas one changes in direction slowly and systematically between Baja California and Japan, the other shows no consistency even for earthquakes in the same area. ambiguity is therefore unimportant in this case. If all the earthquakes between the Gulf of California and Japan are produced by a rotation of the Pacific plate relative to the continental one, any pair of widely spaced slip directions can be used to determine the pole of relative rotation. The two which are used here are the strike of the San Andreas between Parkfield and San Francisco, and the average slip vector of all the aftershocks in the Kodiak Island region16 of the 1964 Alaskan earthquake. A pole position of 50° N., 85° W. was obtained by construction on a sphere. If the paving stone theory applies, all slip vectors must be parallel to the latitudes which can be drawn with respect to this pole. Though this prediction could be tested by tabulating the disagreement with the observations, a simpler and more obvious test is to plot the slip vectors on a map of the world in Mercator projection, taking the

projection pole to be the rotation axis (Fig. 3). The Mercator projection has two advantages; it is conformal, which means that angles are locally preserved and slip vectors can be plotted directly, and also all small circles centred on the projection pole are parallel. Because the upper and lower boundaries of Fig. 3 are themselves small circles, the theory requires all slip vectors to be parallel both to each other and to the top and bottom. This prediction was tested on eighty published. Taket plane solutions for shallow earthquakes during and after 1957. Of these, about 80 per cent had slip vectors with the



Fig. 4. An orthonormal projection of the North Pacific centred on the Mercator Pole. Slip vectors are tangents to concentric circles about the centre.

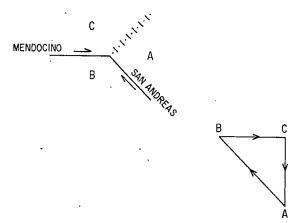


Fig. 5. Both the Mendocino and San Andreas faults can be strike slip if there is a trench to the north or east.

correct sense of motion and within $\pm 20^{\circ}$ of the direction required by Fig. 3. Most of the fault plane solutions for earthquakes before this date also agreed with the sense and direction of motion. Representative slip vectors in Fig. 3 show the motion of the Pacific plate relative to the continental one, which is taken to be fixed. The rotation vector is therefore negative and points inward at 50° N., 85° W. The agreement with theory is remarkable over the entire region. It shows that the paving stone theory is essentially correct and applies to about a quarter of the Earth's surface.

The disadvantage of the Mercator projection is the distortion it introduces around the poles. It is therefore difficult to use Fig. 3 to estimate spreading velocities. For this purpose an orthonormal projection is more useful (Fig. 4), for the spreading rate is then proportional to distance from the centre if this is taken at the pole of rotation. In this projection, which is simply a vertical projection on to a plane at right angles to the rotation axis, rigid rotations of the two plates on a sphere become rigid body rotations on the plane, and all slip vectors must be tangents to concentric circles about the centre of projection (Fig. 4). This projection is useful if spreading rates, rather than angles, are known. There are as yet few such measurements in the North Pacific.

The large active tectonic areas of the North Pacific are now clear from Fig. 3. The fault systems of the San Andreas, Queen Charlotte Islands and Fairweather form a dextral transform fault joining the East Pacific rise to the Aleutian trench. The strike slip nature of these faults is clear from field observations 18-20 and from the fault plane solutions (for example, Fig. 2a). In Alaska the epicentral belt of earthquakes changes direction¹⁰ (Fig. 3) and follows the Aleutian arc. The fault solutions also change from strike slip to overthrust16 (for example, Fig. 2b), and require that the islands and Alaska should override the Pacific on low angle ($\sim 7^{\circ}$) faults. Though the direction of slip remains the same along the entire Aleutian arc, the change in strike changes the fault plane solutions from overthrusting in the east to strike slip in the west (Fig. 2c). A sharp bend occurs between the Aleutians and Kamchatka (Fig. 3). Here the fault plane solutions change back to overthrust (Fig. 2d). motion continues as far as Central Japan, where the active belt divides (Fig. 3) and the present study stops. Thus the North Pacific contains the two types of transform faults which require trenches, and clearly shows the dependence of the fault plane solutions on the trend of the fault concerned.

The variation of trend also controls the distribution of trenches, active andesite volcanoes, intermediate and deep focus earthquakes¹. All these phenomena occur in Mexico, Alaska, the Eastern Aleutians, and from Kamchatka to Japan, but are absent where the faults are of a strike slip transform nature. This correlation is particularly obvious along the Aleutian arc, where all these features become steadily less important as Kamchatka is approached¹o, then suddenly reappear when the trend of the earthquake belt changes. Though it is clear from these remarks that the paving stone theory applies to the North Pacific region as a whole, there are some small areas which at first sight are exceptions.

The most obvious of these is the complicated region of the ocean floor off the coast between northern California and the Canadian border²¹. The difficulties begin where the San Andreas fault turns into the Mendocino fault. Fig. 5 shows that the change in trend of the epicentres is possible only if crust is consumed between C and A (or created in B, which is unlikely). The earthquakes along the coast of Oregon²² and the presence of the volcanoes of the Cascade range, one of which has recently been active and all of which contain andesites, support the idea that crust is destroyed in this area. In the same area two remarkable seismic station corrections which possess a large azimuthal variation²³ also suggest that there is a high velocity region extending deep into the mantle similar to that in the Tonga-Kermadec24 region. These complications disappear when the ridge and trench structures join again and become the Queen Charlotte Islands fault.

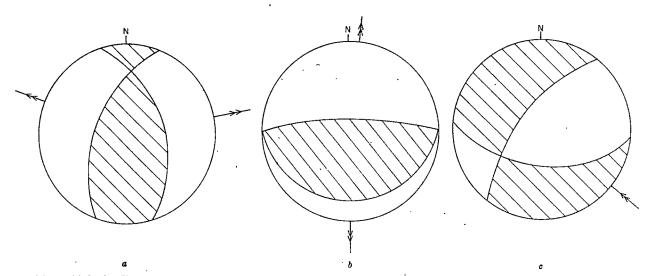


Fig. 6. Mechanism diagrams drawn as in Fig. 2 for three earthquakes in the Kurile Islands. (a) September 15, 1962 (ref. 14), extension by normal faulting. (b) November 15, 1963 (ref. 17), extension by normal faulting. (c) May 22, 1963 (ref. 17), island are overthrust from the Pacific side.

Another complicated area is in Alaska between 147.5° W. and the north end of the Fairweather fault16. In this same area the local uplift after the 1964 earthquake suggested that several faults were active16, and therefore the tectonics cannot be understood without more fault plane solutions.

The third area is in the Kurile Islands where two fault plane solutions (Fig. 6a and b) require dip slip faulting and crustal extension. This motion is completely different from most of the solutions in the area, which agree well with the rest of the North Pacific. Both earthquakes occurred beneath the steep wall of the Kurile trench on the island arc side, and are consistent with gravity slides down into the trench. The terraces which would result from such slides are common features of the trenches of both Japan and the Aleutians^{25,26}. There is also one fault plane solution which requires that the Pacific should be overthrusting the Kurile Islands (Fig. 6c), though the crustal shortening is consistent with the regional pattern.

The two ends of the North Pacific belt may also be discussed with the help of vector circuits. The end in Central Japan gives the trivial result that two trenches can join to give a third. The other end at the entrance to the Gulf of California is the circuit in Fig. 1, and shows how the East Pacific rise and the Middle America trench combine to become the San Andreas transform fault.

The North Pacific shows the remarkable success of the paving stone theory over a quarter of the Earth's surface, and it is therefore expected to apply to the other threequarters. It is, however, only an instantaneous phenomenological theory, and also does not apply to intermediate or deep focus earthquakes. The evolution of the plates as they are created and consumed on their boundaries is not properly understood at present, though it should be possible to use the magnetic anomalies for this purpose. The other problem is the nature of the mechanism driving the spreading. It is difficult to believe that the convection cells which drive the motion are closely related to the boundaries of the plates.

One area where the evolution is apparent lies between the plate containing the Western Atlantic, North and South America¹⁰ and the main Pacific plate. The transform faults in the South-East Pacific are east-west; therefore the ocean floor between the rise and South America is moving almost due east relative to the main Pacific plate. The motion of the Atlantic plate relative to the Pacific is given by the San Andreas, and is towards the south-east. If the motion of the Atlantic plate is less rapid than that of the South-Eastern Pacific north of the Chile ridge, then the crust must be consumed along the Chile trench. The faults involved must have both overthrust and right-handed strike slip components. present motion on the San Andreas is not in conflict with the east-west transform faults of the North-Eastern Pacific if there was originally a plate of ocean floor between North America and the main Pacific plate joined to that which still exists to the west of Chile. This piece of ocean floor has since been consumed, and therefore the direction of spreading in the Pacific appears to have changed in the north but not in the south. This explanation requires changes in the shape of the plates but not in their relative motion, and therefore differs from those previously suggested^{2,6}. This study suggests that a belief in uniformity and the existence of magnetic anomalies will permit at least the younger tectonic events in the Earth's history to be understood in terms of sea floor spreading.

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Solar Oblateness and Magnetic Field

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it has been suggested that the observed Solar oblateness may be caused by a gravitational quadrupole moment. The answer may not be so simple, however, because of magnetic stress and differential rotation between the radiation layer and the core.

This article deals with the interpretation of the experiment recently reported by Dicke and Goldenberg1, which shows that certain optical observations of the Sun indicate an oblateness $\Delta=(R_{\rm eq}-R_{\rm pole})/R_{\odot}$ of $(5\pm0.7)\times10^{-5}$, corresponding to $R_{\rm eq}-R_{\rm pole}\approx35$ km. This exceeds by 27 km the oblateness attributable to the mean apparent solar

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Dicke and Goldenberg suggest that this is evidence for a gravitational quadrupole moment caused by a core flattened by rapid rotation, and that this weakens the agreement between the implications of Einstein's gravitational theory and the otherwise unexplained residual advance of the perihelion of Mercury.

This inference from the observations involves a number of assumptions about processes and conditions in the interior of the Sun. Questions concerning the fluid-dynamical assumptions have been raised recently by Howard, Moore and Spiegel². Our purpose is to assess the role of the Sun's magnetic field—at the photosphere, in the convection zone, in the radiative core and at the interface of these two regions.

The argument of Dicke and Goldenberg rests on the following lemma of the von Zeipel theorem³: If a star is rotating uniformly (with angular velocity Ω), and if all forces other than the pressure gradient are derivable from a potential Φ , then pressure p, density ρ and temperature T have constant values on the equipotential surfaces $\Phi = constant$. If there are no forces other than the pressure gradient and gravity¹, then $\Phi = \varphi - \frac{1}{2}\Omega^2 r^2 \sin^2 \theta$, where φ is the gravitational potential.

Although the phenomenon of solar differential rotation⁴ indicates that there is a force system below the photosphere which is not curl-free, with the consequence that the von Zeipel lemma may not be applied to the interior of the Sun, Dicke and Goldenberg point out that it is sufficient for their purpose that the lemma may be applied, with negligible error, to the "seen layers", that is to the vicinity of the photosphere. Roxburgh⁵ has suggested that the differential Coriolis force associated with differential rotation may invalidate the von Zeipel lemma, but this is open to objection⁶.

Because the Sun's magnetic field represents a force system which is not necessarily curl-free, one may ask whether the photospheric magnetic field can effectively invalidate the von Zeipel lemma. If it cannot, the strong statement of the lemma greatly simplifies the problem¹; if it can, more detailed calculations concerning the photospheric structure are necessary.

If there were no gravitational quadrupole moment, the equipotential surfaces would have a small oblateness, $\delta \approx 10^{-5}$, corresponding to the mean apparent rotation rate. If the most rapid systematic variation of magnetic field is in the radial direction, the component of the force equation in the meridional plane and tangential to the photosphere is

$$-\rho g(\Delta - \delta) \sin 2\theta + \frac{1}{4\pi} B_r \frac{\partial B_{\theta}}{\partial r} = 0$$
 (1)

where the gravitational acceleration (g) is 2.7×10^4 cm sec⁻² and the density of the photosphere at the visible limb of the Sun (ρ) is approximately 2×10^{-8} g cm⁻³. If the magnetic field were randomly oriented, the mean magnetic-stress contribution to equation (1) would be zero. If, however, there were a stress system below the photosphere tending to produce oblateness, this would lead to a systematic distortion of the magnetic field such that the magnetic term of equation (1) would also tend to produce oblateness. If, for the present estimate, the magnetic field is assumed to be almost radial at the photosphere, the magnetic force density can be expressed as

$$\frac{1}{4\pi} B_{r^2} R_{C^{-1}}$$

where R_C is the radius of curvature of the projection of magnetic field lines on to the meridional plane.

The value of 1 gauss is often quoted for the "general" magnetic field of the Sun, based on the work of the Babcocks, but this general field is confined to the polar regions (latitude > 55°). Moreover, as Bumba, and others have emphasized, this value represents a highly integrated measurement, and much greater values are obtained if greater resolution is used. For our purposes, we need an estimate of the root-mean-square magnetic field, in quiet regions of the sunspot zone rather than in the polar regions, derived from measurements made with as fine a resolution as possible. Such measurements have been

made by Howard¹⁰, who finds the root-mean-square value of the photospheric magnetic field to be $8\cdot2\pm4\cdot4$ gauss. It is clear from the work of Severny¹¹ and Sheeley¹² that the polar field also is highly inhomogeneous, so that the root-mean-square value must be substantially greater than the average value. It therefore seems realistic to adopt 8 gauss as the best present estimate of the root-mean-square value of the photospheric magnetic field, but a somewhat smaller value would not change the conclusions which follow.

With the value of the root-mean-square field, the magnetic stress can meet the requirement of equation (1) if the variation of the magnetic field with radius is such that magnetic field lines have a curvature of 2,000 km or Severny¹³ claims, from a comparison of transversefield Zeeman measurements of different spectral lines. that the photospheric magnetic field changes substantially on a radial scale of 100 km. Hence observational evidence, as far as it exists, provides for the possibility that the photospheric magnetic field varies rapidly with depth. If this is so, one must determine the extent to which the visible photosphere can depart from a surface of constant effective gravitational potential by calculations, more detailed than those incorporated in the von Zeipel lemma, based on the force equation and the energy transport equation.

If it is possible that the von Zeipel lemma is not applicable to the solar photosphere, one must examine the possibility that forces other than gravity may be responsible for the observed oblateness, the internal distortion being coupled to the surface distortion by magnetic stresses. It is now possible that the forces responsible for the oblateness are located in the convection zone, a possibility which does not exist if the von Zeipel lemma is applicable to the photosphere. Because a complete theory of the convection zone, which accounts for differential rotation and the solar cycle, has yet to be developed, there is no way of estimating the magnitude and character of the force-field of the convection zone, so that there is no way of determining whether or not these forces lead to photospheric oblateness.

We may now estimate the effect of a "horizontal" magnetic field in the convection zone, such as that envisaged in the Babcock model of the solar cycle¹⁴. The radial component of the force equation may be written as

$$\rho g = -\frac{\mathrm{d}p_G}{\mathrm{d}r} - \frac{\mathrm{d}p_M}{\mathrm{d}r} \tag{2}$$

where p_G and p_M are the gas and magnetic pressures, respectively. Because the magnetic field is "frozen" into the plasma, we should consider a model in which the magnetic pressure increases with gas density. A convenient simple model is to assume that $p_M/p_G=\varepsilon$, where ε is constant throughout the convection zone. Then we see from equation (2) that the scale height H is increased to $(1+\varepsilon)H$ so that the photosphere is raised by a height h given by

$$h \approx \varepsilon D$$
 (3)

where D, the depth of the convection zone¹⁵, is about 10^5 km. To explain the observed oblateness, we need $\epsilon \approx 3 \times 10^{-3}$ which corresponds to a toroidal magnetic field increasing in strength from about 6 gauss at the photosphere through about 2,000 gauss in the centre of the convection zone to about 8×10^5 gauss at the bottom of the zone. Somewhat higher field strengths in the outer layers would obviate the need for very intense fields in the lower layers. The existence of strong magnetic fields in sunspots points to the existence of a magnetic field of some thousands of gauss in the convection zone.

These considerations open the possibility that the observed oblateness may be a consequence of non-

gravitational forces. But even if the oblateness is ascribed to a gravitational quadrupole moment, this does not unambiguously indicate a rapidly spinning core. It is important to note that the core may retain a strong magnetic field, possibly of order 10° gauss, of primaeval origin. If U_M is the magnetic energy of the core and U_{GB} the gravitational binding energy, the departure from sphericity is expected.

$$\Delta \approx 5 \, \frac{U_M}{U_{GB}} \tag{4}$$

For the Sun', $U_{GB} \approx 10^{40}$ erg, from which we find that the observed oblateness might be produced by an internal magnetic field of average strength about 2×10^6 gauss. Thus if the magnetic field of stars is primaeval in origin, and if the original magnetic field of the Sun is now confined primarily to the radiative core, the resulting stresses are likely to produce a deformation of the equipotential surfaces comparable with the photospheric distortion measured by Dicke and Goldenberg.

The recent calculation of Weber and Davis¹⁷ yields 10³¹ dyne cm as an estimate of the torque exerted on the photosphere by the solar wind, through the agency of the magnetic field. This torque is sufficient to halve the angular momentum of the convection zone in a few million years. This implies that the radiative core exerts a torque on the convection zone which is approximately equal and opposite to that of the solar wind on the convection zone. Because part of the torque is caused by viscosity, this imposes an upper limit on the magnetic flux linkage of the radiative core and convection zone.

Using spherical polar co-ordinates, we find that the magnetic torque at the interface of the radiative core and convection zone is approximately $r^3 < B_r B_{\varphi} >$. Hence $\langle B_r B_{\varphi} \rangle$, the average value of $B_r B_{\varphi}$ over this interface, may not exceed 0.05 gauss². Because a velocity shear at the interface will ensure that any magnetic flux crossing the interface will be stretched in such a way that the magnetic stress opposes the velocity difference, we may assume that the magnetic stress had the same sense over the interface. If the three magnetic field components are comparable in magnitude, the average value of the magnetic field must be less than about 0.3 gauss. If the field is normal to the radial vector and of strength 104 gauss, the radial (flux-linking) component must be less than 5×10^{-6} gauss. If it is considered unlikely that the flux linkage between the radiative core and the convection zone is so small, it must be considered unlikely that the angular velocity of the core differs significantly from the mean angular velocity of the photosphere.

In this connexion, we should further note that the solar wind depletes the entire gas-content of the convection zone in about 10° years, so that gas lost from the convection zone at the photosphere is continually being replaced by an outward flow of gas from the radiative core. Such an outward migration of gas must be expected to entail also an outward migration of magnetic field, which adds to the difficulties contingent on the assumption that there is negligible magnetic coupling between the radiative core and the convection zone.

The foregoing considerations imply that the observations of Dicke and Goldenberg may prove more significant in understanding the solar interior than in understanding gravitation. If further analysis of the effects of the solar magnetic field and of solar differential rotation confirms that the photosphere is not necessarily a surface of constant effective gravitational potential, then we must wait for a detailed understanding of all the forces—due to gravity, magnetic fields, differential rotation and convection—before we can interpret the observed oblateness.

If it is determined that factors such as the differential Coriolis force and the photospheric magnetic field do not prevent the photosphere from relaxing to a surface of constant effective gravitational potential, or if the forces in the convection zone produce negligible distortion of the photosphere, then it may be concluded that the observed oblateness provides evidence for a gravitational quadrupole moment. It does not follow, however, that the gravitational quadrupole moment is of such a simple type that it gives rise to a pure oblateness of the photosphere; nor does it follow that the axis of symmetry, if any, of the distortion is coincident with the axis of rotation of the photosphere.

If conclusive arguments can be found which preclude a strong internal magnetic field, or if it can otherwise be decided that the oblateness is caused by a rapidly spinning core, then the gravitational quadrupole moment can be represented by a single second-order spherical harmonic with respect to the axis of rotation of the core. It is possible that detailed understanding of the nature of the internal and external torques exerted on the convection zone will lead to the conclusion that the axes of rotation of the radiative core and of the convection zone differ by only a negligible amount. It will be shown, however, in the subsequent article that a difference of about 3° is crucial in interpreting the residuals of the orbital elements of the planets. Although the data of Dicke and Goldenberg do not readily permit an estimate of the maximum permitted inclination of the rotation axis of the photosphere and the axis of the spheroidal distortion, their graphical results give ±15° as outside limits on the difference in longitudes of the nodes of the equatorial planes of the photosphere and of the spheroidal distortion.

Furthermore, it should be remembered that the angular momentum of the solar system presents anomalies which may be related to the angular momentum of the radiative core. In particular, the rotational axis of the photosphere and the angular momentum vector of the solar system (of which the dominant contribution is caused by the planet Jupiter) have a relative inclination of about 6°. If we admit the possibility that the angular velocity of the radiative core is decoupled from that of the convection zone, it may be that there is a relationship between the angular momentum vector of the core and that of the solar system as a whole, rather than that of the convection zone. If such a discrepancy between the axes of rotation of the radiative core and the convection zone cannot be ruled out on theoretical grounds, it will be necessary to determine by observations of the Dicke-Goldenberg type, or otherwise, the spherical-harmonic coefficient and the three coefficients determining the axis and rotation rate of the core.

If there are no conclusive arguments against the existence of a strong internal magnetic field, it is no longer justifiable to assume that the gravitational quadrupole moment may be represented by a single spherical-harmonic coefficient. In this case, to obtain the information necessary for interpreting planetary orbits, one will need to determine the five second-order spherical-harmonic coefficients with respect to the axis of rotation of the core, plus the three coefficients characterizing the axis and rotation rate of the core.

The observations reported by Dicke and Goldenberg covered a period of four months, but a sequence of observations of the type envisaged in the previous two paragraphs must necessarily extend over quite a long period. In any case, it is clearly desirable that these observations should cover at least one half of a 22 year solar cycle, because variation of the observed oblateness with the solar cycle would indicate that the effect has its origin in the convection zone and therefore has no gravitational significance.

Because the interpretation of the observed photospheric oblateness involves many questions of solar physics which at present seem difficult to answer, one may enquire whether the gravitational quadrupole moment of the Sun may be determined by methods other than

those used by Dicke and Goldenberg. This question is discussed in the following article 18.

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Solar Oblateness and the Perihelion Advances of Planets

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Observations of the oblateness of the Sun suggest that it may have a rapidly spinning core. An experiment to test this suggestion, by comparing the perihelion advance of a small artificial planet of high eccentricity and Mercury, is discussed.

THE suggestion that the radiative core of the Sun may be rotating much faster than the observed surface may affect the agreement of the observed advance of the perihelion of Mercury with the prediction of general relativity1,2. Furthermore, it has implications with respect to the origin of the solar system, the evolution of stars and the role of stellar winds (in particular the solar wind)1,2. visual observations of the photosphere by Dicke and Goldenberg³ make it possible to infer the existence of such a rapidly spinning core of the Sun only on the basis of the validity of the von Zeipel lemma, implying that the observed surface is one of constant gravitational and centrifugal potential, and that the radiative core does not possess a strong internal magnetic field.

It has been pointed out4, however, that the conditions of the von Zeipel lemma may be violated by virtue of photospheric magnetic fields, in which case non-gravitational forces in the convective zone may induce an oblate-(or possibly prolateness) superimposed on the possible oblateness caused by a gravitational quadrupole moment. The optical oblateness measures the sum of these two effects, but only the latter produces a peri-helion advance of a planet. The purpose of this article is to discuss the implications of the preceding considerations on the test of gravitational theories provided by the excess advances of the perihelia of planets (in this context, excess advance means the difference between the observed advance of the perihelion and that computed on the Newtonian theory with the possible effect of solar oblateness ignored). Furthermore, a dynamical method is suggested of checking the existence of a gravitational quadrupole moment of the Sun which is independent of the inevitable uncertainties inherent in the interpretation of an optical oblateness. Finally, it will be pointed out that the possibility of decoupling of the radiative core and the

convection zone of the Sun, raised by the argument of Dicke and Goldenberg, may significantly affect, by virtue of a small angular deviation in the corresponding axes of rotation, the extent to which the discrepancies in the motions of the major planets from their predicted values can be identified as arising from non-Newtonian effects.

If the previous arguments4 are valid, it is possible that the solar oblateness observed by Dicke and Goldenberg provides information on the structure of the convective zone rather than evidence for a gravitational quadrupole moment. In this case, the gravitational quadrupole moment might be insignificant as regards perihelion advances of planets, and the visual indications of oblateness may not change the observational status of gravitational theories at all. It would seem that this possibility could be confirmed or disproved theoretically only by more complete theoretical and observational knowledge of the visible layers and the interior of the Sun, leading to resolution of the uncertainties raised in the preceding article4. Alternatively, the question could be resolved by the dynamical experiment to be discussed later.

A second possibility is that a rapidly spinning core, decoupled from the convective zone, indeed, produces a gravitational quadrupole moment of the Sun and a corresponding oblateness of the core. Externally, the gravitational potential would then depart from spherical symmetry through an axially symmetric component $J_2P_2(\cos\theta)/r^2$ at the radial distance r, where P_2 is a Legendre polynomial in the colatitude θ measured from the rotational axis of the core, and J_2 is a numerical coefficient. In view, however, of the possibility that the von Zeipel lemma may not be applicable to the photosphere and the necessity of decoupling the radiative core from the convection zone inherent in the thesis of Dicke and Goldenberg, one cannot identify a priori the

direction of the axis of rotation of the core with that of the observed photosphere. Thus two cases arise, because the directions of the two axes may coincide or they may diverge significantly as regards perihelion advances of planets. On the basis of the preceding discussion, the decision between the two cases cannot be made on theoretical grounds, and one must invoke an observational criterion.

The case of coincidence of the two axes in question is the one assumed tacitly by Dicke and Goldenberg, although their optical observations clearly are inadequate to verify the assumption. For this case, let $v_{\rm GQM} = v_{\rm GQM}(a,J_{z,\rm Mer})$ be the excess advance of the perihelion of a planet of mean distance a arising from the effect of solar oblateness when the coefficient J_2 is assigned the value $J_{2,Mer}$, which makes this advance for Mercury equal to the corresponding total excess advance of the perihelion. The value of v_{GOM} depends implicitly on known orbital elements specifying the orientation of the planetary orbit relative to the solar quadrupole moment. Furthermore, let $v_G(a)$ be the excess advance of the perihelion of a planet of mean distance a produced by non-Newtonian gravitational effects when the entire excess advance for the planet is ascribed to this cause. Then the assumption of Dicke and Goldenberg implies that the excess advance v of the perihelion of a planet can be represented by

$$v = \alpha v_G + \beta v_{GQM} \tag{1}$$

for every solar planet, with α and β constants satisfying $\alpha+\beta=1$. The constant α measures the fractional contribution of non-Newtonian gravitation (whether by general relativity or the scalar-tensor5 theory) to the perihelion advance, and β is a constant given by

$$\beta = J_2/J_{2,\text{Mer}} \tag{2}$$

where J_2 measures the actual quadrupole moment. Note that v_G on the basis of general relativity and v_{GOM} vary inversely with a as the 5/2 and 7/2 power, respectively.

The dynamical test for solar oblateness permitted by equation (1) is possible only if excess advances of the perihelion are known observationally with sufficient accuracy for two or more planets, say, Mercury and either Venus or the Earth. For the latter two planets, however, the accuracies in the determinations of v are inadequate for this purpose, a circumstance arising in the first instance because of the relatively small advances of perihelion. More importantly, the recent determination of the astronomical unit and the mass of the Earth-Moon system by means of radar echoes from Venus has revealed the existence of systematic errors introduced into conventional orbit determinations by inaccuracy in the previously inferred values of these parameters (determined from perturbations of the orbit of the asteroid Eros at close approaches to the Earth)6,7. Concomitantly, actual errors exceeding the quoted probable error by a factor of three are not uncommon in orbital calculations based on optical observations, and greater factors are possibles. Radar observations of the planets, in progress at Lincoln Laboratory, promise an ultimate accuracy in orbit determinations exceeding the present by at least two or three orders of magnitude (private communication from I. I. Shapiro). The question of the actual accuracy of the figure $43 \cdot 1 \pm 0 \cdot 4$ sec/century for the perihelion advance of Mercury must thus await results from the radar measurements.

Because the accuracies in the determinations of the perihelion advances of Venus and the Earth are inadequate for the purpose, the only natural planet that could be conjoined with Mercury for a test of equation (1) is the asteroid (1566) Icarus⁹. For this minor planet, the advance of the perihelion predicted by general relativity is 10·1 sec per century if the corresponding advance of Mercury arose solely from this cause⁸. The conclusions

of Dicke and Goldenberg imply $\alpha = 0.92$ and $\beta = 0.08$, and thus equation (1) predicts v=10.6 sec per century for Icarus on the basis of the results of these authors. No observational determination of v for Icarus exists, and many years of further observations might be required to obtain it. In view of the high figure of merit¹⁰ for precision in fixing the perihelion advance of Icarus, a probable error in a future determination equal to the low value 0.4 sec per century for Mercury might be achieved, but even then the advance implied by the considerations of Dicke and Goldenberg would exceed that predicted on general relativity by only one probable error. Assuming the possibility of an actual error exceeding the quoted probable error by a factor of three in orbital calculations based on optical observations8, one estimates that a definitive result in favour of solar oblateness could be obtained only for $\beta \gtrsim 0.25$. Thus if the value of Dicke and Goldenberg for β is correct, verifying by means of equation (1) from natural planets does not seem possible in view of present inaccuracies in orbit determinations, and the only recourse would seem to be use of artificial planets of high eccentricity and small mean distance11.

If the axes of rotation of core and photosphere do not coincide, use of artificial planets to infer the solar quadrupole moment dynamically becomes necessary a fortiori, because the analogue of equation (1) then involves at least four constants (an additional two are required to specify the orientation of the axis of the solar core with respect to the ecliptic).

The case of different axes of rotation of the solar core and photosphere has a special importance because, long before the work of Dicke and Goldenberg on the visual oblateness, effects which would arise from the lack of coplanarity of the Sun's equatorial plane and the orbital plane of Mercury were cited as militating against the suggestion that solar oblateness produces the excess advance of the perihelion of Mercury¹². The inclinations of the equatorial plane of the Sun's photosphere and the orbital plane of Mercury are nearly the same (7° and 7° 0', respectively)13, and the angle between the corresponding normals has the low value 3° 22'. This small angle, however, corresponds to a significant difference in the longitudes of the ascending nodes, corresponding to 47° 56′ for the Sun and 75° 18′ for Mercury¹³. As a consequence, the rate of decrease of the inclination of Mercury's orbit would amount to -2.3 sec/century if the entire excess advance of the perihelion of Mercury were ascribed to an oblate solar core with equatorial plane corresponding to the photosphere8. Such a large variation of this orbital element is not admissible, because the residual (observed less computed Newtonian value) is only -0.12 ± 0.16 sec/century⁸. Dicke and Goldenberg infer a solar oblateness less by a factor of about ten, and thus allowable in view of the probable error in the residual.

Because, however, the small angular departure in question between the axis of the solar quadrupole moment and the normal to the orbital plane of Mercury is essentially a matter of presumption from the orientation of the equatorial plane of the photosphere4, one can examine the important possibility where it in fact vanishes. In this case, the equatorial plane of the spinning core coincides with the orbital plane of Mercury, and the perturbing force on the planet depends only on the radial distance r(as is strictly true in general relativity). Thus the perturbation affects neither the inclination nor the longitude of the node of Mercury's orbit. Furthermore, no substantive discord appears between observed and calculated variations in orbital elements for any planet, even if the entire excess advance of the perihelion of Mercury is ascribed to the effect of solar oblateness in this case. Under this assumption, results from equations of Shapiro⁸ are shown in Table 1 for the secular rates of change of the argument of perihelion, the longitude of the ascending node and the inclination of the orbits of Mercury and Venus: corresponding values from general relativity

and residuals are shown for comparison. The agreement with the residual in the motions of both the perihelion and the node for Venus is improved and the motion of the former is now direct as implied by the residual rather than retrograde, in contrast to the corresponding case in which the axis of the solar quadrupole moment is taken normal to the equatorial plane of the photosphere. Every predicted variation in orbital elements (including those for the Earth) falls within a limit of two probable errors from the corresponding residual, a bound which is quite acceptable for those observations⁶⁻⁸.

Table 1. VARIATIONS IN ORBITAL ELEMENTS FOR MERCURY AND VENUS PRODUCED BY A GRAVITATIONAL QUADRUPOLE MOMENT (GQM) OF THE SUN WITH AXIS NORMAL TO MERCURY'S ORBIT AND VALUE NECESSARY TO YIELD THE ENTIRE EXCESS ADVANCE OF MERCURY'S PERHFELION, AS COMPARED WITH RESULTS FROM GENERAL RELATIVITY (GR)

Variation		Me	rcury	Venus		
Orbit element	GQM	$_{ m GR}$	Residual	GQM	GR	Residual
Perihelion argument*	43.1 †	43.0	43·1 ± 0·4 ‡	0.7†	8.6	8·1 ±5·3‡
Node longitude*	0	0	0.4 ± 1.0	3.5	0	0.7 ± 2.2
Inclination*	0	0	-0.12 ± 0.16	0.25	0	-0.07 + 0.12

^{*} In sec/century.
† This work.
† See ref. 8.

The figures for Venus in Table 1 take into account the direction of the normal to the orbit relative to the assumed axis of the solar quadrupole moment. If the small angular difference involved is ignored, the value of Table 1 for the perihelion advance of Venus is grossly overestimated as 4.9 sec/century14. Thus the variation of the perihelion (and also the inclination) of a planet is quite sensitive to the orientation of the gravitational quadrupole moment. The physical reason is that the argument of perihelion is measured from the line of nodes, fixed by an angle of intersection of two planes which is usually small, so that the relevant equations correspond almost to a singular case. This strong sensitivity does not appear in the longitude of perihelion, which is measured from the vernal equinox.

The implication of Table 1 is that any fraction from zero to unity of the excess advance of Mercury's perihelion can be explained by a gravitational quadrupole moment arising from a spinning solar core with axis aligned sufficiently close to the normal to Mercury's orbit, with an observational disagreement in the variation of orbital elements for any planet. Consistency with the result of Dicke and Goldenberg for the visual oblateness, however, requires either that the fraction be small (of the order of 10 per cent) or that the deformation induced in the photosphere by the rotating core be partially compensated by a negative contribution from forces in the convection zone. It must be understood that we are not proposing that the excess perihelion advance of Mercury is indeed caused purely by a gravitational quadrupole moment; our purpose is, rather, to emphasize that the range of possibilities is markedly extended once one relaxes the assumption that the axis of the gravitational quadrupole moment coincides with the rotational axis of the Sun's photosphere.

As a final possibility, it can be mentioned that significant contributions to the excess advances of the perihelia of planets may be produced by a gravitational quadrupole moment arising from distortion of the solar core by a strong magnetic field rather than by rapid spin. In this case, there is no reason to believe that the departure of the gravitational field from spherical symmetry can be represented adequately by only one spherical harmonic of second order, and the data of Dicke and Goldenberg are insufficient to justify such an assumption. Thus the term corresponding to the effect of solar oblateness in the analogue of equation (1) must depend on eight independent parameters, for a total of nine which must be fixed by observation of planetary orbits.

These considerations by no means rule out the possibility of a significant contribution from solar oblateness to the

advance of the perihelion of Mercury. In fact, it has been shown that, at the present stage of knowledge, the observational data admit the possibility (dependent on the orientation of the quadrupole moment) of a contribution from solar oblateness significantly exceeding the value inferred by Dicke and Goldenberg. It would seem that a conclusive test separating the relativistic effect from that of solar oblateness could only be achieved by combining data from the orbit of Mercury with those of artificial planets launched into highly elliptical orbits of mean distance approaching one-half that of the Earth. In this case, the maximum value of the perihelion advance possible for an orbit grazing the Sun becomes 11.6 sec, yr on the basis of general relativity, larger than the value for Mercury by nearly two orders of magnitude. The relatively large value of the excess perihelion advance possible in these conditions, coupled with highly precise radar observations of position and velocity by means of transponders, should permit dynamical evaluation of the solar oblateness within a decade at the most, if a determined effort were made with the resources of modern space technology.

For such orbits permitting close approach to the Sun. many perturbations which ordinarily are negligible would have to be considered and determined in order to exploit the full potentiality of the proposed method. Thus the effects of sunlight pressure and of solar corpuscular radiation, and charge and neutral drag in the extended solar corona, would have to be evaluated15. The reflexion properties and possibly the orientation of the artificial planet, dependent on its characteristics, may have to be known quite accurately in order to compute with sufficient precision the perturbation caused by sunlight pressure.8. Finally, the necessity of cancelling the orbital velocity of the Earth for nearly direct fall of the body into the Sun raises some problems of size for the booster rocket.

In brief, it may be concluded that improved understanding of gravitation may spring either from advances in solar physics or from advances in space science. If this knowledge arises from space science, it may come from the experiment proposed here, from studies of relativistic effects in radar observations of the orbits of Venus and Mercury, as suggested by Shapiro¹⁷, or from analysis of the precession of a gyroscope in a satellite orbiting around the Earth, as proposed by Schiff18 and by Everitt and Fairbank¹⁹.

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Solar Oblateness

by

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Department of Mathematics, Queen Mary College, University of London In this article the author answers criticism, by Dic Goldenberg, on his theory of why the Sun is oblate.

RECENTLY, in an article on the oblateness of the Sun¹, I suggested that the observed difference of 35 km between the polar and equatorial radii² could be caused by the effect of uniform rotation on the solar convective zone. Dicke and Goldenberg, in a reply³ refuting this suggestion, did not consider my principal hypothesis, but concentrated only on the weaker peripheral deductions.

My basic suggestion was that the solar rotation interacts strongly with the turbulent convection in such a way that a steeper temperature gradient is necessary in the polar direction than in the equatorial. If the temperature at the base of the convective zone is approximately the same at pole and equator, then the temperature falls to the surface value in a shorter distance in the polar direction than in the equatorial. Thus the surface is oblate.

In support of this hypothesis the following results can be quoted. (1) Investigations of the onset of instability4 do show that rotation inhibits convection when rotation and gravity are parallel (the polar case), but not when they are perpendicular. (2) Investigations by Weiss⁵ and myself on turbulent convection in a rotating system give the suggested result, although the magnitude of the effect cannot be accurately predicted because there is no satisfactory theory of turbulent convection. (3) Although rotation can only have a small effect in stable zones, because the ratio of centrifugal force to gravity is small, this need not be the case in turbulent convective zones. In some respects gravity is weakened by a factor of the ratio of the superadiabatic temperature gradient to the actual temperature gradient (which is small compared with unity), so that even a slow rotation could conceivably have a large effect.

Certain deductions can be made from the hypothesis which supports the general idea. In particular, the different temperature gradients in the polar and equatorial directions imply a temperature and thus a pressure difference over a surface of constant density. The resulting latitudinal pressure force has to be balanced by motions other than uniform rotation, and the observed equatorial acceleration of the Sun could be taken as evidence of such an effect. Dicke and Goldenberg consider that their model with a rapidly rotating core provides several mechanisms to explain and maintain the equatorial accelerations while the model I proposed does not. In my original paper I considered a radial angular velocity This need not necessarily be the case; the actual variation would depend on the latitude dependence of the excess pressure over an equidensity surface which could only be obtained by a more detailed analysis. As far as the maintenance is concerned, Kippenhahn⁶ and co-workers have found that a circulation in the convective zone (in this case driven by the pressure excess) is

capable of maintaining equatorial acceleration in a envelope.

Dicke and Goldenberg list three of my origin.

for answer. (a) The surface of the Sun is located where
the temperature equals the effective temperature; (b) if
the solar core were rapidly spinning, the polar flux would
exceed by about 1 per cent that at the equator; and (c)
the excess oblateness of 27 km is caused by an internal
temperature gradient and a large positive radial gradient
in angular velocity.

I agree with their statement that the $T=T_{\rm eff}$ is not the observed solar limb, which is at an optical depth of about 0·004. This does not affect my conclusion because wherever the limb is situated the temperature is negligibly small compared with the temperature at base of the convective zone, and can be taken as zero as far as calculating the oblateness is concerned. Admittedly there is much to be done before we understand the structure of the solar atmosphere, and the exact nature of the adjustment of the atmosphere to the oblate convective zones requires detailed examination.

I agree with Dicke and Goldenberg's doubts over the use of von Zeipel's theorem; however, it is more likely to be valid for the interior than for the surface layers.

I do not state that the oblateness is caused by a radial angular velocity gradient. The oblateness is supposed to be caused by the interaction of rotation and convection. This in turn requires some motion other than rotation. Whether this excess motion is differential rotation in a radial sense or an angular sense, or primarily a circulation, has yet to be resolved.

Finally, one should point out that Goldreich and Schubert (work to be published) have recently found that the Rayleigh-Taylor instability occurs in a stably stratified star, and that therefore the angular velocity must be independent of distance from the equatorial plane and that the angular momentum per unit mass must increase away from the rotation axis. This prevents the development of radial variation in angular velocity and ties the rotation of the solar core to that of the envelope. It is therefore difficult to see how the inside of the Sun could be rotating faster than the outside as required in the Dicke model.

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Generation of Radio Sources

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Many radio sources have a double character, and high resolution studies show small components. It is suggested that radio sources may be made up of many small sources.

THE discoveries of the past few years have shown that extragalactic radio sources originate either in galaxies of various kinds or in the quasi-stellar objects. The possibility that there is yet another class of source is not excluded. The bulk of the radiation is emitted by the synchrotron process, and it was shown more than a decade ago that the great distances of the radio sources and their large sizes necessarily imply that a tremendous amount of energy must instantaneously be present in the form of magnetic flux and relativistic particles in such sources. Accurate estimates of the total energy present are not possible because no independent method of measuring the mean magnetic field in the radio sources has yet been found. Thus the most conservative estimates have been made, and these are obtained when there is approximate equipartition between magnetic and particle

energy, $E_p = \frac{4}{3} E_m$. The minimum total energy is then $E = \frac{7}{3} E_m$.

The very large energies which must be released in the radio sources have been a subject of intense investigation, and in nearly all theories it is now argued that this energy is largely gravitational in origin. The mechanism by which this energy is transferred into relativistic particles, however, still remains obscure. Little attention has so far been paid in the literature to the problem of understanding the shapes or brightness distributions of the synchrotron sources. It is this problem that I wish to examine in this article.

There are two major features which need to be explained. The first is the well known double character of the radio sources. The second is the fact that, in a considerable proportion of sources, high resolution studies are now showing that very small components with high surface brightnesses are present. The very small radio components are frequently found in the nuclei of the galaxies with which the radio sources are identified, or are coincident with the optical quasi-stellar objects. Well known examples are 3C 84, various core-halo objects, the small diameter sources investigated by long baseline interferometry or by scintillation techniques, and all the objects which are found to vary. There are a number of cases, however, where there is evidence for small configurations of high brightness far from the nuclear regions of a galaxy. Examples are the structure of Cygnus A recently described by Wade¹, the small high-surface-brightness regions recently discovered in M 87 which may lie along the optical jet (private communication from D. Hogg) and the remarkable double sources 3C 33 (ref. 2), 3C 343 and 343·1 (refs. 3 and 4), and PKS 0453-30, 0456-30 (ref. 5), which have very small sizes when compared with their separations. There are also objects such as 3C 225 and 30 267 (ref. 6) each of which contain a highly compact source. They are both double sources of unequal intensity. In both cases the compact source is of very small angular diameter $\gtrsim 0.2^{\prime\prime}$

Also of great interest are very small sources such as

CTA 21 and 3C 49 (ref. 7) which seem to lie in empty fields. They may be centred on massive objects which are emitting at optical frequencies at very low levels. Alternatively, they may be small sources which have been ejected from a distant galaxy or quasi-stellar object.

It is by now well established that the radio sources are generated by violent outbursts in the nuclei of galaxies or other massive objects. Thus the occurrence of a very small central component associated with an optical object suggests that this is the seat of the outburst which is still taking place. The double nature of the sources and particularly the existence of very small intense sources far from the seat of the explosion, however, have not been satisfactorily explained. It has been generally argued that relativistic particles are ejected freely from the nucleus. because it is clear that if a cloud expanded adiabatically the initial energy would be unreasonably high. To obtain a double source it has been argued that the presence of interstellar matter and magnetic field in a disk-like distribution will lead to ejection normal to the disko, or ejection controlled by the magnetic field configuration of the galaxy¹⁰. Once the particles have escaped from the main confines of the galaxy, however, there is little to confine them, and the problem of the persistence of two discrete components is a severe one.

It has been argued that the particles are effectively moving freely⁶, their trajectories being determined by their passage through the galaxy, that they are moving in a region of enhanced magnetic field and that the time scale of the radio source is determined by the time taken to traverse this region (about 10⁶ years). Such a scheme, however, will not explain the presence of small discrete sources very distant from the explosion centre. Some mechanism of confinement outside the galaxy is required. Gold¹⁶ has therefore suggested that this confinement is caused by the presence of a comparatively strong (about 10⁻⁶ gauss) intergalactic magnetic field. This field must have a cosmological origin. Gold has attempted to show that such a field would exert sufficient pressure on the boundaries of a source such as Cygnus A treated as a simple double, so that it could be confined.

The volume of the Cygnus A source is estimated to be 1069 cm3, while its minimum total energy content is about 3×10^{60} ergs (ref. 11). Here it is assumed that the proton energy is 100 times the electron energy. If the electrons alone are present the minimum total energy is about 1059 ergs. Thus the mean energy density in the source is about 3×10^{-9} to 10^{-10} erg/cm³, the pressure exerted is about 10^{-9} to 3×10^{-11} dynes/cm², and the minimum magnetic field necessary to equalize the pressure inside and outside the source must lie in the range 10-4 to 10-5 gauss. These values are higher than those given by Gold because he assumed a total energy content of about 1058 ergs which is considerably below the minimum total energy which must be instantaneously present to explain the radio source on the synchrotron theory if a single coherent source is involved. Such magnetic fields are unreasonably large. In widely spaced doubles like 3C 33 and PKS 0453-30, 0456-30, the external magnetic fields

to give significant external pressures need to be about 10^{-5} gauss (these estimates are based on the source identifications and thus distances suggested by Moffet² and by Gardner and Bolton³, respectively). For sources which are smaller than these the total energy densities might be higher, because the energy densities of magnetic flux and relativistic particles are proportional to $L^{4/7}r^{-12/7}$, where L is the total power emitted by the source and r is its dimension. Thus it seems that this mechanism of containing radio sources is not likely to be effective.

Another difficulty with the idea that such sources are contained by an external magnetic pressure is the fact that instabilities that give rise to large-scale loss of particles easily develop in the boundary regions. It has been argued that in a rather similar situation cosmic ray particles which attempt to escape from our Galaxy give rise to instabilities which twist together the magnetic lines of force, thus effectively leading to containment¹². As Cowling¹³ has pointed out, however, thermonuclear experiments lead us to believe that magnetic confinement of this type is exceedingly difficult to attain.

Small Source Model

It can be concluded that the radio sources cannot be controlled by outside forces when they appear outside galaxies, and we are then forced to the conclusion that the small sources must be generated and contained by internal forces. What are the requirements of such a model? We first consider the energetics of sources as a function of their linear dimensions. In the calculations that have been made previously14, it has been supposed that the sources are large clouds containing a homogeneous magnetic field. As soon as we admit the existence of small intense sources, however, it is natural to consider a general model in which it is supposed that the flux is emitted from n similar very small intense sources with dimension r and magnetic field H, rather than from a single homogeneous source of dimension R and magnetic field H_0 . If we wish to adhere to the idea that the equipartition condition prevails so that the total energy in particles and field is a minimum, then it is easy to show that

$$\frac{H}{H_0} = \left(\frac{R}{r}\right)^{6/7} n^{-2} \, {}^{7} \tag{1}$$

Also in equipartition conditions, the ratio of the total energy, $E_0 = (E_p + E_m)$, present in the single large homogeneous source to that in a source made up of n small sources each with energy E is

with energy E is
$$\frac{E_0}{nE} = \left(\frac{H_0}{H}\right)^{-3/2} = \left(\frac{R}{r}\right)^{9/7} n^{-3/7} \tag{2}$$

Let us now consider some possible cases. For an object like Cygnus A, in which fine structure has been detected, we might suppose, for example, $n \simeq 10^3$ and $\frac{R}{r} \simeq 10^3$.

Then $H = 51.8 H_0$ and $E_0 = 373 nE$.

Because the equipartition magnetic field for the homogeneous case has been calculated to be about 2×10^{-4} gauss by Maltby et al.¹¹ we see that for this example the equipartition fields in the sub-sources are about 10^{-2} gauss and the total energy necessary to give rise to the source is reduced to about 10^{58} ergs. At the same time the lifetime of the source, calculated by dividing its total electron energy content by the power radiated, is also reduced by the factor (E_0/nE) .

In a large source in which no fine structure can be detected we might argue that there are a very large number of very small sub-sources. For example, we might consider $n = 10^5$, $R/r \approx 10^5$. Then $H/H_0 = 720$ and $E_0/nE = 1930$

If single very small sources are ejected and are found to be comparable in intensity with single large sources, then we have simply that

$$\frac{H}{H_0} = \left(\frac{R}{r}\right)^{6/7} \text{ and } \frac{E_0}{E} = \left(\frac{\dot{R}}{r}\right)^{9/7}.$$

Thus for

$$\frac{R}{r} = 10^6, \frac{H}{H_0} = 1,930, \frac{E_0}{E} = 2.7 \times 10^{6}$$

These examples show that if the radio sources are made up of numbers of small objects, the magnetic fields in them are likely to be much stronger than those usually assumed, the instantaneous energy requirements are much lower, and also the time scales are correspondingly shorter.

In all the calculations that have been made either assuming large homogeneous regions or in models of this type, it has been assumed that the equipartition condition prevails. No physical reason for the development of such a situation, however, has been given and it has been pointed out that even in the case of large homogeneous sources departures from this condition are probable. If a radio source is made up of small coherent sub-regions, there is no reason to believe that equipartition between particles and field develops here, and we can only take the values given here as possible indicators of what the true parameters are. The small objects must be comparatively stable during the period in which they are ejected from the nuclear region of the galaxy or quasi-stellar object.

What are the requirements of such sub-sources? A simple model of such an object might consist of a highly condensed object of mass M, surrounded by a gas cloud of density ρ containing the magnetic field of strength H. The kinetic or turbulent velocity in the gas is v. If the magnetic field is to be controlled gravitationally we must have that

$$\frac{GM}{r'} \gtrsim \frac{H^2}{8\pi} + \frac{1}{3} \rho v^2$$

where r' is the size of the region of magnetic field and gas which is controlled by the central condensed object. (r' is not necessarily the same dimension as r described earlier). We have also assumed that the relativistic particle pressure is small compared with the magnetic pressure so that it has been neglected. Now, for example, if we put v=1,000 km/sec, and $H=10^{-2}$ gauss, then provided that $\rho < 10^{-21}$ g/cm³, the second term on the right-hand side can be neglected and we find that

$$\frac{M}{\tilde{r}'} \gtrsim 6 \times 10^{22}$$

If we suppose that equilibrium is maintained, then for values of r' ranging from 10^{-2} to 1 parsec, $M \sim 10^{6}$ to 10^{8} M $_{\odot}$. At first sight it seems that, if the magnetic field and gas density are lower than the values just assumed, M/r' is reduced. Proper account, however, should be taken of the effect of the relativistic particles. The pressure exerted by these particles may well dominate, because if we assume weaker fields their energies must be correspondingly higher according to the synchrotron theory.

This may very well mean that the small massive objects are simply the centres which are able continuously to maintain the radio source by the ejection of relativistic plasma and magnetic flux and that there is no gravitational stability.

Thus the small source structure far from the explosion centre may not be due to containment by the small massive objects which have been ejected, but to continuous injection from them into the surrounding region.

The mechanism of ejection of such objects is not understood, but we might assume (1) that they are ejected with velocities in excess of the velocity of escape from the parent galaxy or massive object, so that perhaps $v_e > 1,000 \text{ km/sec}$ and (2) that the velocities are not highly relativistic so that v < 0.1 c. With these assumptions, the time scales associated with the ejection lie in the range 10^8 to 3×10^8 years, and the kinetic energy involved is 10^{56} n to 10^{60} n ergs. Because n may range all the way from unity to about 10^5 it is clear that the major part of the energy of the fragments ejected in the explosion, and not the energy instantaneously present in particles and field arising from the fragments.

The dynamic lifetimes estimated here may be short compared with the lifetimes recently estimated by Schmidt¹⁵ from frequency arguments, which range as high as 10° years. It is obvious that lifetimes as long as this require repeated ejection of fragments. If the total number of fragments ejected over the whole lifetime of the source is n, and we consider a strong source ($L \simeq 10^{44}$ erg/sec) which lasts for 10° years, then the mass energy released per fragment, perhaps involving many generations of relativistic particles, is $3 \times 10^{39} \ n^{-1}$ g. For values of n ranging between 1 and 10^5 , we see that the mass converted may be only a small fraction of the total mass of a fragment.

Conclusions

We have briefly explored the small source model because it seems that this kind of model is the only one capable of explaining the existence of small sources of high surface brightness which are found far from the explosion centre, both in larger sources and by themselves. The idea that large sources are made up of many small ones then follows naturally, though it might be possible to show that comparatively small fluctuations in surface brightness might be produced in a large source by instabilities.

If radio sources are generated by the ejection of small massive objects thrown out from the central outburst, severe restrictions are placed on the class of model which is invoked to release energy. None of the proposals that have been made, in which it is argued that particles are ejected or accelerated as a free gas—as is the case in supernova or star collision theories, or in flare mechanisms is satisfactory. We must go back to the models having single massive objects in gravitational collapse¹⁶. It must then be argued that it is the splitting or fragmentation of such an object which gives rise to the energy in the form of smaller massive objects. The double nature of the source must be traced to the distribution of momentum in the initial fission or fragmentation. The fragments which are ejected are then able themselves to eject relativistic particles directly. Such fragments can be likened to miniature quasi-stellar objects, and some of them may have been ejected from such objects.

The overall energetics in such a model may be similar to those invoked in the earlier investigations and it is clear that the initial outburst must involve a very massive object (1010 Mo or more in most cases). however, of no way to make good estimates of the energies of the particles or the strengths of the magnetic fields, or the masses of the material ejected directly from the observations. Thus the figures which have been used only give a very rough indication of these parameters.

We have not discussed the polarization of the radio sources in this preliminary investigation. In some sources,

such as Centaurus A (NGC 5128), Fornax A (NGC 1316) and MSH 13-33 (IC 4296), a fairly high degree of polarization or a regular pattern is seen¹⁷. In very small condensed sources a significant degree of polarization might be expected. If, however, a large source is made up simply of a considerable number of small sources there is no obvious way in which a large net polarization could be obtained, unless the magnetic field between the objects has a regular structure. This might require that there is some gas between the small sources. The most probable source of such gas and magnetic flux is the explosion itself. because there is at present no evidence for any intergalactic matter or magnetic field.

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Note added in proof. Since this paper was submitted for publication, DeYoung and Axford (Nature, 216, 129; 1967) have described a model in which they argue that radio sources may be confined by a dynamical process where the internal pressure of the source is balanced by the ram pressure of the intergalactic gas. If there is enough intergalactic gas, and there is no evidence for this at present, a modest degree of confinement may be achieved by this mechanism. The existence, however, of sources in which the ratio of separation to size is exceedingly large (~ 102-104) leads me to the conclusion that small massive objects stabilized by their own gravitation must be responsible in these cases. It is then not unreasonable to suppose that the major energy sources are, in general, small objects ejected in the explosion.

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Radio Galaxies as Cosmological Probes

by

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Applying the luminosity-volume test to radio galaxies, it is found that for the data to fit the usual relativistic cosmological models there must be strong evolutionary factors affecting the distribution of the radio galaxies. The steady state model is inconsistent with the present data.

This article reports the results of applying a new cosmological test to the radio galaxies: the distribution of radio galaxies in space is strongly affected by evolutionary factors. In particular, the steady state model, in which evolution is not permitted, does not seem to be consistent with the present data for radio galaxies. This confirms the conclusion of Rees and Sciama¹ based on the data for quasars, although some authors2-4 continue to cast

doubt on the cosmological interpretation of the red-shifts of quasars. The classical cosmological tests, the magnitude-red-shift and the number-count tests, are of value only in situations where the dispersion in luminosity of the sources is small. This condition does not hold for the radio luminosities of radio galaxies, or for either the optical or radio luminosities of quasars (assuming their red-shifts to be cosmological). The additional factor of completeness down to some limiting flux level, however, enables a far more powerful test, the luminosity-volume test, to be applied

În earlier communications^{5,6} I have described the application of this test to the quasars, and the determination of various possible forms of evolution which give consistency with the usual relativistic cosmological models. When the cosmological term is included in Einstein's equations, these models can be characterized by two parameters⁷

$$\sigma_0 = 4\pi G \rho_0 / H_0^2 \quad q_0 = -(R\ddot{R}/\dot{R}^2)_0$$

where ρ is the smoothed out cosmological density, H = R/R, R(t) is the scale factor of the expanding universe and the subscript zero refers to the present epoch. I have examined models with $0 \le \sigma_0 \le 3$, $-1 \le q_0 \le 3$, and found that for consistency with any of these models some evolutionary factor must be assumed to be affecting the quasars. For example, the fraction of matter in the form of active quasars might have been greater at earlier epochs, or the typical luminosities of quasars at earlier epochs might have been greater.

Luminosity-volume Test

The principle of the luminosity-volume test is that if the distribution of luminosities of a set of sources is independent of epoch, then in any range of luminosity we expect to find equal numbers of sources in equivalent volumes of space. In ref. 5, the radio luminosity-volume diagram was used to demonstrate that for quasars, "density" evolution (the co-ordinate number-density increases with red-shift, but the relative distribution of luminosities is independent of epoch) is at least as consistent with the present observations as "luminosity" evolution (the co-ordinate number-density is independent of epoch, but the typical luminosity increases with redshift). When proper account is taken of the effects of optical selection, and the optical luminosity-volume diagram is also considered, it is found that only for comparatively empty models ($\sigma_0 \ll 1$) are the quasars consistent with luminosity evolution.

Application to Radio Galaxies

The optical luminosities of radio galaxies show small dispersion, so the effect of the limiting visual magnitude of about 20 is simply to confine our identifications to redshifts smaller than 0.4–0.5. Moreover, we can obtain from the observed visual magnitude of a galaxy a rough estimate of its red-shift in those cases where the red-shift has not yet been determined. At present red-shifts are available in the literature of fifty-nine of the one hundred and forty-seven galaxies identified with radio sources in the ravised 3C catalogue of those identified to date with $V \leq 15$ magnitudes, but only 20 per cent of those identified with $15 < V \leq 20$.

Fig. 1 shows the distribution of red-shift of these galaxies against visual magnitude, as estimated by Wyndham¹⁵: six galaxies with red-shifts less than 0·01 (3C 71, 231, 270, 272·1, 274, 386) are omitted. In what follows I shall confine my attention to "strong radio galaxies", that is, those with luminosity greater than 10²³ W ster⁻¹ (c/s)⁻¹, so that of these six nearby radio galaxies, only 3C 274 will concern us.

Sources of the same luminosity and spectral shape would satisfy a relation of the form

$$V = A + 5 \cdot \log_{10} z + K(z) + 5 \cdot \log_{10} \{D(z)/z\} \dots$$
 (1)

where z is the red-shift, K(z) is the K-correction¹⁶ and D(z) is the "luminosity distance"¹⁷.

For small z, equation (1) can be approximated by

$$V = A + 5 \log_{10} z + B z \dots$$
 (2)

where A and B are constants¹⁶. The locus of this form with A=20.5, B=5, is shown in Fig. 1; this is the best line of form (2) through the data, from a least squares analysis.

The scatter of the points in Fig. 1 is primarily caused by the approximate nature of the magnitude estimates, which are probably not more accurate than ±1^m. Other factors are the dispersion in intrinsic luminosity of the galaxies, the effect of galactic obscuration and differences in spectral shape for different types of galaxy. Corrections could be applied for the latter two effects, but the approximate nature of the data hardly makes this worthwhile at the present. It will be seen that errors of up to a magnitude do not alter the basic conclusions.

Naturally, I shall not attempt to determine cosmological parameters from equation (2), because there is the possibility of systematic error in the magnitude estimates, quite apart from the random errors already mentioned. In what follows we assume only that equation (2), with A=20.5 and B=5, is valid to within a magnitude for the uncorrected visual magnitudes of radio galaxies. For the eighty-eight galaxies identified with 3CR radio sources for which no red-shifts are available, we estimate the redshift from equation (2) with A = 20.5, B = 5. For a total of 142 strong radio galaxies we then investigate the distribution of intrinsic radio luminosity against volume, in various cosmological models: we wish to test whether, in any given range of radio luminosity, equal numbers of sources are found in equivalent volumes of space. The total volume of space in which sources of a given luminosity are visible is limited by (i) the limiting radio flux-level of the 3CR catalogue and (ii) for sources of sufficiently high radio luminosity, a more severe limitation arises from the limiting optical magnitude of about 20 magnitudes, which we assume to be equivalent to the restriction $z \le 0.5$. For each range of radio-luminosity considered, the observable volume of space is divided into two. The numbers found are tabulated in Table 1. The columns labelled (a) refer to the nearer half of the observable volume, and (b) the farther.

Because the properties of the relativistic cosmological models do not differ much out to the red-shifts at which

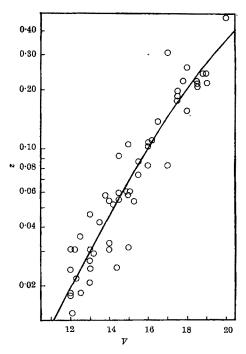


Fig. 1. Red-shift, z, against estimated visual magnitude, V, for fifty-four strong radio galaxies. Solid line: the locus $V = A + 5 \log_{10} z + Bz$, with A = 20.5, B = 5. The majority of the points are within one magnitude of this mean locus.

Table 1. DISTRIBUTION OF RADIO-LUMINOSITIES OF STRONG RADIO GALAXIES IN TWO EQUIVALENT VOLUMES OF SPACE, FOR FOUR COSMOLOGICAL MODELS;
(a) NEARER, (b) FURTHER, HALF OF THE OBSERVABLE VOLUME

Range of $log_{10}P$ W $(c/s)^{-1}$ ster ⁻¹	Einstein- de Sitter		Co. Mi	Cosmological Milne		els itter	Steady	state	
W (0/8) - ster-	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	
27.4-27.9	0	0	0	0	0	1	0	1	
26-9-27-4	2	i	2	ī	š	ō	š	õ	
26.4-26.9	1	0	3	Õ	4	ĭ	3	ĭ	
25.9-26.4	16	19	$1\overline{4}$	24	14	$3\overline{2}$	13	$3\overline{4}$	
25-4-25-9	8	33	8	30	6	23	5	24	
24-9-25-4	10	15	10	13	11	13	11	13	
24·4-24·9	9	7	9	7	8	Š	- 8	5	
23.9-24.4	9	5	9	5	10	5	10	5	
23.4-23.9	5	2	5	2	4	2	4	5 2	
Totals, $\log_{10}P > 23$	60	82	60	82	60	82	57	85	
$\log_{10}\tilde{P} > 24.9$	37	68	37	68	38	70	35	73	

these galaxies are found, it is unlikely that the results will depend very sensitively on the cosmological parameters (the situation is quite different for the quasars, which are visible out to much larger red-shifts). The models we have investigated in detail are the Einstein-de Sitter (σ_0 = $q_0 = \frac{1}{2}$), Milne $(\sigma_0 = q_0 = 0)$ and de Sitter $(\sigma_0 = 0, q_0 = -1)$ models. These are the fixed points in the σ-q plane, and correspond to asymptotic states of homogeneous, isotropic, pressure-free universes. For comparison, the results obtained for the steady state model are given in column 4. From the last row of Table 1 we see that for all these four models the total number of strong radio galaxies found in the farther region (b) is significantly larger than in region (a). The distributions do not differ greatly from model to model.

The incompatibility of the steady state model (in which evolutionary effects are not permitted) with the radio galaxy data is perhaps more serious evidence against that model than the distribution of the quasars¹, because doubts have frequently been raised as to the cosmological nature of the large red-shifts of quasars. Conversely, an important impetus to these doubts, the prospect of attributing the steep radio source counts entirely to the quasars^{18–20}, would be removed if the present results are confirmed by more accurate data.

To test whether the results could be significantly affected by the uncertainties in the estimated magnitudes, the optical magnitudes of the eighty-eight galaxies for which we have estimated the red-shift using equation (2) have been decreased by (i) one magnitude and (ii) two magnitudes (increasing the magnitudes merely reinforces the inequality between the totals for regions (a) and (b)). The corresponding numbers of sources found in regions (a) and (b) are shown in Table 2 for all luminosities greater than 10^{24} W (c/s)⁻¹ ster⁻¹. Because from Table 1 we see that the inequality between the totals in regions (a) and (b) is caused primarily by sources with luminosity greater than $10^{24\cdot9}$ W (c/s)⁻¹ ster⁻¹, the totals are also shown for this restricted range of luminosity.

The numbers of sources found in regions (a) and (b) are still significantly different, even if the optical magnitudes of all the galaxies are in error by 2 magnitudes in the same direction, for the higher range of luminosity.

Table 2. Maximum effect of errors of ± 2 in estimated visual magnitudes, and of varying various parameters, on the totals in table 1

Optical magnitude by 1 ($A = 20.5$,	B=5)							
	Einste de Sit		Mil	ne	de Si	tter	Steady	state
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
$Log_{10}P > 23$ $Log_{10}P > 24.9$	60 35	82 60	60 35	82 61	61 36	81 62	57 31	85 66
Optical magnitud by 2 ($A = 20.5$,	B=5)							
$Log_{10}P > 23$ $Log_{10}P > 24.9$	65 36	77 53	65 36	77 53	65 36	77 54	63 35	79 55
Milne model, $A = 20.5$ B = 4 $B = 6$						(Milne m $A = 20.5$, galaxies	B=5) with
	(a)) (b)		(a)	(b)		$V \leq 19$	only (b)
$Log_{10}P > 23$ $Log_{10}P > 24.9$	58 36			60 37	82 68		50 27	71 57

A more likely situation is that errors of up to 1 magnitude exist, but in either direction, so that the totals found in Table 1 are probably not greatly in error. Rows 5 to 6 of Table 2 show the effect of changing the value of B in equation (2), which again does not alter the basic conclusion. Finally, because it may be argued that, in view of the paucity of red-shifts for galaxies with $19 \le V \le 20$, it is inadmissible to use an extrapolation of the form (2) in this range, row 6 shows the results obtained when attention is confined to galaxies with $V \le 19$ ($z \le 0.3$). Incompleteness of the identifications in the fainter ranges of optical magnitude would merely reinforce our conclusions; against this must be offset the greater possibility of incorrect identifications.

These preliminary results provide the strongest impetus for the completion of red-shift measurements of radio galaxies in the 3CR catalogue. The evidence seems very strong that, for consistency with the usual cosmological models, some evolutionary factor must affect the distribution of the strong radio galaxies. The effect is clearest for galaxies with radio luminosity greater than 1025.4 W (c/s)-1 ster-1, but, because such a small volume of space is visible for the lower ranges of luminosity, no clear effect would be expected for these anyway. Of the models proposed by Longair¹⁹ in his attempt to explain the Cambridge radio source counts, his model (d), density evolution of all sources with radio luminosity greater than $10^{26.8}$ W $(c/s)^{-1}$ ster-1, is definitely unsuitable. But his model (b), luminosity evolution of all sources with luminosity greater than 10^{25.4} W (c/s)⁻¹ ster⁻¹, is entirely in agreement with the present work (though Longair preferred to interpret this evolution as being caused by the quasars only). But it seems unproved that the evolution does not operate down to luminosities a factor of, say, 10 lower than this.

Further evidence of this evolutionary effect is that, if the thirty-four 3CR radio galaxies with luminosities less than $10^{25} \,\mathrm{W} \,(\mathrm{c/s})^{-1} \,\mathrm{ster}^{-1}$ are excluded, a number-count slope of about -1.9 is obtained for 3CR radio galaxies, as opposed to -1.55 reported by Veron²⁰ for all 3CR radio galaxies.

Possible Forms of Evolution

To test evolutionary hypotheses, the luminosity-volume test is easily modified; we investigate the distribution of "corrected" luminosity against "weighted" volume^{5,6}. The evolutionary hypotheses tested in ref. 6 for the quasars were

luminosity evolution

(i) negative power-law dependence on R(t),

$$\bar{P}(z) \propto (1+z)^{QL}$$

(ii) negative exponential dependence on R(t),

$$ar{P}(z) \propto \exp \left\{ (1+z_L) \cdot \left(1-rac{1}{1+z}
ight)
ight\}$$

density evolution:

(iii) negative power-law dependence on R(t),

$$\eta(z) \propto (1+z)^{Q_D}$$

(iv) negative exponential dependence on R(t),

$$\eta(z) \varpropto \exp \left\{ (1+z_D) \cdot \left(1-\frac{1}{1+z}\right) \right\}$$

where $\bar{P}(z)$ gives the behaviour with epoch of the luminosity of any particular class of sources, and $\eta(z)$ is the co-ordinate number-density of sources.

The advantage of evolutions of the form (ii) and (iv) is that they do not require arbitrary truncation of the evolving population to obtain a finite contribution to the integrated background radiation.

The values of the parameters Q_L , z_L , Q_D , z_D necessary to give consistency of the radio galaxy data with various

EVOLUTIONARY PARAMETERS FOR RADIO GALAXIES (RG) AND QUASARS (Q) Table 3.

31		~			
		Luminosity e	volution		evolution
		Q_L	z_L	Q_D	z_D
Einstein- . de Sitter	RG Q	4 ± 1 Not consistent	$\begin{array}{c} 4 \pm 1 \\ 6 \pm 1 \end{array}$	$\begin{array}{c} 9 \pm 2 \\ 6 \pm 1 \end{array}$	$9 \pm 3 \\ 11 \pm 3$
Milne	$_{\mathbf{Q}}^{\mathbf{RG}}$	$\begin{array}{c} 4 \pm 1 \\ 2.5 \pm 0.5 \end{array}$	$\begin{array}{c} 4 \pm 1 \\ 6 \pm 1 \end{array}$	$\begin{array}{c} 9 \pm 2 \\ 5 \pm 1 \end{array}$	10 ± 3 11 ± 3
de Sitter	\mathbf{RG}	5±1 3±1	$\begin{array}{c} 5 \pm 1 \\ 6 \pm 2 \end{array}$	$9\pm 1 \\ 4\pm 1$	10 ± 1 11 ± 4

cosmological models are given in columns 1 to 4 in Table 3, together with the corresponding values for the quasars, taken from ref. 2. There is remarkably close agreement of the parameters required for the exponential evolutions of both quasars and radio galaxies (columns 2 and 4). In view of the arbitrariness of the particular forms of evolution we have considered, this agreement cannot be taken as evidence of a link between radio galaxies and quasars. But if such a link is accepted (for example, ref. 21), then these forms of evolution seem very promising. consistency with radio-source counts to low flux-levels, and with the integrated radio background, will be discussed in subsequent papers.

Although our discussion has been confined to three particular relativistic models, similar results are found for all models in the range

$$0 \le \sigma_0 \le 3, -1 \le q_0 \le 3$$

It seems that for radio galaxies the evolutionary factor far outweighs any differences between the cosmological models. The explanation of this evolution must await more detailed knowledge of the structure of radio galaxies: whether, for example, they correspond to early or late stages in the life of a galaxy, and whether interaction with an intergalactic gas or magnetic field is an essential feature of their activity.

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Note added in proof. The present results, when combined with those of ref. 6, show that it is not possible to attribute the steep radio source-counts to observational bias, as claimed recently by D. L. Jauncey (Nature, 216, 877; 1967).

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Mutagenic Capacity of Adenoviruses for Mammalian Cells

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Adenoviruses are mutagenic for mammalian cells. Viral replication, which results in a cytocidal effect, seems to be independent of a mutagenic effect caused by adenoviruses which initiate, but do not complete, a full replication cycle.

ALTHOUGH the actual frequency with which chromosomal anomalies occur in the human population is still unknown, the evidence in this article clearly indicates that karyotypic anomalies are aetiologically associated with a variety of human diseases. Thus there is a need to understand and to control the factors which are involved in the formation of abnormal chromosome complements. Ionizing and non-ionizing radiation and a great variety of chemicals have repeatedly been considered to be the chief cause of chromosome aberrations, whereas extensive attention has only recently been given to the mutagenic action of mammalian viruses1. Lack of knowledge concerning the mutagenic activity of viruses has been the result of the difficulties encountered in analysing and controlling the multitude of factors which are involved in virus host-cell interactions. In this article it will be shown that, in certain conditions, oncogenic and non-oncogenic

adenoviruses induce chromosomal aberrations which potentially lead to the formation of genetically abnormal cells.

Two different mammalian cell systems have been used in these studies: (a) human cells (AV-3, Hep-2 and HeLa) which favour the formation of infectious adenovirions; and (b) hamster cells (BHK-21 and primary cultures) which do not support a complete replicative cycle of certain adenoviruses²⁻⁵. Human adenovirus types 2, 4, 7, 12 and 18 and simian adenoviruses SA-7, SV-15 and SV-20 were tested. Of these, types 12, 18, SA-7 and SV-20 are highly oncogenic; adenovirus 7 is weakly oncogenic and the remainder, apparently, are not oncogenic.

Regardless of their oncogenic capacity, all adenovirus types which were examined induced a wide spectrum of chromosome aberrations in cultured cells of Syrian hamsters (Table 1). Chromatid breaks and exchanges com-

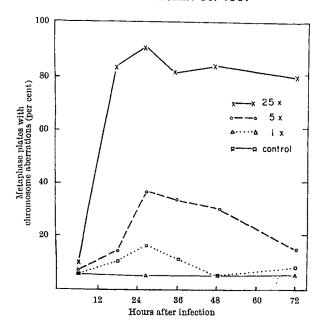


Fig. 1. The effect of viral dose on the incidence of chromosome aberrations. Adenovirus 4, BHK-21 cell line; $\times 25$, $\times 5$ and $\times 1$; control represents uninfected cell culture.

prised the chief types of aberrations found in cells infected with adenoviruses 12 and 18, whereas coiling deficiencies and metaphase arrest (C-mitosis) predominated in cultures infected with adenoviruses 2, 4, SV-15 and SA-7. In human cells, adenovirus infection leads to cessation of mitosis, clumping of interphase chromatin and disintegration of the host cell. Chromosome aberrations can be observed only in those infected human cells which are capable of entering division before onset of viral replication and mitotic inhibition.

An attempt has been made to estimate the influence of the viral dose on the incidence and type of chromosome aberrations (Fig. 1). Viral replication and the possibility of re-infection are two of the factors which usually interfere with quantitative evaluations. By employing cultured hamster cells in which most adenoviruses do not multiply, these difficulties were avoided. In these experimental conditions the incidence of chromosomal aberrations which were induced by each input multiplicity was a direct function of the viral dose. The accuracy of such studies, however, is not comparable with those studies which employ ionizing radiation or chemical mutagens. This is chiefly because of a lack of knowledge concerning,

Table 1. The incidence of metaphase plates with chromosomal aberrations induced by various human and simian adenoviruses in bhe-21 cells

Samples taken	27 h	post-inf	ection	with a	multip	licity o	$f25 \times$	
_	Ad. 18	Ad. 12	Ad. 7	Ad. 4	Ad. 2	SV- 15	8A- 7	Con- trol
Per cent chromatid breaks	44-1	38-0	16.7	13.3	24.0	10.0	11.0	3.4
Per cent fragmenta- tion	39.8	10-0	6.0	2-7	8.0	8.7	2.0	0
Per cent coiling defi- ciences	2.0	1.0	16-0	75.3	20.8	35.3	62.0	0
C-mitosis		_	±	+	+	+	+	

for example, the proportions in a given virus preparation of infectious and of non-infectious virions and of virions unable to adsorb or to be uncoated properly.

Our results do indicate that adenoviruses, which propagate and induce clinical symptoms only in the natural or phylogenetically closely related hosts, are capable of interfering with the chromosome complement in an unrelated host in which viral replication is absent or rare. This observation suggests that chromosomal damage which is detectable at metaphase and which leads to daughter cells with abnormal karyotypes occurs in cells in which the viral replication cycle does not proceed beyond an initial stage. At present it is difficult to point to the precise stage at which the viral replication cycle in hamster cells ceases: T-antigen is formed whereas synthesis of some virion antigens and of viral DNA seems to be lacking2-5,8. If we assume that neither replication of viral DNA nor the transcription of the entire viral genome is necessary for the induction of chromosome aberrations, then adenoviruses with a damaged template should retain their capacity to interfere with the host genome. This assumption has been tested by examining the incidence of chromosomal aberrations induced in human and hamster cells by viruses impaired with ultraviolet light. It is known that UV-exposed adenoviruses lose their capacity to replicate faster than they lose their ability to induce T-antigen. Thus the replication cycle of UV-treated adenoviruses in human cells might be interrupted at a stage similar to that occurring in hamster cells which are infected with non-irradiated virus. UV-irradiated stocks of adenoviruses types 2, 4, and 18, which no longer replicate in human cells, retain their capacity to induce chromosome anomalies in hamster cells (Fig. 2) and human cells (Table 2).

The present claim concerning the mutagenic properties of adenoviruses would seem questionable without mentioning a few pitfalls which, if ignored, could easily lead to erroneous conclusions. A crude or semi-purified virus preparation usually contains various amounts of cell debris and viral sub-units. Chromosomal aberrations

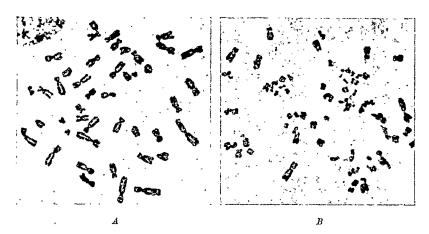


Fig. 2. Chromosome aberrations induced by UV impaired adenovirus 2 in BHK-21 cells. A, Karyotype of normal uninfected cell. B, Karyotype of a cell infected with Ad. 2 exposed 12 min to UV. $\times 10$.

Table 2. THE EFFECT OF UV-IMPAIRED ADENOVIRUS 18 ON CULTURED HEP-2

Samples were taken 48 h post-infection with a multiplicity of 10 x.

	Control	Adenovirus 18*	UV-exposed (6 min) adenovirus 18	Adenovirus 18 plus antibodies
Percentage inclusion bodies	0	45.0	0.2	0
Percentage frequency of mitosis	6.0	0.7	6.0	5.7
Percentage chromatid breaks	3-5	28.6	34.4	4.1
Percentage multipolar mitosis	4.6	17.5	22-2	2.9

[•] Purified by density gradient centrifugation in caesium chloride.

induced by these types of virus preparations could thus be the result of mutagens which are present in the cell debris.

The neutralization of viruses with specific antibodies, and purification of mature virions by density gradient centrifugation, are two useful methods for distinguishing between the mutagenic effect of infectious viruses and that caused by some non-viral elements. The data presented in Table 2 show that preparations of adenovirus 18 which have been treated with viral antibodies lose their capacity to induce chromosome aberrations. Highly purified virus preparations retain their capacity to interfere with chromosome complements. Another possible complicating factor is the presence of viruses associated with adenovirus (AAV) either in the adenovirus preparations or in the host cells10,11. Examination by electron microscopy did not reveal the presence of AAV in purified virus preparation or in infected cells. Thus it can be concluded that the chromosome aberrations mentioned here are the result of adenovirus infection and are not the result of the presence of an associated virus.

Although the mutagenic effect of viruses is comparable in many aspects with that of ionizing radiation or radiomimetic chemicals, there are several features which are unique to viral mutagenesis. First, the replication cycle of adenoviruses must be incomplete or else virus replication leads to cell death. Thus the cytocidal and the mutagenic effects of adenoviruses are mutually exclusive at the individual cell level. Second, chromosomal damage induced by adenovirus cannot be too severe or else loss of proliferative capacity or cell death could result. Finally, the addition of viral information, or part of it, to the infected cell represents a change in the genetic composition of the host cells. Thus cells which survive an adenovirus infection could have an altered genome because of the addition of the viral genome or because of an abnormal chromosome complement or both. In this connexion it is of interest to review antigenic and karyologic analyses of neoplastic cells induced by adenovirus 12 (refs. 12 and 13).

Neoplastic cells of primary or transplanted tumours induced by adenovirus 12 in Syrian hamsters do not yield mature virus particles which can be detected in the electron microscope, nor can infectious virions be recovered from these cells, \$\tilde{s}\$. All primary or transplanted neoplasms induced by adenovirus 12 which have so far been examined contain adenovirus T-antigen. This antigen is localized in fleck-like structures by immunofluorescence microscopy4.8 and is composed of bundles of fibres as revealed by immunoelectron microscopic techniques⁵. It has also been shown that these tumours contain a surface transplantation antigen which is specific for the adenovirus inducing it14. The capacity to synthesize T-antigen, which seems to be coded by the viral genome, is transmitted to most, if not all, daughter cells. This observation suggests that the viral genome, or at least the portion carrying the information for T-antigen, has become an integral part of the host cell. More direct evidence has been obtained from nucleic acid hybridization experiments indicating that the neoplastic cells retain 40-60 per cent of the viral genome¹⁵. Karyological studies have revealed an exceptionally high frequency of chromosomal aberrations in primary, transplanted and cultured neoplasms (Table 3). This persisting instability of the chromosome complement seems to point to the intracellular presence of a mutagen, which must be transmittable over many cell generations and also must be continuously synthesized. The formation of T-antigen in all the cells which show an increased frequency of chromosome aberrations suggests that T-antigen may function as the mutagen. At present it is difficult to prove or disprove this idea, particularly because even the nature of the T-antigen is unknown.

Table 3. THE PERSISTENCE OF CHROMOSOME ABERRATIONS IN AD. 12 INDUCED NEOPLASTIC CELLS OF SYRIAN HAMSTERS

	Chromosome aberrations (%)	Abnormal karyotypes (%)	No. of neoplasms analysed
Primary	33.7-62.5	64-89	10
Transplanted	32.0-46.8	100	-5
Cultured	50-0-59-5	100	6

At this point the old and controversial question concerning the significance of chromosomal aberration in the process of neoplastic transformation arises 18-19. controversy concerns the claim that chromosomal aberrations leading to abnormal genotypes are of primary importance in oncogenesis and the counter claim that chromosomal anomalies represent phenomena independent of, or superimposed by, the oncogenic process. The neoplasms induced by adenovirus 12 seem to exemplify a case which does not fit precisely into the chromosome concept of oncogenesis, nor does it prove the absence of any chromosome involvement. The great variety of abnormal karyotypes in this neoplasm and the continuous appearance of new ones12,13 suggest that the maintenance of the malignant property is not a function of a particular chromosome complement. Similarly one could argue that the formation of the T-antigen cannot be dependent on a particular karyotype. This absence of any detectable role of particular karyotypes in the established neoplasms does not exclude the possibility that chromosome aberrations could be involved in one of the initial stages of oncogenesis. The "open ends" of chromatid breaks, which tend to associate with other "open ends". could function as acceptors of the viral genome or part of it. This idea would be in agreement with the generally accepted concept that breaks are required for the incorporation of one DNA segment into another DNA molecule. In this connexion it is of interest to note the suggestion that viral DNA fragments could be more easily incorporated into the host genome than would the entire viral DNA (ref. 20).

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Haemolytic Efficiency of Rabbit IgG Anti-Forssman Antibody and its Augmentation by Anti-Rabbit IgG

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Complement dependent lysis of an erythrocyte can be achieved in optimal conditions by reaction of a suitable anti-lgG with a single molecule of lgG antibody on the surface of an erythrocyte. Optimal conditions require special precautions to avoid antibodies which prevent C' activation. For lysis of a sheep erythrocyte without augmentation, about 800 molecules of an lgG antibody were needed.

EVIDENCE has been published^{1,2} that a single molecule of rabbit IgM antibody against the Forssman antigen on the surface of sheep erythrocytes can suffice to activate guinea-pig complement (C') so as to lead to a site of irreparable damage and cell lysis. In the case of IgG antibodies it was suggested¹ that C' activation and lysis only occur when two antibody molecules become attached to the cell surface at adjoining sites, and indirect evidence has confirmed that interaction of two such antibody molecules is needed³. The maximum number of IgG antibodies which can attach to a sheep erythrocyte is about 600,000 and, with certain assumptions, the number of molecules which must be attached so as to give an even chance that two will be on adjacent sites can be calculated to be about 800 (ref. 4). Direct measurements of the number of IgG anti-Forssman molecules which are required for C' dependent lysis of an erythrocyte gave figures of this order though somewhat larger¹.

Knowledge concerning the number of IgG antibody molecules which can attach to an erythrocyte with or without causing C'-dependent lysis is also of interest in connexion with the development of plaques of haemolysis around cells making antibody in gels or gums containing erythrocytes^{5,6}. Lysis is normally caused mainly or wholly by IgM antibody, but, by additional treatment with a suitable antibody against IgG, erythrocytes coated with IgG antibody became susceptible to lysis by C' (refs. 7 and 8). If several molecules of anti-IgG interact with each molecule of attached antibody and so activate sufficient C' at each site of attachment to cause a local haemolytic "hit", a single IgG antibody on the cell surface should suffice to lyse the cell by this technique. On this assumption, comparison of the number of IgG antibody molecules/cell required for haemolysis with and without augmentation by anti-IgG, would yield an independent estimate of the number of attached IgG molecules which are required to lyse a cell by means of C' alone. The experiments which are described later were designed to test these assumptions.

Fresh guinea-pig serum which had been absorbed three times at 0° C with sheep erythrocytes was used as the source of C'. Purified rabbit IgG anti-Forssman antibody was prepared from serum of rabbits which were hyperimmunized by intravenous injections of boiled sheep cell stroma⁹, by absorption on to formolized sheep erythrocytes, elution at pH 10·6, and further separation from

IgG by gel filtration through 'Sephadex G-200' (Pharmacia, Uppsala) followed by repeated centrifugation through a gradient of 10-30 per cent sucrose⁴. After trace labelling with ¹²⁵I, more than half of the IgG specifically bound to sheep erythrocytes. No detectable IgM or IgA was present.

The purified antibody was titrated for its capacity to lyse sheep erythrocytes in a standard system used for all these tests, containing 10° erythrocytes/ml. and a 1/100 dilution of C' in veronal buffer saline with 0·1 per cent gelatine and 0·0005 molar magnesium and 0·00017 molar calcium (VBS)°. The dilution which caused 50 per cent lysis after incubation for 1 h at 37° C was estimated by centrifugation of the mixtures in the cold and measurement of the haemoglobin in the supernatant by its OD at 540 mμ.

Antisera were obtained from horses, goats and guineapigs which had been immunized with purified rabbit IgG (in Freund's complete adjuvant, followed in some instances by IgG adsorbed on aluminium hydroxide). These sera were not absorbed with rabbit Ig light chains, and contained antibodies to light as well as to IgG heavy chains. They had no demonstrable haemolytic antibody against Erythrocytes were mixed with a sheep erythrocytes. dilution of IgG anti-Forssman antibody 1/50th of that sufficient to lyse the erythrocytes by itself. After incubation at 37° C for 10 min, C' was added, followed by varying dilutions of the antisera. Incubation was continued for 1 h and the degree of haemolysis was measured. control tubes with IgG antibody and C' alone, lysis was less than 5 per cent. The horse and goat antisera caused either no or only partial additional haemolysis over a wide range of dilutions. Some, but not all, of the guinea-pig antisera caused complete lysis, however. It seems probable that the variable potency of the antisera to augment haemolysis depended on the extent to which they contained antibodies capable of activating guinea-pig C'. Most horse and goat antibodies are known to activate guinea-pig haemolytic C' poorly or not at all, and even guinea-pig 7S antibodies have been shown to be separable into two distinct immunoglobulin components, γ_2 - and γ_1 -, of which only γ_2 - activates C' (ref. 10). These components were therefore separated from the guinea-pig antisera by chromatography on DEAE cellulose¹¹. The γ_2 -immunoglobulin preparations were apparently pure as shown by immunoelectrophoresis against an antiserum against mixed guinea-pig globulins, but the γ₁- preparations contained several contaminating proteins other than Y2immunoglobulin.

Haemolysis tests with guinea-pig γ₂-anti-rabbit IgG were set up as follows using a separate pipette for each dilution step in order to avoid dilution errors. Sets of tubes containing washed sheep erythrocytes and suitable two-fold dilutions of the rabbit IgG anti-Forssman antibody were incubated for 30 min at 37° C with occasional shaking. To each set C' was added next, followed immediately by one of the chosen dilutions of guinea-pig γ_2 anti-rabbit IgG. After further incubation for 1 h the tubes were chilled and the degree of haemolysis was measured. Controls were included to allow for absorption at 540 mu of complement alone and to exclude any haemolysis of unsensitized cells by anti-rabbit IgG. The results of three experiments using different preparations of guinea-pig γ_2 anti-rabbit IgG, prepared from separate sera, are shown in Table 1. The augmentation of lysis increased with increasing concentration of anti-rabbit IgG to a maximum of 425-fold in the first experiment, 1,300fold in the second and 670-fold in the third.

Table 1. Augmentation of C'-dependent haemolysis by anti-igg

	Dilution of gpig antibody	Dilution of rabbit anti- Forssman IgG required for 50% lysis	Augmentation ratio
Experiment 1	1/∞ 1/200 1/50 1/20 1/10	1:1,000 1:64,000 1:190,000 1:400,000 1:425,000	64 190 400 425
Experiment 2	1/∞ 1/100 1/30 1/15	1:1,000 1:490,000 1:1,250,000 1:1,300,000	490 1,250 1,300
Experiment 3	1/∞ 1/30 1/15	1:570 1:280,000 1:380,000	490 670

The 50 per cent haemolytic end-point for sheep erythrocytes of rabbit anti-Forssman IgG was measured in the presence of varying dilutions of the γ_2 -globulin fractions prepared from guinea-pig anti-rabbit IgG. Different preparations of anti-rabbit IgG were used in the separate experiments. The final concentration of erythrocytes was 10^8 per ml. and of C' was 1/100.

According to the assumptions made earlier, anti-rabbit IgG was not expected to augment lysis by rabbit IgM anti-Forssman antibody. This was confirmed by pretreating sheep erythrocytes with an amount of purified IgM antibody which was sufficient to cause 65 per cent haemolysis in the presence of C' alone. It was found that the extent of lysis was unaffected by the addition of sufficient anti-Ig to augment almost 500-fold the lysis by IgG anti-Forssman antibody.

Unlike γ_2 antibody no preparation of γ_1 guinea-pig antirabbit IgG caused haemolysis of erythrocytes which had been sensitized with sublytic amounts of rabbit IgG Forssman antibody, even though the concentration of antibody in the γ_1 -globulin was considerably greater than that in the γ₂-globulin when compared in terms of precipitation with rabbit IgG in gel diffusion tests. Furthermore, if the γ_1 - and γ_2 - anti-rabbit IgG from the same serum were mixed, the haemolytic effect of the Y2-antibody on erythrocytes sensitized with rabbit IgG was inhibited. In some guinea-pig antisera the γ₁-antibody present was sufficient to prevent completely the haemolytic augment-

ing effect of γ_2 -antibody present in the same serum. The results obtained with goat and horse anti-rabbit Ig are set out in Table 2. Most of the antisera were able to cause partial augmentation of lysis, and there was a definite optimum concentration for this effect; there was no evident correlation, however, with the total amount of precipitating antibody present. view of the evidence presented here that guinea-pig γ_1 -antibody inhibits the augmenting effect of γ_2 -antibody, these findings strongly suggest that the goat and horse antisera also contain a mixture of antibodies, part of which can activate guinea-pig haemolytic C' and part cannot, but are rather inhibitory. It has indeed been

reported by Nakamura and Katsura¹² that γ₂-globulin but not T-globulin from horse anti-diphtheria toxin will fix guinea-pig haemolytic C' in the presence of antigen, and this has been confirmed by Raynaud and his colleagues 13 .

Table 2. Augmentation of haemolysis by sublytic amounts of rabbit igg anti-forssman antibody produced by goat and horse anti-rabbit ig

	Anti-RGG	%	Lysis (of sheep c	ells in pre	sence
Nature of	content	of various dilutions of antiserum				
${f antiserum}$	(mg Ab/ml.)	1/50	1/500	1/5,000	1/50,000	1/500,000
Goat 3/55	8	0	0	0	0	0
Goat 2/62	2	44	83	68	17	0
Goat "Bishop"	3	65	92	75	50	Not tested
Horse 253	Approx. 8	1	34	42	27	9
Horse R.P.	Approx. 8	0	34	25	13	0

Sheep erythrocytes were sensitized with 1/50th the amount of rabbit IgG anti-Forsman antibody required for 50 per cent haemolysis, and were then incubated in the presence of C' for 1 h at 37° C. None of the antisera caused lysis of unsensitized erythrocytes under the same conditions.

The haemolytic potency of rabbit IgG anti-Forssman antibody could be augmented 470-1,300 (mean 820) fold by various preparations of guinea-pig γ_2 anti-rabbit Ig under otherwise identical conditions. If two molecules of rabbit IgG antibody, adjacent on the erythrocyte surface, are required for activation of C' in order to produce a site of damage, whereas a single molecule suffices when it acts as a focus for subsequent attachment of anti-IgG, this finding would imply that there must be about 800 molecules on the surface for two to be effectively adjacent. An assumption implicit in this calculation is that the addition of anti-IgG does not alter the number of Forssman antibody molecules which are attached to the erythrocyte surface. This is probably justified because the IgG Forssman antibody used in this study was highly avid4 and a very large excess of Forssman sites was present, so that virtually all of the antibody will have been bound to erythrocytes before the anti-IgG was added. When another and less avid purified IgG Forssman antibody was used, the maximum augmentation of lysis by the same anti-IgG preparations was only 80 fold.

The very close agreement between the mean value of 820 and the calculated value of approximately 800 attached IgG antibody molecules/cell required for lysis without augmentation is gratifying but probably fortuitous and the highest value, 1,300, may be the most significant. Nevertheless, taken together, the findings indicate that lysis of an erythrocyte can—under optimal conditions—be achieved by reaction of a suitable anti-IgG with a single molecule of IgG antibody on the surface. In view of the presence which has been shown of an inhibitory antibody in antisera to IgG prepared in guinea-pigs, and of its probable presence in goat and horse antisera, optimal conditions are unlikely to be achieved without special precautions. Fortunately rabbit antisera, which are often used as the source of antibody against immunoglobulins in studies on augmented haemolytic plaque formation, contain predominantly antibodies which can activate guinea-pig C'.

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LETTERS TO THE EDITOR

ASTRONOMY

Cosmological Models containing both Radiation and Matter

JACOBS¹ has recently compared two types of cosmological models which change from a radiation-like model for early time to a matter-like model for recent time. The radiation in these models is in thermal equilibrium with the matter and the photons, neutrons and electrons are not treated separately (as they are, for example, in ref. 2). In a flat space (k=0), the function R(t) of the line element

$$ds^{2} = c^{2}dt^{2} - R^{2}(t) \left\{ \frac{dr^{2}}{1 - kr^{2}} + r^{2}(d\theta^{2} + \sin^{2}\theta d\varphi^{2}) \right\}$$
(1)

changes from being like $At^{1/2}$ for early time to being like $At^{2/3}$ for recent time. The two models Jacobs discusses will here be called model I and model II. R(t) is given in model I by

$$t/\tau = [(R/R_0) - 2S_0] [(R/R_0) + S_0]^{1/2} + 2 S_0^{3/2}$$
 (2)

where

$$\tau \equiv \left(\frac{3}{2} \times c^4 \,\rho_{m,0}\right)^{-\frac{1}{2}}, \, S_0 \equiv \rho_{r,0}/\rho_{m,0} \tag{3}$$

and in model II by

$$\log R(t) = 2 \left([3t + \beta^{-1}(1 - e^{-\beta t})]^{-1} dt + \text{constant} \right)$$
 (4)

where β is a constant3. Here the density and pressure are

$$\rho = \rho_r + \rho_m, \ p = p_r = \frac{1}{3} \rho_r c^2 = \frac{1}{3} a T^4$$
 (5)

where ρ_r and ρ_m are the density of radiation and matter, respectively, p_r is the pressure of radiation, the pressure of matter is put equal to zero, a is Stefan's constant and T is the radiation temperature. Also, for a Hubble parameter

$$H = \dot{R}/R \tag{6}$$

we define

$$E_m = (\dot{\rho}_m + 3H\rho_m)c^2 \tag{7}$$

and

$$E_r = (\dot{\rho}_r + 4H\rho_r)c^2 \tag{8}$$

so that the conservation equation gives

$$E_m + E_r = 0 (9)$$

 E_m and E_r were first defined by Davidson⁴ and are the total net rate of transfer of radiation energy per unit volume into matter energy and vice versa. A zero subscript denotes the present epoch. The present value of Tis of the order of 3° K from the measurement of the isotropic microwave background first found by Penzias and Wilson⁵ and Roll and Wilkinson⁶. This is believed by many to be a remnant of the initial "big-bang" or fireball''.

For the model I,

$$E_{\tau} = E_{m} = 0 \tag{10}$$

so that

$$\rho_m R^3 = \text{constant}, \ \rho_r R^4 = \text{constant}$$
 (11)

and for the model II,

$$E_r = -\rho_r/\chi, \ \chi^{-1} = \frac{3\beta t - 1 + e^{-\beta t}}{3\beta t + 1 - e^{-\beta t}}. \ \beta$$
 (12)

so that E_m is greater than zero for all t. As Jacobs points out, the model I is given by Chernin' in a different form.

As far as I know, the first analytical solution, with equation (10) holding, was given by Alpher and Herman⁸ for the space of negative curvature, and this solution is re-used by these authors together with Gamow in a recent communication⁸. The corresponding k=0 solution in their notation is (R. A. Alpher, private communication)

$$t = \frac{2}{3\gamma \rho_{m}^{2}} \left[(\gamma \rho_{r}^{*} + \gamma \rho_{m}^{*}L)^{3/2} - 3\gamma \rho_{r}^{*} (\gamma \rho_{r}^{*} + \gamma \rho_{m}^{*}L)^{1/2} + 2(\gamma \rho_{r}^{*})^{3/2} \right]$$
(13)

(which is the same as equation (2)) and the k=+1 solution is also given in ref. 9, when $K_2^{1/2}=ic/R_0$ is used in equations (13)-(15) of that communication, though the authors do not mention this because they are interested only in the k=-1 case. It can also be shown that these $k=\pm 1$ solutions are the same as those given by Chernin. A solution similar to that of equation (2) was also given by Dautcourt (private communication). Practically all writers in this field and in the field of cosmological element production assume equation (10).

For the model I, Chernin obtained formulae for the red-shift, z, and for other observable quantities but in inexact form. I have been able to find another way of expressing the solutions so that exact forms of z and of the [m,z] relation can be easily calculated 10. This is done by writing the solutions in terms of the parameter

$$\varepsilon = p/\rho c^2 \tag{14}$$

so that

$$R = A (1 - 3\varepsilon)/\varepsilon \tag{15}$$

(A = constant) from (5) and (11). For example, for k = 0, the [m,z] relation is

$$m_{\text{bol}} = 5 \log_{10} (1+z) \frac{2}{1-3\varepsilon_0} \left\{ 1 - \left(\frac{1+3z \, \varepsilon_0}{1+z} \right)^{1/2} \right\} + c \quad (16)$$

For small z, and for ε_0 small compared with 1, this reduces to

$$m_{\text{bol}} = 5 \log_{10} 2(1+z) \{1 - (1+z)^{-\frac{1}{2}}\} + c$$
 (17)

which is the equation given by Mattig¹¹ $(q=\frac{1}{2})$. It is interesting to compare the value of the red-shift, z, for the Einstein-de Sitter model $(R(t) = At^{2/3})$ with the value given by this model for a particular (non-recent) value of t_1 (the time of light emission). Partridge and Peebles¹² assess the general possibility of observing distant, newly formed galaxies which go through a stage of high luminosity at an epoch of

$$t_p \sim 1.5 \times 10^8 \text{ yr} \tag{18}$$

and they state that this epoch corresponds to a red-shift in the range 10 to 30 for various cosmological models. We will use a value of $t_1 = 4.8 \times 10^{16} \sec \sim t_p$. For the Einstein-de Sitter model

$$1+z = \frac{S_0}{S_1} = \left(\frac{t_0}{t_1}\right)^{2/3} \tag{19}$$

 $H_0t_0=2/3$ and, for $H_0=3\cdot2\times10^{-18}~{\rm sec^{-1}}$ and the assumed

$$z = 11.3 \tag{20}$$

For model I

$$t = \frac{2}{3} \frac{AB}{c} \left\{ e^{-3/2} \left(1 - qe \right) + 6\sqrt{3} \right\}$$
 (21)

 $(\beta = constant)$

$$1+z = \frac{S_0}{S_1} = \frac{1-3\varepsilon_0}{1-3\varepsilon_1} \cdot \frac{\varepsilon_1}{\varepsilon_0}$$
 (22)

and

$$H = \frac{c}{AB} \frac{\varepsilon^{3/2}}{(1 - 3\varepsilon)^2}$$
 (23)

For $T_0 = 3^\circ$ K

$$\rho_{r,0} = 6.8 \times 10^{-34} \text{ g/cm}^{-3} \tag{24}$$

and, with

$$\varkappa \rho_r c^4 = q H^2 \varepsilon \tag{25}$$

$$\varepsilon_0 = 1.24 \times 10^{-5} \tag{26}$$

so that, from equation (22)

$$c/AB = 1.53 \times 10^{-4} \tag{27}$$

With t_1 as before, equation (21) now gives

$$\varepsilon_1 = 1.53 \times 10^{-4} \tag{28}$$

so that, from equation (23)

$$z = 11.3 \tag{29}$$

Thus the zero pressure, Einstein-de Sitter model, is accurate enough for observations down to a red-shift of light from the youngest galaxies. Also, for $z \leq 11.3$, there is no real difference in the two [m,z] relations (16) and (17).

The model II fits in with equation (4.29) of Davidson and Narlikar's communication13, that is

$$\dot{\rho}_r + 4H\rho_r = S - \rho_r/\chi \tag{30}$$

where S(t) is the rate of emission of radiation from unit volume, and $\chi(t)$ is a frequency averaged mean free time between the emission and absorption of a photon, though only with S=0. Thus the model may be claimed to be one for a universe with radiation from a primeval bigbang, but one in which the matter does not emit radiation. With $E_r = E_m = 0$,

$$S=0, \chi=\infty \tag{31}$$

or

$$S = \rho_r/\chi \tag{32}$$

What is wanted is either a model with $E_m < 0$ for all t, or, better still, one with $E_m > 0$ for early t and < 0 for recent t. Many models of this type for spaces with $k=0, \pm 1$, and also the general theory of models with $\frac{1}{3} \ge \varepsilon \ge 0$ and $E_m \ge 0$, have been developed, and work in this field will be described in a future paper. Part of this work leads to

$$E_m \propto - \left[\dot{\mathbf{\epsilon}} + \varepsilon (1 - 3\varepsilon) H \right]$$
 (33)

which gives a condition on ε for the required sign of E_m . The solution of

$$E_m = 0 = \stackrel{\bullet}{\varepsilon} + \varepsilon (1 - 3\varepsilon)H \tag{34}$$

is equation (15) as expected.

The most physically satisfactory form of a seems to be

$$\varepsilon = \frac{1}{3} (1 + \mu t)^{-n} \tag{35}$$

(μ , n positive constants). Here, for all t, $E_m>0$ for small μt . For k=0 and n less than about 2/3, $E_m<0$ for recent t.

Jacobs comments on the late stage of domination by the matter in the model II. Any model with $E_m > 0$ will become dominated by the matter at a time after the corresponding time in the $E_m = 0$ model and this will itself be after the corresponding time in an $E_m < 0$ (for recent t) model.

Davidson⁴ showed that for $R \propto t^n$

$$E_m \varpropto n(2n-1) \ (3n-2) \tag{36}$$

which is <0 for $\frac{1}{2} < n < \frac{3}{3}$. from which it would appear that, as R(t) changed from an $n = \frac{1}{2}$ like model to an $n=\frac{2}{3}$ like model for t increasing, E_m would always be < 0, but this is by no means true. He also points out13 that a disappointing feature of this work is that "the physical character of cosmic evolution seems to be very sensitive to the chosen R(t) function.

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PHYSICS

Ultrasonic Attenuation in the Pre-melting Region

THE solid-liquid transition, a co-operative phenomenon, should be quantum mechanical in origin. There have been a number of phenomenological treatments but so far no quantitative microscopic description of the interactions responsible. Plausibly, the source of the transition may be sought in the lattice dynamics solution for the eigenvalues in the anharmonic solid near the melting point. In the pre-melting region, as the anharmonicity of the interatomic forces increases, phonon-phonon scattering will be enhanced. Experimental evidence for this is Ultrasonic waves, although much lower in frequency, have the same nature as lattice vibrations; the attenuation of an ultrasonic beam, directly measur-

able, provides detail of phonon interactions.

The attenuation of longitudinal 10 Me/s ultrasonic waves has been studied by the pulse-echo technique in the pre-melting region of polycrystalline In₂Bi, a convenient material (melting point, 89°C) for preliminary measurements. Some details of the apparatus are given in ref. 1.

From room temperature to 87.5°C the attenuation rose by only 0·1 dB/μsec, but over the last degree before the melting point a rapid increase was extant, as shown in Fig. 1. On cooling, some hysteresis was observed; but the original value of attenuation was regained within about 2° C of the melting point. Results were reproducible. At no time was the temperature raised high enough for melting to ensue. Similar results have been observed during experiments, still in progress, on indium single crystals of 99.9999 per cent purity; the effect does not seem to result from specimen inhomogeneity or grain boundary melting.

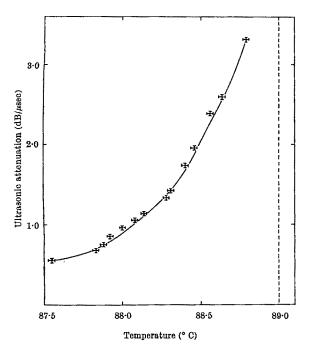


Fig. 1. Ultrasonic attenuation at 10 Mc/s near the melting point (89° C) in ${\rm In_2Bi.}$

Insight into the phenomenon can be gained by considering the contribution to the ultrasonic attenuation from the viscous drag of the lattice phonons^{2,3} in the condition that the thermal phonon mean free path is small compared with the sound wavelength ($\omega \tau_{th} \ll 1$). The phonon frequencies are modulated by the sound

Table 1. Velocity of Longitudinal sound waves in In₂Bi
Temperature (°C)
$$-269$$
 -198 20.5 77 85 [88.5
Velocity 2.63 2.56 2.43 2.40 2.38] 2.38 ($\times 10^5$ cm/sec)

wave; phonon-phonon interactions, dependent on anharmonicity, then bring the system back to thermal equilibrium; the sound wave driving the process is damped. This seems to be the predominant mechanism for ultrasound absorption in the pre-melting region. Woodruff and Ehrenreich's find that the attenuation al of an ultrasonic wave of angular frequency ω is given by

$$\alpha_L = \frac{8.68 C_{\nu}. T. \gamma^2 \tau_{th} \omega^2}{3 \rho v^3} dB/em \qquad (1)$$

where \gamma is an average Gruneisen constant of the order of unity. In terms of the thermal conductivity $K = 1/3 C_V$ $v^2 \tau_{\rm th}$) this becomes

$$\alpha_L = \frac{8.68 \,\gamma^2 \,\omega^2 \,KT}{\rho v^5} \,dB/em \qquad (2)$$

The velocity of longitudinal sound waves shown in Table I does not alter within the experimental accuracy of ± 0.5 per cent in the pre-melting region. At temperatures greater than the Debye temperature the thermal conductivity is approximately proportional to T^{-1} ; therefore KT and, in consequence, α_L (~0-1 dB/cm) should be independent of temperature. A sharp rise in the thermal conductivity of both pure metals and alloys, however, has been observed near the melting point, and the specific heat anomaly is well known^{5,6}. Thus the ultrasonic attenuation reflects the rise in the specific heat. The interaction takes place within the entire assembly of the thermal phonons, rather than with the individual thermal modes. Enhancement of ultrasonic damping probably results from a rapid increase in anharmonicity of the lattice forces near the melting point.

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THE SOLID STATE

Breakdown of Passivity of Stainless Steel by Halide lons

METALS and alloys which are resistant to corrosion usually depend for their resistance on the formation and maintenance of thin films (commonly between 5 and 200 Å thick) of passivating oxide. The breakdown of such films by "aggressive" anions such as chloride, at sufficiently positive anode potential and at sufficiently high temperature, is often responsible for the failure of such alloys, because it usually leads to serious pitting of the bared metal.

Many mechanisms for the breakdown action of chloride ions have been suggested: they include (1) the penetration of the small chloride ions through pores1 in the oxide film—if actual pores are not present, weak places2 might suffice; (2) the displacement of the (very thin) film by adsorption of chloride ions^{3,4}; (3) anion exchange between oxide ions and chloride ions; (4) migration of chloride ions inwards through the oxide lattice5; and (5) peptization of the oxide film by a strong negative charge caused by adsorbed anions. None of these mechanisms has been shown by experiment to be more than plausible for any particular case, and in particular there is very little kinetic evidence about the rate of breakdown and the factors influencing it.

We have studied the kinetics of breakdown of anodically formed passivating films on 18 chromium-8 nickel austenitic stainless steel by chloride and bromide ion. Anodic passivation was first achieved by polarizing the alloy specimen in slow flowing deaerated 0.01 molar sulphuric acid (pH about 2) or 0.15 molar phosphate buffer solution (pH 7) at a fixed potential controlled by a poten-When the anode current density had become steady (about 10-6 amp/cm2), the flow was replaced by a solution similar in all respects except for an addition of sodium chloride or bromide. We expected that a sufficient addition would lead to either a slow, though accelerating, increase of anode current density caused by slow contamination of the oxide lattice by anion exchange (suggestion (3)) or by migration (4), or an almost immediate large increase caused by penetration of pores or weak places (1), adsorption displacement (2) or peptization (5). Neither of these kinds of change occurred; in fact, the overall anode current density after the chloride addition remained constant for an induction period, τ , of from a few seconds to several hours, depending on the chloride concentration, $c_{\rm Cl}$ -, the anode potential, E, and the absolute temperature, T, and then very rapidly increased by several times (Fig. 1) with the onset of visible pitting. Evidently, the anode current density at the very small pit or pits increased by several orders of magnitude compared with the current density of the passivated surface.

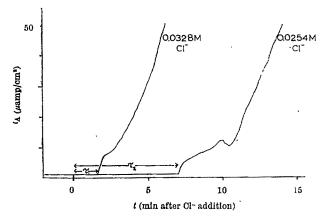


Fig. 1. Typical current-density/time curves, 18-8 stainless steel in sulphuric acid, showing effect of chloride addition. 25° C; pH 2-05; $e_H+800~\rm mV$.

The relation between log τ and log c_{Cl} at 25° C for three anode potentials is shown in Fig. 2. If we suppose that $1/\tau$ is an approximate estimate of the rate of the breakdown process, whatever it may be, then the slope of the log $\tau/\log c_{\text{Cl}}$ plots shows that the rate is proportional to the *n*th power of the chloride ion concentration, where n lies between 2.5 and 4.5. From the separation of the three lines, we find that $(-\partial E/\partial \log \tau) c_{\text{Cl}}$, τ is in the range 250 to 450 mV. Similar experiments at 40° C showed that τ is decreased by more than two orders of magnitude by the modest temperature rise from 25° C. or

$$(-\partial \log \tau/\partial \frac{1}{T})c_{\text{Cl}}^{-},_{E}$$

is about 1.2×10^4 , leading to an experimental apparent energy of activation of some 60 kcal/mol. Experiments with bromide additions gave very similar results; the rate dependence on $c_{\rm Br}^-$ showed n between 4 and 4.5, and $(-\partial E/\partial \log \tau)c_{\rm Br}^-$, T was around 30 to 70 mV.

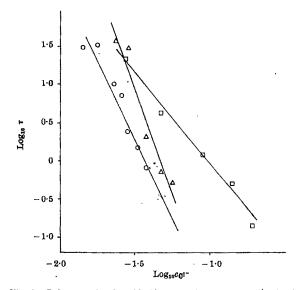


Fig. 2. Influence of added chloride concentration, c_{Cl}-, on induction period before breakdown, τ. 25° C; pH 2·05. Ο, 800 mV (nhe); Δ, 600 mV (nhe); Π, 400 mV (nhe).

The sharp dependence of the rate of the breakdown process on halide-ion concentration, anode potential and temperature shows at once why notional values of these factors have often been estimated, and indeed quoted in the specifications of resistant alloys; a particular alloy is resistant, say, up to "a chloride concentration of 0·1 molar", "a breakdown potential of +0·8 V (nhe)", "a temperature of 50° C", when the other two factors are kept at constant values. The sharp dependences of the rate of the breakdown process imply (in this illustrative example) that the onset of breakdown, although long delayed at the values given, could be rapid at, say, 0·2 molar chloride, or at +0·9 V (nhe), or at 60° C.

Further, the linear dependence of rate on $c_{CI-,Br-}^n$ where n=2.5 to 4.5, and the very high apparent activation energy, about 60 kcal/mol, show that the transition state in the breakdown process involves 2.5 to 4.5 halide ions* and has a very high energy. This rules out simple anion exchange or migration as rate-determining stages in breakdown. We therefore think that the breakdown process can best be described as follows. Three or four halide ions jointly "adsorb" on the oxide film surface around a lattice cation—one next to a surface anion vacancy for preference. The transitional complex thus formed will be of high energy and the probability of its formation at any instant will be very small. But, once formed, the complex can readily and immediately separate from the oxide ions in the lattice, the cation dissolving in the solution, very much more readily than the nonor aquo-complexed cations present in the film surface in the absence of halide ion. Under the anodic field, a further cation comes up through the film to replace the dissolved cation—the field at constant anode potential increases at the "thinned" point of the film; but arriving at the film/solution interface, it finds, not stabilizing oxide ion formed from water (nor, in de-aerated solution, oxygen molecules2), but several halide ions, so that the "catalytic" process, once begun, has a strong probability of repeating itself, and of accelerating because of the increasing electrostatic field. Thus, once localized breakdown starts with the initial transitional complex, it accelerates "explosively".

The process we have just suggested contains elements from hypotheses (1), (2), (3) and (4), and depends primarily on the high polarizability (in the electrostatic sense) of chloride or bromide ion, which promotes adsorption.

^{*} Note that values of n such as 20, which could be given no physical meaning, are not found.

The activation energy of the process is, of course, reduced by more positive anode potentials (much as in a simple charge-transfer process) and the probability of formation of the high-energy transitional complexes increases with rise of temperature, in the usual way.

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CHEMISTRY

Microwave Spectra and Structures of Isothiazole, 1,2,4-Oxadiazole and **Imidazole**

ROTATIONAL spectroscopy has proved a powerful method of studying the structures of heterocyclic molecules 1-6. We have extended work of this type to molecules of isothiazole, 1,2,4-oxadiazole and imidazole.

Spectral assignments followed established lines, absorptions being observed for J values between 0 and 18 and in general for both A and B components of the dipole To observe the spectra of imidazole, the moments. sample and absorption cell were maintained at about 50° C to provide sufficient vapour pressure. Rotational constants were derived as follows (in Mc/s)

	\boldsymbol{A}	$\boldsymbol{\mathit{B}}$	c
Isothiazole 1,2,4-Oxadiazole Imidazole	8,275·52 ± 0·1 10,337·37 ± 0·2 9,725·06 ± 0·2	$5,846 \cdot 24 \pm 0 \cdot 05$ $10,092 \cdot 66 \pm 0 \cdot 1$ $9,373 \cdot 92 \pm 0 \cdot 2$	3,424·21 ± 0·05 5,103·33 ± 0·1 4,772·02 ± 0·2
N-Deutero- imidazole	9.668-31 + 0-2	8.699-39 ± 0-2	$4.578 \cdot 43 \pm 0 \cdot 2$

The moments of inertia show that all the molecules are planar, the small inertial defects being close to those found for furan¹, pyrrole², isomeric oxadiazoles³,⁴, thiazole⁵ and pyrazole⁶. The moments of inertia of imidazole are somewhat larger than for the molecular dimensions found by X-ray diffraction in the crystalline state?. This lack of agreement is not unexpected in view of the strong intermolecular interactions, and the short CH distances, reported in the solid. The effect, on the constants, of deuteration of the imino group allows the imino hydrogen to be located in the inertial axis frame; for any acceptable model this means that the A axis in imidazole makes an angle of about 28° with the NH bond. A study of numerous models with reasonable structure parameters suggests that this axis intersects the NC bond joining the 1 and 2 positions.

Nuclear quadrupole hyperfine structure was resolvable in all these spectra. For isothiazole, the coupling constants in the inertial axes are (in Mc/s):

$$\chi_{a\dot{a}} = 1.05 \pm 0.2$$
 $\chi_{b\dot{b}} = -2.42 \pm 0.1$ $\chi_{cc} = 1.37 \pm 0.3$

These are acceptable values in view of the findings for similar cases and the approximately known directions of the A and B axes in the molecule. For 1,2,4-oxadiazole, well resolved splittings, caused by the two non-equivalent nitrogen nuclei, occur. Although the coupling constants for the different nuclei may clearly be of comparable magnitude, an analysis, in terms of individual coupling constants, should be possible and is proceeding. expect that the hyperfine structures for imidazole will be

similarly analysed with anticipated improvements in resolution.

Analysis of Stark effects for a number of transitions of each substance leads to the following preliminary values of the dipole moment components (in \bar{D})

	$\mu_{\mathbf{a}}$	μъ	μ (total)
Isothiazole	1·1 ± 0·1	$2 \cdot 2 \pm 0 \cdot 1$	$2.4_4 \pm 0.2$
1,2,4-Oxadiazole	1.2 ± 0.2	0.2 ± 0.2	1.2 ± 0.3
Imidazole	3.7 ± 0.3	0.0 ± 0.3	3.8 ± 0.1

Because in isothiazole the A axis must approximately bisect the C-S-N angle, these data roughly define two possible lines of action of the total dipole in the molecule. The total moment agrees well with a recent determination from dielectric measurements. For 1,2,4-oxadiazole the moment found is notably less than those of the other oxadiazoles. Unpublished molecular orbital calculations by Davies and Mackrodt show that this difference is theoretically predictable and give good agreement with the moment found here. The dipole moment of imidazole has been given rather scattered values from dielectric experiments. Our result is close to the lower limit of the values obtained in solution and agrees closely with the moment calculated theoretically by Brown and Coller¹⁰. From the experimental A and B components of the moment in imidazole, the line of action of the moment is found to make an angle of approximately 13° with the A axis. This line of action can only agree well with that predicted by Brown and Coller¹⁰ if the A axis intersects the N-C bond joining positions 1 and 5, but this evidence may not be strong enough to compel a preference for this orientation of the axes.

It is clear that much fuller information about the orientation of axes of inertia, lines of action of dipole moments and axes of quadrupole coupling tensors will follow from extended studies, in progress or planned, involving isotopic forms of these molecules. Refinement of the Stark measurements will require allowance for quadrupole splittings and, in the case of 1,2,4-oxadiazole and imidazole, for quartic Stark effect corrections. Such studies promise exceptionally full data on both electron distributions and internuclear distances for comparison with the results of extensive theoretical treatments.

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Thermal Conductivity of Glycine

RECENTLY it has been suggested 1-3 that electron spin resonance studies of irradiated amino-acids reveal thermal effects as well as directly induced free radicals. model proposed is that absorption of radiation energy may lead to a localized heating of small regions; this may allow radical reactions to take place at a rate characteristic of a much higher temperature than the average temperature of the sample. Evidence for this model includes the fact that there are differences in the spectra from irradiated glycine and valine after very high doses and lower doses of radiation energy1; the effects of different linear energy transfer radiations2; and the changes in the glycine spectra as functions of dose and time3. The validity of such a model depends on the assumption that the energy deposited by the radiation cannot diffuse by a conduction process, as only then can a local "hot spot" be created. A theoretical examination of this problem is hampered, however, by a lack of knowledge of the processes involved; in particular, no measurements have been made, as far as is known, on thermal conductivities. As a first step, an investigation was therefore made of the thermal conductivity of a single crystal of glycine.

The method adopted was the thermal-comparator technique (ref. 4, Fig. 7) developed by Powell. A 'Chromel' probe block was used, and a 100 g weight, which could be fixed on the top, ensured a constant pressure by the comparator on the system. The potential difference from the thermocouple was measured with a microstep potentiometer (Cambridge Instrument Co., Ltd.). Three reference materials of known conductivity (0.0037, 0.010 and 0.018 Joules cm/cm²sec °C) were used to

calibrate the comparator.

The single crystals of glycine were grown by slow evaporation from aqueous solution. They are monoclinic in form, with an elongation along the c-axis. Surfaces parallel to the a-c plane had the largest dimensions (approximately $1 \text{ cm} \times 0.5 \text{ cm}$) and their thickness was 2-3 mm. Most of the measurements were made with the probe resting on the a-c plane surface, although a few were tried on the b-c plane surface. None were possible on the a-b plane surface as the dimensions were too small to support the probe. No significant difference was found between the two surfaces investigated.

Measurements were made with the comparator at 60° C and 82° C, the temperatures being taken with a mercury-in-glass thermometer monitoring the oven used to heat the comparator. Results are plotted in Fig. 1, where the circled points refer to the glycine. Comparator readings have standard deviations of about ±2 per cent, and to this degree of accuracy it can be seen that there is no difference in the thermal conductivity of glycine at the two temperatures used. It is concluded that the thermal conductivity of glycine is 0.013 Joules cm/cm² see °C.

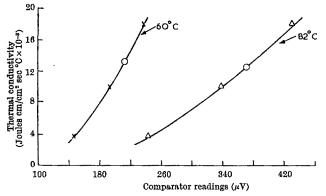


Fig. 1. Relationship between thermal conductivity and comparator readings. Circles denote glycine readings.

The implication of such a low value is that conduction in this material is predominantly by means of the lattice. (It is reasonable to assume that other aliphatic amino-acids will have comparable thermal conductivities.) Thus the absorbed energy cannot readily be evenly distributed throughout the whole sample, but tends to remain close to the point of deposition. This results in the local heating and radical reactions postulated to account for the electron spin resonance data.

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MOLECULAR STRUCTURE

Bonding Radii of 80S Ribosomes

THERE are now clear descriptions of the packing of ribosomes from two different nucleated organisms into two different crystal forms. One form, the chromatoid bodies of *Entamoeba invadens*, is a hexagonal array of helices¹. The other form, found in mitotic cells of chilled chick embryos, consists of tightly packed layers of plane lattices². At first sight there is no apparent relation between the packing of ribosomes within these two forms. We will show, however, that there is a way of looking at the bonds which these ribosomes form which closely relates one packing scheme to the other.

Byers² has shown that the ribosome crystals found in chick embryos belong to the space group P422. His micrographs show that the ribosomes are not in the general positions of this space group, but rather in very special ones. Fig. 1a gives a view of this packing perpendicular to the four-fold axes. Nothing in the symmetry of the space group demands that the distances marked d_1 and d_2 in this figure should be equal. Yet they are. By using either this property, or the equivalent one that ribosomes fall along mutually perpendicular families of lines, one can find, by algebraic geometry, that the special positions occupied by ribosomes in this particular lattice are described by the co-ordinates x=3/10a and y=1/10a, where a is the length of the square face of the unit cell, 540 Å. As a consequence α , the angle between centres of the ribosomes and the cell edge, is arc $\tan(y/x) = 1/3$ or 18.4°, as Byers has observed.

Fig. 1b shows a section of the same packing parallel to the four-fold axes and through the ribosomes marked I in Fig. 1a. Again note the symmetry of the space group does not demand that the distances marked d_3 and d_4 should be equal. Yet they are, and so we deduce that the special value of the z co-ordinate is $z=\frac{1}{4}c$, where c, the

height of the unit cell, is 700 Å.

Given these co-ordinates of any ribosome in the crystal, it is easy to compute the centre-to-centre distances between nearest neighbours. In the planes (Fig. 1a), these neighbours are 240 Å apart, as Byers has noted², while botween planes (Fig. 1b) nearest neighbours are 366 Å apart, and situated along lines making an angle β of $17\cdot1^{\circ}$ (that is, are $\tan=y/z$) with the long edge of the cell. The ratio of these two closest approaches is $1\cdot5$.

When we use these numbers to define the lengths and directions of what we call the "effective bonding radii" of these ribosomes, the resulting pattern is pictured in Fig. 2a. We may describe its construction as follows. (1) A bonding site of radius 120 Å occurs along the equator of three meridians: 0° , 90° , 180° . Call these s_1 , s_2 , s_3 .

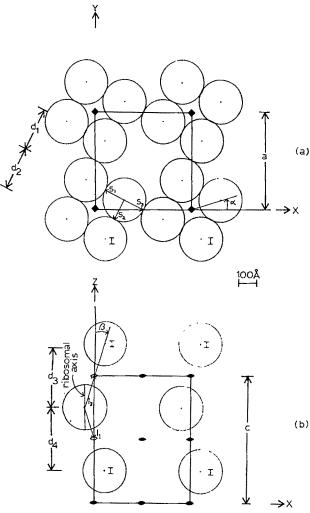


Fig. 1. Crystal packing of ribosomes within chilled chick embryos. Data from Byers*. a, Section perpendicular to four-fold axis; b, section parallel to four-fold axis.

(2) Along the fourth such meridian, 270°, there is no bonding site. Moreover, the electron micrographs give an indication of "clefts" here (see ref. 2). (3) Along meridian 90°, and at angles of $17 \cdot 1^{\circ}$ to each pole, there are bonding sites of radius 180 Å. Call these l_1 and l_2 .

The rules governing ribosome packing within the crystal form of chick embryos are therefore: (a) s_1 and s_2 bond with each other (these are the "tetramer" bonds); (b) s_3 bonds with s_3 (the "dimer" bonds). (Note that $|s_1| = |s_2| = |s_3|$.) (c) l_1 bonds with l_2 , and l_2 bonds with l_2 (the bonds holding successive planes of ribosomes together). Ribosomes of the shape described joined by these rules will form the observed crystal.

We have applied the same procedure to the helical packing found in chromatoid bodies¹. In 1966 when this packing was described it was noted that two types of bonds between ribosomes were probably effective here. One bond joined every other ribosome along the helix of twelve nodes in five turns (that is, nodes n and n+2, see Fig. 3c). This resulted in two sets of chains, each having six nodes in one turn. The other bond was proposed to join the chains to each other in dimers (node n to n+1). The radius proposed for the centres of ribosomes was 150 Å.

Using this radius, and the helical parameters found for this packing (rotation of 150° , translation of 75 Å), we calculate that the distance between nearest ribosones (n and n+2) is 212 Å, while that between next nearest (n and n+1) is 300 Å. The ratio of these approaches is 1.4. In the plane formed by any three successive ribosomes (say n-1, n, and n+1), their centres form an isosceles triangle with apex at ribosome n (see Fig. 3n). In this plane, the angle between the long bonds $(l_1 \text{ and } l_2)$ formed by this ribosome is 42° .

There are short bonds $(s_1 \text{ and } s_2)$ between ribosomes in this helical form. These lie in the plane formed by centres of ribosomes n-2, n, and n+2 (see Fig. 3b). Here they subtend an angle at the ribosome n of 132°. The angle between the planes of the long bonds and short bonds is 86°. Thus this form can be given a description similar in many ways to that given for the chick ribosomes but with different angles for the amoeba ribosomes. But in the amoebal ribosome, the mid-portion of meridian 90° is now occupied by the two long bonds, leaving no room for

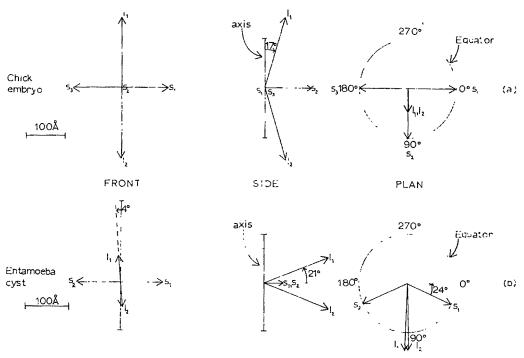


Fig. 2. Bonding radii of ribosomes from (a) chick embryos and (b) cysts of Entamoeba invadens.

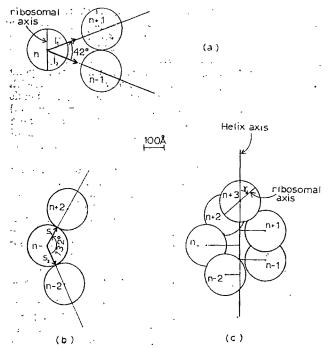


Fig. 3. Crystal packing of ribosomes within chromatoid bodies of *Entamoeba invadens*. Data from Morgan and Uzman¹. a, Plane through centres of ribosomes n-1, n, n+1; b, plane through centres of ribosomes n-2, n, n+2; c, projection onto a plane through ribosome n and the helix axis.

a third short bond such as the chick ribosome possesses. Perhaps as a consequence of this arrangement, amoebal ribosomes only have two short bonds. Again, the packing is formed by a similar rule which may be condensed to "long bonds to long" and "short bonds to short". The bonding radii of amoebal ribosomes are shown in Fig. 2b. In the helix, the ribosomal axis is inclined to the helix axis at an angle γ of 49° (see Fig. 3c).

Thus to deform an amoebal ribosome into a chick ribosome we must first straighten out the bend between s_1 and s_2 , so that they are diametrically opposed, and then increase the angle between l_1 and l_2 from 42° to 146°, thus disclosing a third short bond on the equator of meridian 90°.

So far our description has simply rephrased the geometry which relates the centres of electron-dense stain seen in electron micrographs of sections through ribosome crystals to each other. What physical structures underlie these vectors? Indeed, do parts of the ribosomal RNA or protein extend from the centre of the ribosome to the full length of these bonding radii? These questions, involving resolution beyond that given by the micrographs, cannot be answered by electron microscopy. Nor can these micrographs give an unequivocal answer to a third question: what is the relation between these bonding radii and the subdivision which divides all ribosomes into two-thirds and a third?

One possible way in which this subdivision could occur is suggested to us by the number and lengths of the bonding radii of the chick ribosomes: assume that mass is distributed along these radii in proportion to their length. Then l_1 , s_2 and l_2 will collect a mass of 4, while the remaining bonds s_1 and s_3 will collect a mass of 2. Thus l_1 , s_2 and l_2 will constitute two-thirds of the total mass, and could represent the larger (60S) sub-unit, while s1 and s3 will constitute one-third and could represent the 40S sub-unit. Even though a third short bond is not seen in the amoebal ribosome, a similar mass distribution could easily occur.

Another way in which these ribosomes could be subdivided is suggested by the electron micrography of extracted particles, either negatively stained3,4

shadowed⁵. From such images, one gets the impressions (1) that the larger sub-unit should include bonds s_1 and s_2 , as shown in Fig. 2b or Fig. 3b for the amoebal ribosomes, and (2) that the smaller sub-unit should include only those portions of bonds l_1 and l_2 which extend beyond a distance from the centre of the ribosome approximately equal to s_1 or s_2 . We can draw an envelope around s_1 and s_2 which will include twice the volume (and therefore twice the mass) of an envelope drawn around only the outer portions of l_1 and l_2 , but the manner in which the envelope is drawn is not specified by the crystallographic data in any exact way. The resultant shape is exactly that shown by Shelton and Kuff⁴ in their Plate I, c, d and f.

We can relate this second conception of ribosomal structure to two other pieces of evidence concerning ribosomes from *E. coli*. Langridge and Holmes⁶ observed that gels of 50S particles from this organism gave spacings corresponding to orders of 212 A. We would attribute this spacing to s_1 - s_1 bonding of the large sub-unit. Huxley and Zubay³ observed (see their Plate VIII, b, bottom row) dimers of 70S particles, in which we measure the centreto-centre spacing between the larger sub-units to be close to 300 Å. This we interpret as l_1-l_1 bonding. correspondence between these distances and those observed for the amoebal ribosomes is striking.

Our analysis of the two known crystalline forms of ribosomes shows that both ribosomes have a pair of long contacts or bonds in a plane very nearly perpendicular to the plane of a pair of short bonds, that the ratios of these bond lengths is very close to 1.5 and that within their planes each bond occurs at a defined angle.

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MICROBIOLOGY

Sorption of Water Vapour and Nitrogen Gas by Bacterial Spores

As a result of their high refractility and their resistance to stains and to lethal agents such as heat and chemicals, it has long been thought that bacterial spores are relatively dry and impermeable. There is conflicting evidence, however, about the degree of desiccation and impermeability which may exist. Refractive index measurements on spores in aqueous suspensions indicate very low water contents1, and the surface area of lyophilized spores available to adsorption of nitrogen gas is so small that little or no internal porosity can be postulated2. On the other hand, it has been demonstrated that spores have a significant affinity for water3. In aqueous media it is known that germinants enter spores rapidly and evidence has been presented for considerable porosity and a water content as high as 75 per cent of the dry weight4. effort to account for these discrepancies, we have employed standard gas adsorption techniques to measure water vapour sorption of intact and crushed spores and the nitrogen gas adsorption of spores which were dried in various ways. The results confirm the hydrophilic nature of spores and indicate that a considerable fraction of the volume occupied by water in wet spores can be preserved

in the dry state by employing dehydration methods which minimize the collapse of hydrophilic, macromolecular structures.

Clean refractile Bacillus subtilis var. niger spores with a viability of 70–100 per cent were obtained by centrifugation in density gradients of sodium bromide solutions followed by washing in water until the washings were free of bromide. Spores were dried by freeze-drying and by a solvent replacement procedure. The latter was carried out by washing the spores six times in absolute ethanol in centrifuge tubes which were provided with airtight caps. The alcohol was replaced by at least four washes of dry pentane. In the final alcohol wash and in all the pentane washes, the removal of supernatant and the addition of fresh solvent were carried out in a dry nitrogen atmosphere. The pentane was finally removed from the spores by evaporation at its boiling point (36° C). The viability of the spores was not significantly affected by the solvent replacement process.

Crushed spores were prepared by shaking an absolute ethanol suspension of clean spores with 0.2 mm diameter glass beads for 4 min at 4,000 r.p.m. in an MSK Braun homogenizer (Bronwill Scientific, Inc.). After removing the glass beads on a coarse frit and washing the suspension with ethanol, the crushed spores were spun down and dried directly from alcohol for water vapour sorption studies. For nitrogen adsorption, the alcohol in the suspension was replaced with pentane and evaporated as described. Less than 6 per cent of the dry weight of the original spores was lost during the entire process. Electron micrographs of crushed spores which were dried directly from alcohol suspension and shadowed with platinum showed recognizable spores with extensively ruptured coats and some spore fragments of unidentified origin.

Water vapour sorption was determined gravimetrically on well dispersed, 20-25 mg samples of spores using a quartz spring balance (Microchemical Specialities Co.) with a sensitivity of 1 mm/mg. The experiments were carried out in an air-conditioned room at 23° ± 2° C. After introducing the sample, the apparatus was pumped down to a residual gas pressure of 0.05 mercury before introducing water vapour. Equilibrium vapour pressures were measured to ± 0.05 mm mercury or less with a mercury manometer. During adsorption, water vapour was admitted to the system in doses, by momentarily opening a stopcock leading to a storage bulb of liquid water. During desorption, water was removed in successive stages by freezing in a cold trap. The time required to reach a constant weight at each pressure was longer at low than at high pressures, but in no case was it more than 2 h. After two or more complete adsorptiondesorption cycles (reproducible to about ± 8 per cent or better at all points), the sample was heated to constant weight at about 110° C. This weight was used to compute the water uptake for the sorption curves plotted in Fig. 1. The heating process causes a weight loss of about 4 per cent compared with the weight at room temperature and zero partial pressure of water. After rehydrating the heated samples, however, it was found that the sorption curves obtained before heating could be reproduced. The weight loss is thus caused by a small amount of tightly bound water.

Nitrogen adsorption measurements were made on spore samples $(0\cdot2-1\cdot0$ g) by the American Instrument Company. Spores were degassed by evacuation at room temperature until a pressure of less than $0\cdot05\mu$ mercury was maintained. The surface area ascribed to a monomolecular layer of nitrogen on the sample was calculated by the Brunauer, Emmett and Teller (B.E.T.) procedure using 16·2 Ų as the area of the adsorbed nitrogen molecule.

The water sorption of both intact and crushed spores (Fig. 1) is similar to that of natural polymers such as polysaccharides and proteins; the adsorption isotherms almost match those for dewaxed, washed wool⁶. Some of

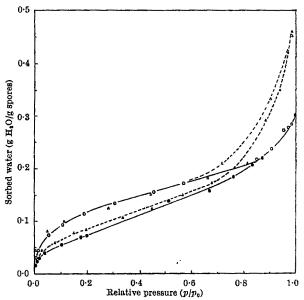


Fig. 1. Water sorption of Bacillus subtilis spores as a function of relative pressure of water vapour (relative humidity). Filled points represent adsorption, open points represent desorption. ——, Freeze dried spores, ---, crushed spores.

the interpretations derived from the extensive studies of these simpler materials seem appropriate here7. The sigmoid shape of the curves is typical of solids which have a high affinity for water and can swell as water uptake progresses. The steep slope of the water uptake curves at low relative humidities (p/p_0) may be attributed to strong binding by specific sites accompanied by the evolution of heat. At intermediate relative humidities, water is held less energetically and, subsequently, the adsorbent swells in the process of forming a solid solution. The ultimate amount of water taken up in this latter process seems to be limited by the swelling constraint imposed by the molecular structure of the adsorbent and by the capillarity of the sample. Hysteresis is also typical of swelling adsorbents. It is not caused by failure to reach a stable water uptake but rather by different macromolecular constraints in the adsorbent, depending on whether a particular water content is approached by desorption or adsorption8,9.

A striking feature of the curves for crushed spores is that they are very similar to those for the intact spores at low and intermediate relative humidities in spite of the extensive exposure of internal structures. This indicates that the water uptake must be attributed to sorption by structures in the bulk of the spore and cannot be effected merely by the external surface. It also shows that no significant number of primary water-binding sites have been made available by rupturing the spore coat. This is an argument against the existence of water permeability barriers which could maintain an anhydrous spore core. Crushed spores show a slightly narrower hysteresis and an increased water uptake at higher humidities compared with intact spores; this signifies a decreased constraint on swelling. Whether or not the core substance is responsible for most of this swelling as some workers have suggested10 cannot be conclusively decided until water sorption data are available on isolated parts of the spore anatomy.

Typical surface areas of the various spore preparations as determined from nitrogen adsorption measurements are summarized in Fig. 2. The surface area of freezedried spores (7 m²/g) is nearly the same as the external geometrical area (6 m²/g) calculated from the average dry weight of each spore and the spore dimensions obtained from electron micrographs assuming an ellipsoidal shape. This is in agreement with the results which Berlin, Curran and Pallansch² obtained for a variety of freeze-dried spores.

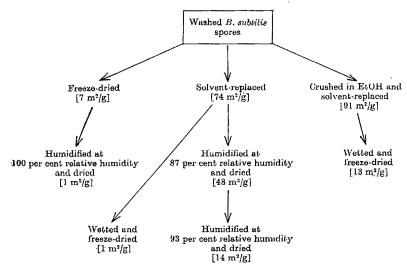


Fig. 2. A summary of the drying procedures for *Bacillus subtilis* spores and the corresponding surface areas (in m²/g) as calculated from the adsorption of nitrogen at -195°C using the B.E.T. procedure. Humidification was accomplished in the sequences indicated by the arrows, by passing air at the indicated relative humidity through the spore samples and then drying by evacuation or by wetting with liquid water and freeze-drying.

If the drying is carried out by solvent replacement, however, an approximately ten-fold increase in area is available. This additional area is most readily accounted for by the presence of accessible surface area in pores existing in the bulk of the spore. It is not the result of extraction of substituents by the organic solvents because the expanded structure can be collapsed again by contact with water. Thus the area lost on equilibration of the expanded spores with humidified air or liquid water followed by drying by evacuation or freeze drying, respectively, increases in proportion to the degree of humidification (Fig. 2). Areas less than that calculated for the geometrical surface area of the spores (< 6 m²/g) can occur as a result of agglutination. The areas of crushed spores parallel those for similarly dried intact spores; the somewhat larger value for crushed spores is consistent with their smaller particle size.

The wide variation in nitrogen surface area with different methods of sample preparation is well known, especially in the literature on cellulose¹¹, and the interpretations offered there seem applicable here. On this basis we suggest that the following model is consistent with the data on bacterial spores. In the water-wet state, the spore (or at least the part of the spore external to the core and possibly the cortex) is swollen and a water-filled porous structure exists in accordance with the findings of Black and Gerhardt4. On drying in air or even freezedrying, this porous structure collapses completely. Many of the hydrogen-bonding sites on the macromolecular constituents of the spore, initially interacting with water, are likely to be satisfied by interaction between adjacent chains as the water moves out. These intermolecular spaces are closed during the drying process until, in the completely dry state, non-polar molecules, even those as small in size as nitrogen, are unable to penetrate the exterior surface. Voids in the interior of the dry spore appear highly unlikely, but they cannot be ruled out on the basis of our data. If the water is removed by the solvent-replacement procedure, however, considerable porosity is retained. That this porosity represents only a part of the porosity existing in the water-wet state is indicated by the fact that the area available for water sorption computed from the water adsorption isotherm by the B.E.T. procedure amounts to 240 m²/g (taking 10.5 Å² as the area of a water molecule). We cannot at present specify which of the several steps in the solvent-replacement process is responsible for this shrinkage. In the case of cellulose, the surface area losses seem to occur chiefly

during the final evaporation and are reduced as the surface tension of the evaporating hydrocarbon liquid is lowered11.

A more quantitative discussion of such questions as the pore size distribution of spores dried by solvent-replacement and of a correlation of these pore sizes with those found in the water-wet state must await an exploration of conditions for the preparation of spores with maximal surface area. A reconciliation of the results of optical methods of determining water content¹ with the higher values obtained by other methods here and elsewhere3,4 awaits the availability of optical and water content data on the individual anatomical regions of the spore. The mechanism of the striking loss of surface area occurring during the freeze-drying process, both with spores and with cellulose¹¹, seems worth investigating in view of the widespread use of this method to dehydrate biological and other hydrophilic materials with the intention of preserving structure.

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Novel Approach to studying Relationships between Mycoplasmas and Tissue Culture Cells

Most continuous cultures of tissue cells are contaminated with mycoplasmas1, which can be detected by staining2, immunofluorescence3, isolation3 or biochemical studies4. Furthermore, the interaction of mycoplasma organisms with HeLa cells and other types of mammalian cells has been studied with the electron microscope^{5,6}. Electron microscopy, in particular, shows that there is an intimate relationship between mycoplasmas and infected cells, which might explain why it is so difficult to eliminate these organisms from tissue culture cell lines and strains.

Del Giudice and Pavia, used a completely different macroscopic technique to study the relationship between Mycoplasma pneumoniae and erythrocytes. They observed that guinea-pig erythrocytes poured over M. pneumoniae colonies on agar medium adsorbed to the colonies after a short period of incubation at 37° C. This phenomenon of haemadsorption has since been observed with a number of other mycoplasma species and with erythrocytes from other mammalian and avian species. Furthermore, we have observed adsorption of bovine and human spermatozoa to colonies of several mycoplasma species and to sheets of M. gallisepticum organisms attached to plastic Petri dishes. It was logical to use this technique

to study the association between mycoplasmas and tissue culture cells. Thus we have not infected cells in culture, but we have observed the adsorption of tissue culture cells in suspension to mycoplasma colonies and sheets.

Details of the mycoplasma growth medium have been presented previously^{8,10}. We used mycoplasma colonies on pH 6.5 agar medium, and sheets of mycoplasma organisms adherent to the base of plastic Petri dishes11. These mycoplasma sheets were washed three times with 5 ml. of Difco PPLO broth before use. The tissue culture cells used in these experiments were M-HeLa12. They were grown as monolayers in Roux bottles in Eagle's medium containing 10 per cent ox serum. The cells were collected by treatment with 0.05 per cent versene at 37° C for 10 min and were resuspended in a small amount of the cell culture medium. Immediately before use the cells were centrifuged at 900g for 10 min, washed once with 0·1 molar phosphate buffered saline (PBS), pH 7·2, and resuspended in PBS at a concentration of about 106 cells/ml. Then 1.0 ml. of the cell suspension was poured over the mycoplasma colonies on agar or over the mycoplasma sheets adherent to plastic and these preparations were incubated at 37° C for 15 min. The colonies and sheets were then examined under a plate microscope for adsorption of the HeLa cells and again after excess cells had been removed by washing with PBS.

We chose to study M. pneumoniae and M. gallisepticum because haemadsorption and spermadsorption had been demonstrated previously⁷⁻⁹, and M. hominis because this mycoplasma is a frequent tissue culture contaminant. Fig. 1 shows the appearance of a colony of M. gallisepticum before and after the addition of HeLa cells; the surface of the colony is covered by adherent cells. Table 1 shows results obtained when HeLa cells were added to the three different mycoplasma species mentioned compared with those obtained in haemadsorption and spermadsorption tests. Colonies and sheets of M. gallisepticum and M. pneumoniae which extensively adsorbed both erythrocytes and spermatozoa also adsorbed HeLa cells extensively. Moreover, colonies and sheets of M. hominis adsorbed HeLa cells extensively in spite of the fact that they adsorbed neither erythrocytes nor spermatozoa. This is of particular interest in relation to the frequent finding of this mycoplasma as a contaminant of tissue cultures. In addition, the cells could not be dislodged from any of the colonies or sheets by normal washing procedures.

The simplicity of this test system is valuable for two reasons. First, it is possible to examine the adsorption of cells derived from various avian and mammalian tissues to different mycoplasmas, and so obtain further information on mycoplasma host specificity and tissue affinity. Second, the system affords an opportunity to determine the effect on adsorption of treating both mycoplasma and cell with various reagents and so define the nature of possible receptors. Gesner and Thomas¹³ have indicated that sialic acid receptors on erythrocytes are involved in the agglutination of such cells by M. gallisepticum. In our experiments, the affinity noted between M. hominis and HeLa cells was in contrast to the lack of affinity previously observed between M. hominis and erythrocytes or spermatozoa. This suggested that some unusual sort of receptor mechanism was involved in the case of the HeLa cells. We therefore treated HeLa cells by incubating them

Table 1. Adsorption of Hela cells, erythrocytes and spermatozoa to mycoplasma colonies and sheets Adsorption* of indicated cell to mycoplasma

		Colonies	_	ea cen to	Sheets	114
Mycoplasma species	M-HeLa	Erythro- cytes	Sperm.	M-HeLa	Erythro- cytes	Sperm.
M. gallisepti- cum (A5969) M. pneumoniae	++++	++++	++++	++++	++++	++++
(F.H.) M. hominis	++++	++++	++++	++++	++++	++++
(V2785)	+++	_	-	+++	-	-
			1			_

*+++++, +++=100 per cent and 75 per cent, respectively, of mycoplasma colony or sheet covered by adherent cells.

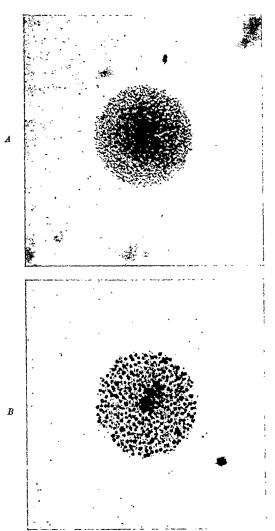


Fig. 1. A, A colony of the S6 strain of M. gallisepticum. B, A colony with adherent M-HeLa cells; the cell suspension was poured over the colony, the preparation incubated at 37° C for 15 min and excess cells removed by washing.

for 1 h at 37° C with neuraminidase, and then tested for adsorption. Neuraminidase treatment of cells inhibited their adsorption to sheets of M. hominis organisms, indicating that neuraminic acid receptors on the HeLa cells were involved in this reaction. Such a bond between HeLa cell and mycoplasma organism would be one possible explanation for the difficulty encountered in climinating the organism from cells in continuous culture.

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Ageing Effect on Phage Particles leading to an Increase in Burst Size

In constant conditions, the burst size of a particular bacteriophage is a characteristic of that bacteriophage. We have found aged P4/2 phage particles yield larger

burst sizes than young phage particles.

The bacteriophage P4/2 was isolated from Salmonella potsdam (NCTC 3206) and propagated on another strain of S. potsdam, SP2, which seems to be non-lysogenic1. Phage lysates were stored in a refrigerator, and at periodic intervals one-step growth experiments were performed in which the multiplicities of infection were between 0.01 and 0·1. A steady increase in the average burst size was found with an initial low value of 34 from cells infected with fresh phage particles to a high value of 786 for cells infected with phage particles which were I month old. The burst sizes from cells infected with phage stored for times longer than 1 month were approximately the same as those obtained with cells infected with month old phage. The rate of adsorption, determined by measure of both unadsorbed phage and of infective centres, was found to be independent of the age of the infecting phage particles. Neither the rate of adsorption nor the burst size itself was affected by variations in the multiplicity of infection. The burst sizes produced by phage lysates kept at both higher and lower temperatures were like those yielded by phage kept for the same time in a refrigerator even though there was a drop in the plaque forming titre of lysates kept at higher temperatures.

In a series of single burst experiments, the proportion of infected cells which gave rise to small numbers of phage progeny was high when young phage particles were used for infection but low when old phage particles were used. The proportion of cells infected with aged phage particles, which yielded large bursts, was also significantly higher. These results support those obtained in the one-step growth experiments but give the added information that the differences in the average burst size are directly related to the size of the burst from individual cells rather than to differences in the proportion of cells which

release phage progeny.

A series of premature lysis experiments was performed with lysates which gave bursts of different sizes. The length of the eclipse phase was found to be shortened as the burst size increased although the latent period itself remained constant at 40 min. The shortest eclipse phase was 20 min for a burst of 700, the longest was 35 min for a burst of 34. Thus the length of the eclipse phase was

inversely proportional to the size of the burst.

If there is the same relationship between phage DNA and the appearance of mature, intracellular phage with this phage and its host as there is with T2 infected cells of E. coli, a delay in the appearance of intracellular phage would mean a delay in the synthesis of DNA². Attempts to test this directly have so far failed because, at the low multiplicities of infection necessary to prevent lysogenization, the net DNA synthesis was not representative of phage infected cells. We cannot therefore determine whether the delay which results in initial, small average burst sizes attributed to one or more phage regulated functions is associated with a delay in the onset of phage DNA synthesis or in the maturation process with a consequently larger DNA vegetative pool.

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Distribution of the Soluble Dehydrogenases and Diaphorases of Brucei Subgroup of Trypanosomes in Starch-gel Electropherograms

Antigenic variation in the brucei subgroup of trypanosomes considerably limits the development of a suitable anti-trypanosomal vaccine, and this limitation is likely to continue until we have more knowledge of the nature of antigenic variation. Until then, however, more information about the nature of trypanosome antigens would be useful.

Some of the variable antigens are soluble proteins and the variation seems to be associated with changes in their immunological specificity¹⁻³. We have separated soluble trypanosome proteins into at least twenty-nine components by starch-gel electrophoresis, and in an attempt to characterize these components we tested for glucose-6-phosphate dehydrogenase which was known to be present. This was found in the region occupied by the major 4S variable antigens of Williamson and Brown³ (bands 1-7 in Fig. 1). This suggested that the antigens might be dehydrogenases, and so we made a survey of some of the dehydrogenases and diaphorases of carbo-hydrate metabolism. We present here the results of the distribution of these enzymes in starch-gel electropherograms and discuss their possible role in metabolism.

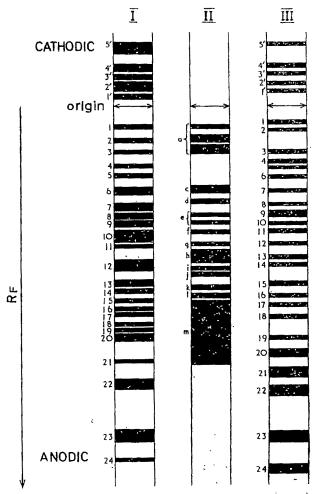


Fig. 1. Starch-gel electropherogram of the rat serum and soluble trypanosome proteins. I, EATRO-691; II, normal rat serum; III, T. rhodesiense, SS 518. The figure is a combined scale drawing from different preparations run at different times. The rat serum notation is that of Beaton et al. 19. 0-05 molar glycine/sodium hydroxide buffer, pH 8-9, gel; 0-05 molar borate buffer, pH 8-58, electrode chambers; 8 V/cm, 2-5 h. Proteins stained in 1 per cent amido black 10B in methanol/water/acetic acid (50/50/10, v/v):

^{*} Nutter, R. L., and Sinsheimer, R. L., Virology, 7, 276 (1959).

A brucei subgroup isolate EATRO-691 and T. rhodesiense isolate, SS 518, were used for this study and prepared soluble proteins from these organisms as previously described. The protein solution was loaded onto strips of filter paper which were then inserted into a starch gel previously prepared in glycine-sodium hydroxide buffer. An uninfected rat serum control was run at the same time and bromphenol blue was applied as a tracking dye to a hole on one side of the gel. Horizontal zone electrophoresis was carried out in the cold room at 2°-4° C, for 2·5 h at a volt gradient of 8 V/cm. The gel was then sliced horizontally, one slice stained for proteins and the remainder for enzymes.

The enzyme test system for dehydrogenases and diaphorases was essentially that of Fildes and Parr⁷ and consisted of an "overlay" made up of agar (45 mg) in

0.3 molar tris hydrochloric acid buffer (pH 8.0, 1.6 ml.), to which was added 0.1 molar magnesium chloride (1.6 ml.) and water (1.3 ml.) mixed at 45° C with the tetrazolium salt 'Nitro BT" or 'MTT" (0.9 mg), phenazine metho sulphate (PMS, 0.2 mg), NAD or NADP (0.4 mg), EDTA (disodium salt, 12 mg), and the particular test substrate at a final concentration of 1.5–2.5 mmolar. In the case of diaphorases, NADH or NADPH (0.5 mg) were the substrates and NAD, NADP and PMS were omitted. The enzyme was located as a bright blue zone. The protein and enzyme bands were correlated by comparing their R_{FS} (ref. 8).

Fig. 1 shows a combined scale drawing of an electropherogram of several different preparations run at different times. Although not all components appeared in each preparation on each occasion, the bands from dif-

Table 1. DISTRIBUTION OF DEHYDROGENASES AND DIAPHORASES IN STARCH-GEL ELECTROPHEROGRAM

Enzymes	Specificity		1	2	ND 1		HORA 4			faroi 6		Band 7		9	10	11	12	13	14
Isocitrate	NA NA	ADP								++						_‡			
G-6-P	T. rhodesiense	ADP AD ADP	+	++	+ •	+ + + +	+	+	+		+	+ +			+	+	÷ ÷		
6-P-G	T. rhodesiense	ADP AD ADP AD ADP AD	+		+	+	+		+	+	÷								
Ethyl alcohol	T. rhodesiense	ADP ADP ADP ADP ADP									+	4					+		
Glutamate	T. Thodesiense	ADP ADP ADP ADP ADP ADP		+	+	+	+		+	+	+	4	-						
3-P-Glyceraldehyde	T. rhodesiense	IADP IAD IADP IAD IADP IAD		+	++++++					++	+	÷ †	4-	+		÷ +			
:-Gtycerol-phosphate	T. rhodesiense N Rat N	IADP IAD IADP IAD IADP IAD			++	+	+ + +			+ +									
Lactate	T. rhodesiense	IADP IAD IADP IAD IADP IAD				+			+	+ +		++	t	+		+		÷	
Malate	T. rhodesiense	NADP NAD NADP NAD NADP NADP NAD		+	+ + + +	+	+ + ±	+ +	<u>+</u> -	+ + + + +	+	<u>+</u>	+ + +	+ + + + +	+	+	+		<u>.</u>
Succinate	T. rhodesiense	NADP NAD NADP NAD NADP NAD		+	-		+	+		+		+	*}-				+		
NADPH diaphorase	(EATRO-691 T. rhodesiense Rat									+		÷ +	+	+	+				
NADH diaphorase	EATRO-691 T. thodesiense Rat						4	÷		+	+	÷	<u>+</u>	+	+ +	+	- +		

The numbers refer to the bands in Fig. 1. In the case of rat serum proteins, the numbers refer to their corresponding band numbers on the trypan-aome component. +, Enzyme present; + + +, the formazan appeared as three sharp bands joined by weakly staining regions.

ferent preparations could be reproduced to within one R_F value. Some of the protein bands had the same mobility as the rat serum proteins, but two observations suggested that these were not contaminants from the rat serum. First, the method of preparation9 gives trypanosomes which are about 99 per cent pure and the concentration of the serum proteins is so low that they are unlikely to be detected by the protein stain. Second, in the enzyme histochemical localization, the mobility of the trypanosome enzymes does not correspond with that of the serum enzymes.

NADH and NADPH diaphorases and isocitrate (EATRO-691 only), ethyl alcohol, glucose-6-phosphate (G-6-P), 6-phosphogluconate (6-P-G), glutamate, 3-phosphoglyceraldehyde, α-glycerol-phosphate, lactate, lipoate, malate and succinate dehydrogenases were tested for and, apart from lipoate, they were all detected. distribution of these enzymes among the bands is shown in Table 1. Some of the enzymes have mobilities falling between the stained bands. These could be highly active enzymes in very low concentration which are not therefore stained by amido black 10B or, more likely, the assigned mobility may result from an error in measurement. In general, the enzyme pattern was the same for both isolates. but in some cases the number of bands differed. Usually, the mobility of the trypanosome enzymes differed from the rat serum enzymes, but when they coincided the trypanosome ones were assumed to be contaminants from the rat serum.

All the enzymes seemed to be isoenzymes except α-glycerol-phosphate and lactate dehydrogenases in EATRO-691 and 3-phosphoglyceraldehyde in both trypanosome isolates. They all had a low specificity for NAD and NADP with the exception of glutamate, ethyl alcohol and succinate dehydrogenases which were specific for NAD. Although the enzymes reacted with both NAD and NADP, not all the bands did so. Several of the bands of various isoenzymes seemed to have the same mobility, but it is not clear whether one band is an enzyme with more than one activity or whether the band is composite. With some of the enzymes-malate dehydrogenases, for example—the bands reacted with NAD in both rat serum and trypanosomes. With NADP, however, except for one band in the rat, only the trypanosome enzyme reacted.

All glycolytic dehydrogenases have been found in this study, which can be taken as a further confirmation of the accepted10 classical Embden-Meyerhof pathway of glucose catabolism. Work on the distribution of carbon-14 on labelled metabolites in these organisms suggested that the Krebs cycle is inactive^{11,12}. Both isocitrate and succinate dehydrogenases have been found and confirmed cytochemically (our unpublished results). Shaw et al.12 did not detect the formation of 2-oxoglutarate from carbon-14 glucose, and Ryleys found that the blood forms did not utilize 2-oxoglutarate so it can only be concluded that there is a by-pass in the pathway from isocitrate to succinate. It is, however, difficult to explain the absence of 2-oxoglutarate (usually a product of glutamate transferase) when the presence of glutamate dehydrogenase has been confirmed.

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Distribution of Experimentally Induced Scrapie Lesions in the Brain

Lesions of scrapie are confined to the central nervous system. Differences in the distribution of these lesions have been described for the two strains of scrapie agent used in goats1 and for the lesions which arise as a result of injecting mice with boiled and unboiled material2. We have also collected unpublished data showing wide variation in the distribution of lesions which result from using different strains of the agent in mice or by inoculating different genotypes of mice with the same agent.

In order to estimate the concentration of scrapic agent in various tissues of four strains of mice, the tissues were removed from animals which had been killed at intervals between the time of challenge with the ME7 strain of agent and development of the clinical disease. One of the four mouse strains, C57BL, also served as the test strain in bioassay of the scrapie titre. As a result of the routine use of a system which we have developed3 for measuring the degree and spatial distribution of scrapie lesions in the brain, it has been possible to examine a number of potential sources of variation in these lesions, such as sex of the test animal, titre of scrapie in the inoculum, strain differences between donor and host, and the finer specificities associated with the particular donor tissue which forms an integral part of the inoculum. So far we have data for scrapic agent in the brain and in the spleen.

All the donors which we used were females of the strains C57BL, SM, LM and VM; the latter differed notably from the others in the alleles of the gene sinc, which controls ME7-scrapie incubation period4. Rigorous aseptic precautions were used when tissues were removed, to prevent cross contamination between different organs. For injection, the tissues were ground and made up in a saline suspension to give an overall concentration of 10-2. This was then spun at 300g for 10 min and 0.02 ml. of the supernatant was administered intracerebrally into mice. An elaborate bioassay design involving 273 C57BL mice was used to minimize random or fluctuating sources of error, both at the time of preparation of the inoculum and during measurement of the incubation period. The brains were removed for histological examination at the same stage in the progression of the clinical disease. The degree of vacuolation in the various regions of the brain was scored on a scale ranging from 0 (no vacuolation) to 5 (vacuoles confluent throughout the defined brain location)3.

As seen in Table 1, the degree and distribution of lesions are unaffected by sex. When, however, the degree and distribution of lesions from each tissue source are compared, either within the same sex or on an overall basis as in Fig. 1 and Table 1 (col. 3), differences can be seen. Thus the lesions are more advanced in the anterior midline cerebral cortex, hippocampus and paraterminal body and less advanced in the cerebellum when scrapie brain is used as the inoculum than when scrapie spleen is used as the source. These differences are present in tissues taken from donors in all stages of the disease (Table 1, columns 4-9). It has been established that the scrapie titre in spleen is uniformly high in all strains at 08 07 08 08 04 07

	κā	Sex	Tissue used for inoculum			Stage of	Stage of disease			Don	or strain—hos	Donor strain—host strain difference	nce
Location	Female	Male	Prob. of difference between spleen and	<40% of incubation period	% of in period	55–75% of incubation period	% of n period	Terminal scrapie phase	rapie phase	C57 tissues into C57 recipionts	ics into ipients	SM, LM tissues i recip	SM, LM and VM tissues into C57 recipients
			orain (see Fig. 1) (%)	Spleen	Brain	Spleen	Brain	Spleen	Brain	Spleen	Brain	Spleen	Brain
Medulla	1.91 ± 0.05	1.78 ± 0.07	> 10	1.58 ± 0.11	$2 \cdot 04 \pm 0 \cdot 06$	1.88 ± 0.12	$1 \cdot 74 \pm 0 \cdot 12$	1.95 ± 0.22	$2 \cdot 09 \pm 0 \cdot 11$	$1\text{-}61\pm0\text{-}10$	1.97 ± 0.14	1.82 ± 0.07	1.95 ± 0.08
Cerebellum	1.55 ± 0.07	1.37 ± 0.08	< 0.1	1.64 ± 0.10	0.99 ± 0.11	$1 \cdot 72 \pm 0 \cdot 14$	1.10 ± 0.15	1.81 ± 0.14	1.21 ± 0.12	1.92 ± 0.13	$1{\cdot}07\pm0{\cdot}12$	1.68 ± 0.08	1.13 ± 0.08
Tectum of midbrain	2.28 ± 0.05	2.30 ± 0.06	25	2.12 ± 0.10	$2 \cdot 42 \pm 0 \cdot 10$	$2 \cdot 26 \pm 0 \cdot 11$	2.21 ± 0.11	$2 \cdot 22 \pm 0 \cdot 14$	2.54 ± 0.09	$2{\cdot}14\pm0{\cdot}08$	$2{\cdot}31\pm0{\cdot}13$	$2 \cdot 22 \pm 0 \cdot 06$	2.45 ± 0.07
Hypothalamus	1.55 ± 0.07	1.55 ± 0.07 1.65 ± 0.07	5-10	$1{\cdot}41\pm0{\cdot}05$	1.70 ± 0.11	1.58 ± 0.05	1.67 ± 0.14	1.64 ± 0.19	1.71 ± 0.07	1.48 ± 0.14	1.59 ± 0.16	1.53 ± 0.07	1.73 ± 0.08
Thalamus	2.55 ± 0.05	2.56 ± 0.06	> 20	$2\text{-}41\pm0\text{-}07$	2.65 ± 0.13	$2\cdot 46\pm 0\cdot 14$	2.61 ± 0.08	2.69 ± 0.10	$2 \cdot 59 \pm 0 \cdot 20$	2.44 ± 0.10	2.54 ± 0.16	2.53 ± 0.05	2.65 ± 0.09
Hippocampus	2.68 ± 0.04	2.69 ± 0.05	< 0.1	$2 \cdot 43 \pm 0 \cdot 08$	2.89 ± 0.00	2.44 ± 0.08	2.83 ± 0.06	2.72 ± 0.14	3.00 ± 0.05	2.67 ± 0.09	2.93 ± 0.08	$\textbf{2.48} \pm 0.05$	2.89 ± 0.04
Paraterminal body	2.65 ± 0.04	2.69 ± 0.05	< 0.1	$2{\cdot}43\pm0{\cdot}00$	2.83 ± 0.05	2.51 ± 0.04	2.87 ± 0.05	2.62 ± 0.15	2.91 ± 0.04	2.50 ± 0.10	2.83 ± 0.07	2.50 ± 0.05	2.92 ± 0.04
Cerebral cortex (posterior midline) 2.09 ± 0.04	midline) 2.09 ± 0.04	$2 \cdot 10 \pm 0 \cdot 06$	> 40	$2 \cdot 03 \pm 0 \cdot 08$	$2 \cdot 11 \pm 0 \cdot 08$	2.09 ± 0.09	$2 \cdot 11 \pm 0 \cdot 09$	$2{\cdot}11\pm0{\cdot}10$	2.14 ± 0.11	$2{\cdot}14\pm0{\cdot}10$	$2 \cdot 10 \pm 0 \cdot 11$	2.06 ± 0.06	2.12 ± 0.07
Cerebral cortex (anterior midline) 2.53 ± 0.05	nfdline) 2.53 ± 0.05	2-64 ± 0-05	v 0·1	$2 \cdot 33 \pm 0 \cdot 07$	2.78 ± 0.01	2.41 ± 0.03	$2 \cdot 72 \pm 0 \cdot 02$	2.55 ± 0.12	2.88 ± 0.07	2.42 ± 0.09	2.77 ± 0.08	2.41 ± 0.05	2.80 ± 0.04

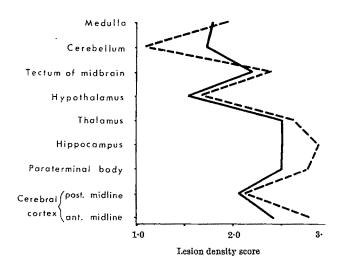


Fig. 1. Scraple lesion density in nine regions of the brain resulting from intracerebral challenge with inoculum of scraple spleen (solid line) or scraple brain (broken line). The order of brain regions is arbitrary.

these stages of incubation; the stages do, however, represent an increasing titre in the brain, over a 10,000 to 100,000-fold range, as incubation advances. Final levels in the brain and spleen are about the same (ref. 5 and our unpublished results). The general pattern of differences shown in Fig. 1 is independent of the presence or absence of isogeny between donor and host (Table 1, cols. 10-13).

These findings are the unexpected results of an experiment which was designed with an entirely different aim in view and we had no initial intention of analysing the lesion distributions in the assay animals. The results seem to be entirely consistent throughout the data. Other experiments are now needed to discriminate between two chief possibilities, either that the scrapie agent may have been modified in the different donor tissues, or the described effect stems from accompanying organ-specific constituents which could involve some immunological process.

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PATHOLOGY

Induction of a Neurological Disorder by Cycasin in Mice

SINCE an unusually high incidence of amyotrophic lateral sclerosis, a neurological disease, was found on the island of Guam by Kurland and Mulder¹, the search for the cause of the disease has been focused on Cycas circinalis, which the Guamanians have long utilized as a source of food starch. Ingestion of cycad plant material in the tropics and subtropics where eyeads are indigenous has been reported to cause posterior weakness and ataxia, with a characteristic "goose-stepping" gait in cattle?. Numerous feeding experiments have been conducted to evaluate the disputed conditions in which cycad paralysis

Table 1. MORTALITY OF NEWBORN RATS WHICH RECEIVED A SUBCUTANEOUS INJECTION OF CYCASIN

Mother No.	No. of newborn	5	6	7	8	13	14	15	20	Days aft 21	er inject 23	ion of 24	cycasin 25	26	27	28	29	30	31	32	33
1 2	6 5	,				1 .									1		1		1	2	
4 5	7 5					1			2							2		1	n	-	
7 8	4 8	1*		1						•	1	1	1	1	. 1		1	1	2		2
10 11 Total	6 6 60	1	1	1	,	2	1	2 2	2	i 2	2	q	1	1	2	1 3	2	2	R	9	2

^{*} Each figure denotes the number of animals which were dead or killed at moribund condition.

occurs, but one cannot be sure that the test animals were not exposed to other risks which could produce locomotor difficulties, except for the experiments which were carried out in controlled conditions. Recently, Anderson and Hall³ reported that cattle which had been fed fresh cycad leaves showed a slight lack of co-ordination in the hindquarters which resulted in a swaying gait. None of the glycosides which are contained in cycads, however, has been shown to be responsible for producing such neurological disorders.

Laqueur et al.4.5 found that cycad meal and cycasin, a methylazoxymethanol glycoside which is contained in cycads, produce a carcinogenic effect in rats. The same effect of cycad material in guinea-pigs and mice was reported by Spatz⁶ and O'gara et al. 7, respectively. The neurological disorder was not, however, observed in these

In the course of the experiment to investigate the carcinogenicity of cycasin, we accidentally found that mice which were treated with cycasin showed a high incidence of neurological disorder. Mice used in this experiment were supplied by the National Institute of Genetics. Sixty newborn mice, less than 24 h old, from the eleven mothers of the inbred strain C57BL/6 received a single injection of 0.5 mg of cycasin/g body weight. Cycasin was used in a 2.5 per cent solution of sterile physiological saline. Thus the volume of cycasin solution injected was 0.02 ml./g body weight. The solution was filtered before use and administered under the skin of the back to each mouse. Another group of twenty-one newborn mice, less than 24 h old, from the three mothers of the same strain, served as a control group. These mice were subcutaneously injected with 0.02 ml. of saline/g body weight in the same manner. The litters were kept in the same cage with their own mothers, which were maintained on a diet of CLEA (Central Laboratory of Experimental Animals) and water.

In the group which received a single subcutaneous injection of cycasin, nine out of sixty newborns (15 per cent) died 5-15 days after the injection and thirty-three (55 per cent) died 20-33 days after the injection (Table 1), while mice in the control group were healthy and developed normally. Forty animals (80 per cent) out of fifty-one animals which survived for more than 20 days showed ataxia and posterior paralysis. The sex of the affected animals was unrelated to the incidence of the disease. It was difficult to diagnose the neurological disorder in mice which died before they could walk.

The severity of locomotor difficulties was tentatively classified into the following several grades. Grade 1: slight posterior weakness with excessive swaying of the hindquarters and gait is slow. Grade 2: relatively slight ataxia, that is, a lack of co-ordination and exaggerated lifting of the hind feet with a characteristic "goose-stepping" gait. Grade 3: marked ataxia; animals stagger and stumble or fall down frequently when trotted, but are able to recover and stand up. Grade 4: complete paralysis of the hindquarters, but the animal is able to move, dragging its paralysed hindquarters.

These symptoms were irreversible and closely resembled those symptoms in cattle which had been fed cycad plant material². There was no appreciable loss in bladder, anal or tail functioning. Animals affected with the more severe ataxia usually died. During observation for 6 months after newborns were injected, the mothers were completely free of such disorders. Autopsies were performed on all mice which were dying or which had been killed in a moribund condition and which had survived for more than 20 days and showed symptoms of the neurological disorder after the injection of cycasin. Tissue from all the organs as well as the complete central nervous system was fixed in 10 per cent formalin. Routine methods of cutting, paraffin embedding and sectioning were used. The embedded tissue was sectioned and stained with haematoxylin and eosin. Duplicated slides of the central nervous system were stained with Luxol fast blue. The gross findings at autopsy were essentially negative. Histological changes were not found in any organs except the liver: animals which died a relatively short period after the injection of cycasin showed degeneration of the liver cells. In animals which survived for about 1 month after the injection, irregularity of liver cells in shape and size was a common finding. These histological changes in the liver were attributed to the toxicity of cycasin. Although detailed histological observations of the central nervous system are now in progress, a definite lesion to explain the neurological disorder has not yet been detected.

In another experiment, a total of fifty-eight adult male mice, of strain C57BL/6, 2 months old, received a single oral administration of cycasin by stomach tube. Eighteen mice received 1.0 mg cycasin/g body weight and ten mice received 0.5 mg/g. Cycasin was used as a 10 per cent solution dissolved in physiological saline. The cent solution dissolved in physiological saline. remaining thirty mice were given 0.3 mg cycasin/g body weight using a 5 per cent solution of cycasin. Most animals which had been given a large dose of cycasin died of an acute toxicity without neurological disorder within about 2 weeks after the administration of cyeasin. Follow-up observations have been carried out for more than 8 months in thirty-four survivors, but they have not shown any sign of the locomotor difficulties.

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BIOCHEMISTRY

Electron Spin Resonance and Optical Spectra of Haemoglobin-M Hyde Park

HUMAN methaemoglobins, M, form a class of variants in which the haems of two of the polypeptide chains contain ferric ions at all times, while the haems of the other two chains contain iron ions in either the ferric or the ferrous state. Haemoglobin-M Hyde Park (Hb-M HP) belongs to this group of methaemoglobins. It is characterized by a substitution of tyrosine for histidine in position 92 of the β chains. This is the fifth co-ordination position of iron. Only α -chains can be oxygenated, even though the oxygen affinity and Bohr effect are similar to those in Hb-A³.

In order to study the behaviour of the two pairs of chains further, I have analysed the electron spin resonance and optical spectra of Hb-M Hyde Park in two different forms. In one, all four chains are in met form—the ferric form of haemoglobin with a water molecule occupying the sixth co-ordination position of iron. In the other, the abnormal chains are in met, while the normal ones are in oxygenated condition. Iron of the latter is ferrous and an oxygen molecule is present in the sixth position of iron. We shall call these two forms met—met and met—oxy, respectively. When compared with measurements on met Hb-A and oxy Hb-A, information concerning the presence, or lack, of interactions between α and β chains of this variant is obtained.

Hb-M HP was separated from Hb-A by Dr Helen Ranney in the manner previously described (to be published). The preparation contained 80-85 per cent Hb-M HP in the met-oxy state. The transformation to met-met state is achieved by adding 1.3 potassium ferricyanide per haem, followed by dialysis against appropriate buffer.

Electron spin resonance measurements were performed in a Varian V-4500 spectrometer operating at about 9,000 Mc/s with a modulation frequency of 100 kc/s. All the electron spin resonance measurements were obtained in frozen solutions at 77° K. Optical spectra were taken at room temperature in a Spectronic 505 spectrometer.

The absorption signal of electron spin resonance in haemoglobin originates from the iron in the ferric form

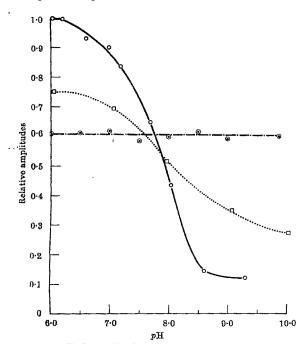


Fig. 1. Amplitudes of the electron spin resonance absorption signal at g=6 as a function of pH. O—O, Met Hb-A; O—·O, met—oxy Hb-M HP; $\square \dots \square$, met—met Hb-M HP.

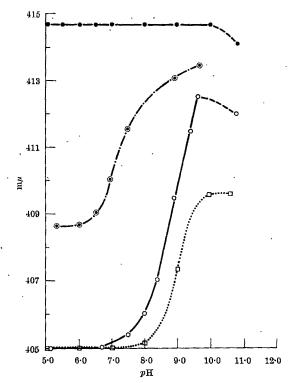


Fig. 2. The wavelength of the Soret band maximum as a function of pH.

One is a function of pH.

The wavelength of the Soret band maximum as a function of pH.

The wavelength of the Soret band maximum as a function of pH.

alone. The signal in methaemoglobin as A is characterized by a spectroscopic splitting factor $g_{\perp}=6$ (ref. 3). The signal from Hb-M HP appears at the same value of g_{\perp} but consists of a split absorption line, in contrast to the single line in met-Hb-A⁴, indicating a lower symmetry of the ligand field at iron in Hb-M HP than in Hb-A. The amplitudes of the absorption lines at different pH are directly proportional to the concentrations of ferric ions because no changes in shape of electron spin resonance lines are observed.

Fig. 1 summarizes the results of the electron spin resonance experiments. The amplitudes of the g=6 signal are plotted as a function of pH for three different samples, met Hb-A, met—met Hb-M HP and met—oxy Hb-M HP. Data are normalized with respect to the amplitude of the signal in Hb-A at pH 6.0, taken arbitrarily to equal one. All data refer to concentrations of $2\cdot 4g$ per cent.

The sharp decrease of the amplitude of electron spin resonance signal with pH in met Hb-A is a well known phenomenon⁵ and is caused by a transition from high to low spin state of ferric ions associated probably with the formation of hydroxide ions at the position occupied by water molecules at lower pH^5 . The pK of this transition occurs at about 7.8.

Only the ferric ions of the abnormal β chains contribute to the electron spin resonance absorption signal in metoxy Hb-M HP. It is apparent that no change in the amplitude of the signal, and therefore no change in the spin state of the ferric ions, occurs between pH 6.0 and 9.7.

The behaviour of met—met Hb-M \overrightarrow{HP} is intermediate between that of met Hb-A and met—oxy Hb-M HP. This strongly suggests that the normal α chains, when in met state, undergo a high to low spin transition similar to Hb-A. The β chains continue in the high spin state, as indicated by the remaining amplitude of the electron spin resonance signal at pH 9 and 10.

For optical spectra, the wavelength of the maxima of the absorption bands near 400 m μ , Soret bands, is plotted as a function of pH in Fig. 2. Met—met Hb-M HP shows a transition to longer wavelengths with increasing pH

which is less pronounced, however, than in met Hb-A. The width of the band, at half absorption maximum, is 30 m μ at pH 7.0 or less, and 33.5, 50 and 75 m μ at pH 8, 9 and 10, respectively. The width of the Soret band in met Hb-A is 30 mu below pH 8.5, and 38 mu at higher pH. The behaviour of met-met Hb-M HP indicates a superposition of two Soret bands, one arising from a chains, identical to met Hb-A, and the other one from β chains with a maximum at about 405 m μ at all pHbetween 5.0 and 10.4.

The behaviour of the Soret band in met-oxy Hb-M HP is intermediate between that of oxy Hb-A and met Hb-A. The width of the band is about 34 to 35 m μ at all pH, as in oxy Hb-A. There is no indication of a splitting of the band. Based on our previous interpretation, a superposition of Soret bands with peaks at 405 and 415 m μ would be expected. This should have resulted in a very broad band, or a split one with a maximum, independent of pH.

Fig. 3a is a plot of the extinction coefficient of the 541 mu band as a function of pH. The transition from a low to a high extinction coefficient in met Hb-A is associated with the transition from high to low spin⁸. Its pK is identical with the pK of the electron spin resonance transition. Met—met Hb-M HP has a less pronounced

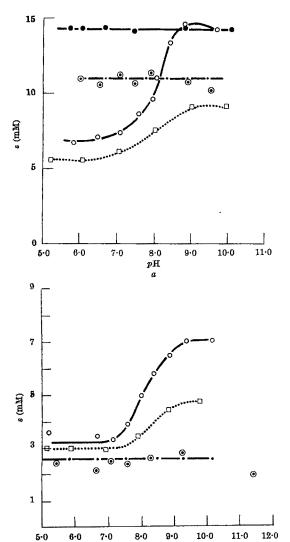


Fig. 3. a, Extinction coefficients at 541 m μ as a function of pH. • Oxy Hb-A; O—O, met Hb-A; O—·O. met-Oxy Hb-M HP; \square ··· \square , met-met Hb-M HP. b, Extinction coefficients at 600 m μ as a function of pH. O—O, Met Hb-A; O—·O, met-oxy Hb-M HP; \square ··· \square , met-met Hb-M HP.

pH

transition at the same pK. No transitions are observed in oxy Hb-A and met-oxy Hb-M HP. These results are entirely consistent with the independent behaviour of α and β chains in this abnormal haemoglobin and with the conservation of the high spin state in β chains at all pH. Identical results were obtained for the 576 mu band² (not shown).

The behaviour of the 600 mµ band is also consistent with our picture (Fig. 3b). The small amplitudes of the 630 mµ band made numerical comparisons difficult.

It can therefore be concluded that: (1) Ferric state of low spin is not formed in the haems of B, abnormal, The substitution of tyrosine for chains of Hb-M HP. histidine in the fifth co-ordination position of iron affects the charge distribution at the position of the sixth ligand, preventing a low spin complex forming at pH as high as 10. (2) There is no indication that the α , normal, chains in Hb-M HP behave any differently than in Hb-A, as far

as their magnetic and optical properties are concerned.

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Synthesis of Macromolecules and Maturation of Starfish Ovocytes

DURING the maturation of amphibian eggs, 'Feulgen positive bodies appear in the cytoplasm^{1,2}: they become visible when the nuclear membrane breaks down and are undoubtedly of nuclear origin. Their appearance is not prevented by treatment of the eggs with actinomycin, puromycin or cycloheximide3, which block the maturation of amphibian eggs^{3,4}. According to Dettlaff⁴, actinomyoin suppresses maturation only during the initial hormone-dependent phase in Rana. In Xenopus we In Xenopus we found³ that all stages of maturation were sensitive to actinomycin.

The present investigation was undertaken in order to answer two questions. First, is an elimination of material which stains with Feulgen detectable during the maturation of starfish ovocytes? Second, is this maturation, which occurs spontaneously when ripe ovocytes are exposed to sea water, blocked by the inhibitors of RNA and protein synthesis?

Ripe ovaries of Asterias ruhens were placed in sea water from the North Sea. In April or May, more than 95 per cent of the ovocytes underwent maturation within 30 min. They were treated continuously with either pure sea water (controls), actinomycin D (20 $\mu/\text{ml.}$), puromycin (50 μg/ml.) or cycloheximide (20 μg/ml.). At various intervals of time, ovocytes were fixed with acetic Zenker and sections were stained in the usual way, with Feulgen and Unna. In two experiments, the incorporation of



Fig. 1.

 $^3H\text{-uridine}$ (6.6 \times 10-3 $\,\mu\mathrm{moles/ml.})$ into ovocytes undergoing maturation has been studied by autoradiography.

Results show some variability because of the heterogeneity of the egg population: for example, at zero time, 33 per cent of the ovocytes already have a degenerate germinal vesicle. As a result of an invasion of the nuclear sap by pre-existing cytoplasmic ribosomes, the region where the nuclear sap is mixed with the cytoplasm stains intensely with pyronine. No incorporation of uridine can be detected by autoradiography in this basophilic region. Such an invasion has been observed by electron microscopy during the maturation of Xenopus ovocytes (results in preparation).

The chromosomes become visible when the germinal vesicle has lost its membrane. In addition, an annular body which stains positively with Feulgen can be seen (Fig. 1). It is easily recognizable by its shape and by the fact that it stains more lightly with Feulgen than the chromosomes. It differs in two respects from the "large chromosome" described by Dalcq^{5,6} in starfish ovocytes undergoing maturation. Thus it can only be seen when maturation proceeds in a hypotonic medium, when it is found attached to the spindle. In contrast, the annular body is found during maturation in normal sea water and it always lies at some distance away from the spindle. In the ovocytes with intact nuclei, phase contrast observation of preparations stained with Feulgen shows that the nucleolus is often formed of two distinct zones; Feulgen positive material is associated with the denser of the two.

No appreciable changes occur during the first 10 min after treatment. After 20 min, more than 90 per cent of the ovocytes have undergone maturation and the chromosomes and the annular body are very visible in the basophilic Ovocytes which have been treated with evtoplasm. puromycin and cycloheximide are identical with the controls. After actinomycin treatment, a paradoxical effect is observed: the segregation of the granular and fibrillar constituents of the nucleolus is facilitated by actinomycin in unripe starfish ovocytes7, but is delayed by the same inhibitor in ovocytes undergoing maturation.

After 40 min, the structure of the cytoplasm has changed and, as described by Dalcq^{6,7}, cytasters are frequent. As in amphibian eggs8, their formation is not prevented by actinomycin, puromycin or cycloheximide. The chromosomes and the annular body migrate towards the cortex; the body always lies outside the spindle, at some distance from the chromosomes. Its frequency is higher in the eggs treated with actinomycin (63 per cent) than in the controls (36 per cent). This difference could be caused either by a condensation of the chromosomes and the annular body, induced by actinomycin (as we observed in amphibians3, or by a delay in the maturation produced by this drug. It is not uncommon to find one or two Feulgen

positive bodies in the central cytoplasm, in the control as well as in the treated eggs. Inhibition of the formation of the spindle and the migration of the chromosomes towards the animal pole is found when protein synthesis is blocked by cycloheximide or puromycin.

After 90 min, the majority of the controls have a spindle which is anchored in the cortex; some of them have already expelled their first polar body. Feulgen positive bodies can sometimes still be found in the centre of the egg but the annular body has usually disappeared. Actinomycin has definitely delayed or blocked meiosis; no polar bodies are seen and the condensed chromosomes are still in metaphase. The annular body is usually present. Maturation in eggs treated with puromycin is completely blocked and the chromosomes, which are often slender, usually remain in the centre of the eggs.

There are many similarities between the maturation of amphibian^{2,3} and starfish ovocytes: in both cases, maturation is blocked by inhibitors of RNA and protein synthesis. We have, however, been unable to demonstrate by autoradiography, in Asterias, the RNA synthesis which occurs in amphibians9. This failure could be a result of poor permeability to the precursors or to loss of radioactive material during the autoradiography procedure. The effects of the various inhibitors on the chromosomes are also the same in the two materials: actinomycin produces a condensation of the chromosomes, while puromycin and cycloheximide tend to induce their despiralization. both cases, Feulgen positive bodies appear in the cytoplasm during maturation and cease to be visible when the first polar body is expelled. There are many more in amphibian than in the starfish ovocytes, where the annular body probably represents a unique equivalent of the many granules found in amphibians1-3. The origin of these Feulgen staining bodies remains enigmatic. If one takes into account the facts that they have probably no centromere (because they are never attached to the spindle) and that they differ greatly in number in Asterias and Xenopus, one can venture the hypothesis that these bodies might correspond to the DNA of the cistrons of the nucleolar organizers, which are no longer needed when the nucleoli break down during maturation. This hypothesis derives from the fact that Xenopus ovocytes have hundreds of nucleoli and Asterias ovocytes only one.

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Subcellular Localization of 5-Hydroxytryptamine and Histamine in Blood Platelets

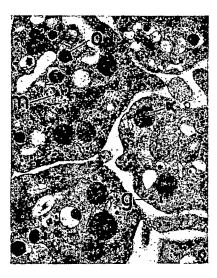
BLOOD platelets of various species contain particular, dense, osmiophilic organelles (dense bodies) which are somewhat smaller (500 to 2200 Å) than the a-granules. By combining electron microscopy and 5-hydroxytryptamine determinations, including experiments with reserpine, we have provided strong evidence that the

osmiophility of the organelles is caused by an accumulation of 5-hydroxytryptamine¹⁻⁴. Experiments with tyramine confirmed this view⁵. Here, we report attempts to isolate the dense osmiophilic bodies of platelets by density gradient centrifugation. Furthermore, the distribution of 5-hydroxytryptamine and histamine in various subcellular platelet fractions, including the dense bodies, was determined.

Blood platelets of rabbits, which contain a large number of dense bodies, were isolated, as previously described, and disrupted by ultrasonication. The incompletely destroyed platelets were removed by differential centrifugation and the remainder was centrifuged in a continuous density gradient (results in preparation for publication). In the various fractions of the homogenate, 5-hydroxytryptamine and histamine were measured by spectrophotofluorimetric procedures, and related to the protein content (determined colorimetrically). Furthermore, three principal fractions were submitted to electron microscopy after fixation with glutaraldehyde-osmium

tetroxide.

The three fractions, examined by electron microscopy, contained (from top to bottom) mitochondria, α-granules



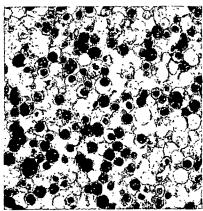


Fig. 1. Electron micrographs of intact isolated blood platelets of rabbits (above) and of isolated dense bodles (bottom layer) obtained by density gradient centrifugation from rabbit platelets (below) (\times 20,000). O, Osmiophilic organelle (dense body); a, a-granule; m, mitochondrium; g, glycogen granules.

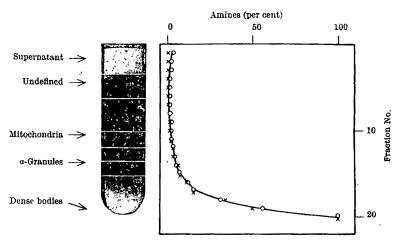


Fig. 2. Distribution of 5-hydroxytryptamine (5HT) and histamine in the various fractions of rabbit platelets. Each fraction, indicated on the abscissa, consists of nine drops of the centrifugate. The bottom layer, attached as a fine film to the tube wall, was dissolved in distilled water. The ordinate indicates the amine content (gmolar 5HT or histamine)µg protein) of each fraction as a percentage of that contained in the bottom layer (5HT or histamine of dense bodies = 100 per cent). Typical experiment. O, 5HT; ×, histamine.

and dense osmiophilic bodies. The fractions of α -granules and mitochondria were contaminated with other particles, including some dense bodies. The bottom layer, which was attached as a fine film to the tube wall, did not contain α -granules or mitochondria; it consisted of virtually 100 per cent vesicle-like structures. These measured 500–2200 Å in diameter and were surrounded by a single membrane. A large number of the vesicles were filled with a dense osmiophilic material, others were incompletely filled or empty. Ultramorphology and size of the organelles were similar to those of the dense bodies described earlier in intact platelets (Fig. 1).

The amount of 5-hydroxytryptamine in the bottom layer, containing the dense osmiophilic bodies, was much greater than in the other fractions (Fig. 2). In relation to the amount of proteins, the bottom layer contained over fifty times more 5-hydroxytryptamine than the total homogenate of platelets. In agreement with previous findings¹⁻⁴, these results therefore indicate a localization of platelet 5-hydroxytryptamine (which reduces osmium tetroxide¹) in particular, dense, osmiophilic organelles which, contrary to other views¹⁰, do not belong to the α -granules. The occurrence of empty organelles in the bottom layer is possibly caused by an artefact, because of the technical procedures such as ultrasonication, ultracentrifugation and fixation. It might, however, also reflect the true conditions in vivo, because in intact rabbit platelets empty and partly filled vesicles seem to be present besides completely filled ones (Fig. 1).

As previously found¹¹, histamine showed the same distribution as 5-hydroxytryptamine in the various

As previously found¹¹, histamine showed the same distribution as 5-hydroxytryptamine in the various fractions (Fig. 2). In the bottom layer which, in the present experiments, contained the highest amount of both amines, the molar ratio of histamine to 5-hydroxytryptamine was 0.42 ± 0.03 (eleven experiments).

The close parallel of the distributions of the two amines indicates that they are located in similar particles which are found chiefly in the bottom layer. It is conceivable that 5-hydroxytryptamine and histamine are present in the same organelles, which would be in contrast to previous assumptions. The possibility remains to be excluded, however, that in the bottom layer the osmiophilic organelles contain only 5-hydroxytryptamine, while the histamine (not reducing osmium tetroxide¹) is localized in the "empty" vesicles.

In conclusion, the high content of 5-hydroxytryptamine in the fraction of isolated dense osmiophilic bodies confirms the existence of particular platelet organelles storing 5-hydroxytryptamine. The results furthermore indicate that in rabbit platelets histamine and 5-hydroxy-

tryptamine are localized in the same or in similar subcellular structures.

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Distribution of Platelet Hexokinase and the Effect of Collagen

THE interaction of platelets with the subendothelial collagen, which is exposed when blood vessels are damaged, is believed to be of fundamental importance in haemostasis.

It has recently been shown that when collagen is added to washed human platelets there is a 30 per cent increase in platelet lactate production (in preparation). This increase occurs immediately, and in the presence of glucose is linear over 60 min. To investigate the mechanism of this increased lactate production, we have studied the effect of incubation of platelets with collagen on the activity of a number of platelet glycolytic enzymes. Particular attention has been given to the study of hexokinase because this enzyme is thought to have a rate-limiting role in glycolysis in platelets² and other tissues³⁻⁸.

Human platelets were obtained from the blood of normal volunteers. The blood was drawn through a 19-gauge non-siliconized needle into a 50 ml. siliconized syringe and rapidly mixed with 2 per cent ethylene diamine tetra-acetate (EDTA), 9 parts of blood to 1 part of EDTA. Blood from each donor was processed separately within 30 min of venepuncture. Platelet-rich plasma was prepared by centrifugation at 200g for 10 min at 4° C; this was then centrifuged at 2,000g for 20 min at 4° C. The supernatant was removed and the platelet pellet washed once in cold EDTA-Tyrode solution and resuspended finally in Krebs-Ringer bicarbonate buffer, pH 7.4, modified by replacing the calcium chloride and magnesium sulphate with 0.9 per cent sodium chloride. The final platelet suspension was free of aggregates and contained approximately 1×10^6 platelets per mm³. Erythrocyte and leucocyte contamination was less than 1 cell per 2,000 platelets. Two ml. aliquots of platelet suspension were incubated with either 0.2 ml. of collagen suspension, prepared as described elsewhere (Loder, Hirsh and de Gruchy, in preparation), or 0.2 ml. buffer for 10 min at 37° C. After cooling the incubation mixture in melting ice, the platelets were disrupted by adding 1 ml. of aqueous digitonin solution (1.5 mg/ml.) and gently

agitating the mixture at 4° C for 1 h. To obtain further disruption the suspension was then frozen and thawed three times in methanol at -70° C. It was found that phosphofructokinase was largely inactivated by the freezing and thawing procedure; with assays of this enzyme this step was therefore omitted. Enzyme assays were performed on either the whole cell lysate (total activity), prepared as described previously, or on the supernatant fraction (soluble activity), prepared by centrifugation of the whole lysate at 30,000g at 4° C for 20 min. The solutions for assay were kept at 0° C at all times and assays were performed within 3 h of disrupting the platelets. No significant loss of enzyme activity was observed over this period. All enzyme assays were carried out spectrophotometrically at 25° C using systems linked to the oxidation or reduction of pyridine nucleotides under conditions where the enzyme to be measured was ratelimiting. Optical measurements, all at 340 m μ , were made with a Gilford model 2000 multiple sample absorbance spectrophotometer or a Perkin Elmer UV137 with Leeds Northrup linear absorbance recorder. Assays were performed in a total volume of 2.5 ml. in a cuvette of 1 cm path-length. Enzyme activities were calculated from the change in optical density over a period during which the reaction rate was linear and expressed as µmoles of substrate utilized per min per 1011 platelets. The methods used were basically as previously described for red cell glycolytic enzymes, with minor modifications. The final concentrations of reagents in the assay systems are given

Hexokinase. Triethanolamine-hydrochloric acid buffer, pH 7·5 (TRAP), 50 mmolar, EDTA 5 mmolar, magnesium chloride 8·0 mmolar, NADP 0·5 mmolar, glucose 2 mmolar, sodium fluoride 2 mmolar, glucose-6-phosphate dehydrogenase 0·003 mg, lysate 0·1 ml., ATP 1·5 mmolar. Phosphohexose isomerase. TRAP 50 mmolar, EDTA

Phosphohexose isomerase. TRAP 50 mmolar, EDTA 5 mmolar, magnesium chloride 8.0 mmolar, NADP 0.5 mmolar, glucose-6-phosphate dehydrogenase 0.007 mg, lysate 0.02 ml., fructose-6-phosphate 4.0 mmolar.

Phosphofructokinase. TRÅP 50 mmolar, EDTA 5 mmolar, magnesium sulphate 5 mmolar, NADH 0·26 mmolar, ATP 0·4 mmolar, aldolase 0·1 mg, triose phosphate isomerase 0·0013 mg, α-glycerophosphate dehydrogenase 0·006 mg, lysate 0·1 ml., fructose-6-phosphate 4 mmolar.

Aldolase. TRAP 50 mmolar, EDTA 5 mmolar, NADH 0.26 mmolar, α -glycerophosphate dehydrogenase 0.006 mg. triose phosphate isomerase 0.0013 mg, lysate 0.100 ml., fructose-1,6-diphosphate 4.0 mmolar.

Enclase. TRAP 50 mmolar, EDTA 5 mmolar, NADH 0-26 mmolar, potassium chloride 75 mmolar, magnesium sulphate 7-5 mmolar, ADP 0-2 mmolar, pyruvate kinase 0-013 mg, lactic dehydrogenase 0-025 mg, lysate 0-02 ml.. 2-phosphoglycerate 0-6 mmolar.

Lactic dehydrogenase. TRAP 50 mmolar, EDTA 5 mmolar, NADH 0.2 mmolar, lysate 0.05 ml., pyruvate 3.8 mmolar

The results of enzyme assays performed on the supernatant fraction are shown in Table 1. There was a significant increase in hexokinase activity after platelets were exposed to collagen (t=7.36, P<0.01), the mean increase being 31 per cent. No such increase was observed with the five other enzymes examined.

Assays performed before and after centrifugation of the whole cell lysate revealed that activity of hexokinase, unlike that of the other glycolytic enzymes examined, was considerably greater in the preparation before centrifugation (Table 2). Furthermore, the difference in activity could be accounted for by assay of the washed precipitate. This suggests that some of the platelet hexokinase is attached to sedimentable cellular components.

The effect of collagen on total and soluble hexokinase activity is shown in Table 3. It is apparent that there is an increase in soluble activity but no corresponding increase in total enzyme activity. This suggests that collagen

causes release into the supernatant fraction of some of the hexokinase which is bound to sedimentable cellular com-

The existence of particle-bound hexokinase has been demonstrated in a number of other tissues¹⁰⁻¹³, and more recently mitochondrial hexokinase has been reported14-17. The exposure of platelets to collagen results in marked ultrastructural changes which are associated with disappearance of mitochondria and other cytoplasmic granules¹⁸. These changes are accompanied by a sudden increase in lactate production (Loder, Hirsh and de Gruchy, in preparation). The results presented here show that the interaction between platelets and collagen is also accompanied by an increase of about 30 per cent in soluble hexokinase activity in the platelet lysate. It seems possible therefore that collagen causes a release of particle-bound hexokinase into the cytoplasm and that this redistribution of enzyme activity contributes to the increased lactate production.

Table 1. ENZYME ACTIVITIES IN THE SUPERNATANT FRACTION BEFORE AND AFTER EXPOSURE OF PLATELETS TO COLLAGEN

AFTER EXPUSURE	OF PLATELETS TO CO	LLAGEN
Enzyme	Activity of 30,000 Platelets without collagen	y supernatant Platelets with collagen
Hexokinase	9.5 7.4 7.0 9.1 8.4	12·3 10·1 9·1 13·0 10·2
Phosphohexose isomerase	Mean 8-3 107 99 89	10-9 105 100 89
Phosphofructokinase	Mean 98 22 17 13 17	98 22 19 13 14
Aldolase .	Mean 17 8.3 7.5 5.2	17 8·2 8·0 5·3
Enolase	Mean 7.0 28 42	7·2 26 39
Lactic dehydrogenase	Mean 35 209 150 193 197 196 187 189	33 211 152 183 194 195 187
	Mean 189	188

Activities of six glycolytic enzymes estimated in the 30,000g supernatant fraction of platelet lysates after incubation in the presence and absence of collagen. Values are expressed as μ moles of substrate utilized per minute per 1011 state. collagen. Val. 1011 platelets.

LADIC Z. TOTAL AN	D SOPORPE HEXOR	LINASE ACTIVITIES OF	LYSED PLATELET
Experiment	Lysate (total activity)	Supernatant (soluble activity)	Per cent in supernatant
1	11.7	9.6	82
2	13.3	9.5	71
3	8-4	6.8	81
4	12.5	7.4	59
5	10.2	7.0	69
Mean values + S.D.	11.2 + 1.0	8-1 + 1-4	79 ± 0

Values are expressed as μ moles of substrate utilized per minute per 10^{11} platelets. There is a significant difference between total and soluble hexokinase activities ($t=6.01,\,P<0.01$).

Table 3. EFFECT OF COLLAGEN ON TOTAL AND SOLUBLE HEXOKINASE ACTIVITY

	Platelets w	ithout collagen	Platelets	with collagen
Experiment	Lysate	Supernatant	Lysate	Supernatant
1	12.5	7.4	12.4	10.1
2	10.2	7∙0	10.3	9.1
3	13.3	9.5	13.2	12.3
Mean values	12.0	8-0	12.0	10-5

Values are expressed as μ moles of substrate utilized per minute per 10^{11} platelets.

There is no significant difference in total enzyme activities of control platelets and platelets incubated with collagen but a significant difference in soluble activities (t=11.4,~P<0.01). The mean percentage increase in soluble activity after incubation with collagen is 31 per cent.

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Involvement of a Membrane Potential in the Synthesis of ATP by Mitochondria

Cockerell, Harris and Pressman¹ recently reported that when mitochondria which had been poisoned with rotenone were suspended in a potassium-free sucrose medium buffered with tris phosphate, the addition of valino-mycin led to a rapid loss of potassium, a rapid but smaller uptake of hydrogen ions, and a simultaneous net synthesis of ATP. This synthesis of ATP, apparently at the expense of a downhill movement of potassium ions, is extremely interesting, but it may be interpreted in more than one way. Cockerell et al. compared the ATP synthesis in their experiments with the ATP synthesis which seems to accompany reversal of the "sodium pump" in the red cell membrane², and suggest that the synthesis in mitochondria is similarly brought about by the reversal of an ATP-driven cation pump. The outward movement of potassium ions is, they suppose, directly coupled to ATP synthesis. This explanation may be correct, but it does require that a significant part of the efflux of potassium should take place through the pump pathway, and therefore—given that only the inner mitochondrial membrane serves as an effective barrier to small ions-valinomycin should act specifically at the pump sites, for example, by facilitating the access of potassium ions to those sites.

An alternative hypothesis is that valinomycin merely confers on the inner mitochondrial membrane the high permeability to potassium ions which it is known to confer on lipid bilayers3,4, lipid micelles5 and on red cell membranes6.7, so that in the presence of valinomycin the potassium gradient sets up an electrical potential. By driving hydrogen ions inwards, or anions outwards, through a mechanism which coupled ion movements to phosphorylation⁸, this electrical potential could serve as the immediate source of energy for ATP synthesis. If the potassium concentration within the mitochondrial inner membrane is 145 mmolar, and the external potassium concentration is 0.4 mmolar, the potassium equilibrium potential is 149 mV. Not enough is known about the permeability of the inner mitochondrial membrane, either to potassium or to other ions, to predict the potential which would in fact be reached after treatment with valinomycin, but it could easily be 100 mV. At this level, and assuming that there was sufficient buffering to prevent the development of a substantial pH difference across the membrane, the inward movement of 4 g equiv. of hydrogen ions-or the outward movement of 4 g equiv. of hydroxyl ionscould make available about 9,000 calories of free energy.

The proposed hypothesis requires that the inward current driven by the electrical potential should be chiefly carried by ions traversing a pathway coupled to phosphorylation, rather than by ions penetrating the valinomycin channels. In experiments on lipid bilayers treated with valinomycin, Lev and Buzhinsky's have shown that when potassium and hydrogen ions are present in equal, low concentrations it is the hydrogen ions which penetrate more readily. In the mitochondrial experiments, however, the difference of perhaps six orders of magnitude between the concentration of potassium ions and the concentration of hydrogen ions makes it reasonable to suggest that the hydrogen flux through the valinomycin channels could be small compared with the potassium flux. The flux of hydrogen ions through the valinomycin channels might also be limited by the curious interaction between less and more-penetrating ions noticed by Mueller and Rudin in experiments on lipid bilayers, although the behaviour of hydrogen ions was not investigated in those experi-

Cockerell et al. point out that exit of potassium and entry of hydrogen ions are not necessarily associated with the formation of ATP because no ATP synthesis accompanies the large loss of potassium and gain of hydrogen ions which take place when nigericin is added to suspensions of mitochondria in conditions similar to those used in the valinomycin experiments. The way in which nigericin acts to promote this exchange of cations is not understood, but nigericin is a monobasic acid. If part of the molecule behaves as a negatively charged cation-carrying shuttle unable to penetrate the membrane in the charged form (compare the role of guaiacol in Osterhout's classical model¹⁰), the catalysed exchange of potassium and hydrogen ions would be strictly 1:1 and would not lead to a transfer of charge. An exchange of this kind could not generate an electrical potential capable of maintaining an inward current, and therefore, by hypothesis, could not lead to ATP synthesis. It follows that the absence of ATP synthesis when potassium: hydrogen exchange is brought about by nigericin cannot be regarded as evidence that the action of valinomycin is at specific sites.

Interpreted in this way, the experiments of Cockerell et al. provide the first evidence that a membrane potential might be involved in the synthesis of ATP by mitochondria.

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Chemical Radioprotection of mRNA in a Nirenberg Cell Free System

In 1964, Wacker and Chandra¹ showed that the incorporation of polyU-directed 14C-phenylalanine into the acid soluble fraction of a Nirenberg cell free system² was inactivated by gamma irradiation. Atter a dose of about 80 krads, inactivation reached the 50 per cent level. Experiments of Eckert³ provided data which showed a steeper dose/response curve for irradiated polyU.

Following our recent investigation on radiosensitivity of mRNA synthesis4-6, we decided to study the behaviour of mRNA with respect to the possibility of its chemical radioprotection when subjected to ionizing radiation in a Nirenberg cell free system. In non-dividing yeast cells⁵, doses of X-rays of 120 krads do not cause more than 48 per cent suppression of mRNA synthesis and for this reason they are not suitable for radioprotection experiments. As the data presented in this communication indicate, however, Nirenberg's cell free system is a very suitable model for radioprotection investigation at the molecular level. In Fig. 1 is shown the effect of chemical radioprotection of polyU when 0.015 per cent concentration of cysteamine hydrochloride is used. Further dilution leads to a decrease in radioprotective activity: 0.0015 per cent cysteamine hydrochloride in the system is almost completely ineffective and the corresponding dose/response curve goes down and approaches the control curve. Several experiments were carried out using 0.15 per cent cysteamine hydrochloride, but a marked suppression of up to 70 per cent was found in this case in non-irradiated samples.

The strong dependence of radioprotective activity on the concentration of cysteamine hydrochloride in the range 0.015-0.0015 per cent seems to support deoxygenation of the irradiated polyU-solutions caused by cysteamine. This, however, is not in accord with Eckert's observation3 that a decrease of the oxygen content in polyU solutions during irradiation lowers the radioresistance of polyU, as checked by 14C-polyphenyl-alanine synthesis in a cell free system. This suggests that a specific cysteamine" radioprotection cannot be excluded.

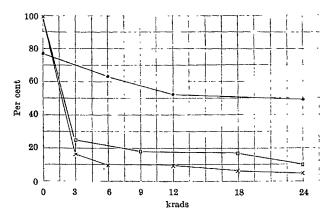


Fig. 1. Cysteamine radioprotection in irradiated polyU solutions. E. coli B was cultivated on an aerated glucose-bullion medium. The incubated S-30, cell free fraction (Inc. S-30) was prepared according to the classical technique of Nirenberg*. Samples of 0-5 ml., each containing 3,000 µg of Inc. S-30, were incubated for 40 min at 35° C after addition of 0-004 µmoles of ¹*C-phenylalanine (specific activity 121 mc./mmole). Water solutions of polyU (100 µg/ml.) were irradiated with cobalt-60 gamma rays (4,000 g equiv. of radiation) at a dose rate of 600 r./nin (ferrous sulphate dosimeter); the temperature during irradiation was maintained at 4° C. Samples were treated using a filter disk technique according to a modification given to us in personal communication by A. Wacker and P. Chandra: 0-1 ml. from each test tube was transferred onto 3 cm filter disks and was then immersed as follows: 2 h in 10 per cent TCA (5° C). 20 min in 5 per cent TCA (5° C), 20 min in 5 per cent TCA (5° C), 20 min in 5 per cent TCA (5° C), 20 min in 5 per cent TCA (5° C) and twice in ethanol/ether (1:1). Radiometric determinations were made using a scintillation mixture consisting of 0-4 per cent diphenyloxydiazol, 0-01 per cent POPOP in xylene. Each point on the curves represents an average of three independent samples. ×, PolyU; \(\preceq\), polyU and 0-015 per cent cysteamine hydrochloride;

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Steric Course of the Succinic Dehydrogenase Catalysed Exchange of Hydrogen between Succinate and Water

Succinic dehydrogenase (succinate: (acceptor) oxidoreductase E.C. 1.3.99.1) catalyses the oxidation of succinate by various electron acceptors to fumarate. Although the mechanism of the reaction is not yet understood, it has been shown¹ that the overall process involves removal of one H_R- and one H_S-atom² from adjacent carbon atoms of the substrate (Fig. 1a); this corresponds to a trans elimination from the antiperiplanar conformation of succinate, as in Fig. 1b.

In anaerobic conditions, the same enzyme is known to catalyse the exchange of hydrogen atoms between water and the methylene groups of succinate3, with fumarate acting as an accelerator of the process. The stereochemistry of this exchange has been the object of conflicting reports. Using Keilin and Hartree particle bound enzyme preparations, one group of workers claimed that incubation of succinate in deuterium oxide leads to a preponderance of monodeuterated molecules possessing (S)-configuration, as inferred from the comparison of the positive plain optical rotatory dispersion (ORD) curve of the recovered succinic acid with that of an authentic In similar conditions, fast exchange with sample⁶. the medium was also reported for the HR-atom* of the methylene group in (S)-chlorosuccinate, Fig. 1c, although in a later paper, it was stated that the methine hydrogen of this substrate exchanges with the medium faster than HR on the adjacent carbon atom. More recently a second group, working with the same enzyme preparation, reporteds the almost exclusive formation of monodeuterated succinate molecules and maintained on the basis of ORD measurements that the exchanged succinic acid is devoid of optical activity, thus implying lack of stereospecificity for the exchange reaction.

Preliminary experiments carried out by the late Mr U. Steinemann in Zurich (unpublished results) led us

* Note that, notwith standing the different indices, Hz of Fig. 1c is sterically equivalent to Hz of Fig. 1a.

to reinvestigate this problem. Our results differ considerably from those previously reported.

Anaerobic incubations of succinate in deuterium oxide were carried out in the presence of an enzyme preparation purified by the method of DerVartanian and Veeger*. Different batches of the enzyme showed specific activities between 200 and 240 U/mg and were almost free of fumarase activity. In order to follow the time dependence of the exchange reaction, samples were withdrawn at different time intervals and the reaction was stopped in each case by heating to 100° for 5 min. After removal of the denatured protein by centrifugation, the reaction mixtures were chromatographed on Dowex 1 (formate form) according to Busch et al. 10. Clean separation of succinic, malic (whenever present) and fumaric acid was achieved. The samples of succinic acid were further purified by repeated crystallization from water.

Determinations of isotopes were carried out with a Hitachi-Perkin Elmer RMU-6A mass spectrometer at low voltage. Succinic acid was admitted to the normal inlet system of the instrument at 180°. The amount of the different deuterated species was evaluated on the basis of the ion peak at m/e = 56 arising by the loss of carbon dioxide from the thermally preformed anhydride. Optical rotations were measured with a Bellingham and Stanley-Bendix 'Polarmatic 62' spectropolarimeter, using a cell of 1 cm path length and aqueous solutions containing 20-50 mg/ml. of succinic acids. The ORD curves of authentic (-)-(R)-succinic acid (and, as a check, of unlabelled succinic acid) were obtained at the same time.

Data from a typical experiment are recorded in Table 1. All the samples with a substantial deuterium content showed a negative plain ORD curve, similar to the one which is characteristic for (-)-(R)-succinic acid- d_1 (ref. 6). This is true not only for the five samples of the table but also for twenty-three additional samples obtained from eight further incubations. It is evident from the data of the table that relevant amounts of d2-molecules are formed beside the monodeuterated ones even at an early stage of the reaction. The initially observed ratio $(d_1:d_2 \equiv 2:1)$ rules out the possibility that the early appearance of d2molecules is caused by a further exchange of preformed monodeuterated molecules. If reversible trans dehydrogenation of succinate is operative in the formation of these d₂-molecules, the species produced would be (optically inactive) meso-succinic acid-d₂. This is supported by the observed satisfactory agreement between di-content and percentage of optical activity for samples 2F and 3F. Comparison of sample 3F with an artificial mixture of succinic acids (22 per cent d1, 78 per cent d0) by infrared spectroscopy¹¹ revealed that the d_2 -molecules in 3F were indeed preponderantly (but not exclusively) of the meso-The consistently and remarkably high value for the optical rotation of samples which have been allowed to interact with the enzyme for a longer time (for example, 5F and 6F) requires that, as the exchange reaction proceeds, a relevant amount of d1-molecules is converted to (R,R)-succinate-d2 through the exchange of the remaining labile HR-atom. By the same exchange, optically active (-)-(R)-succinate- d_3 will accumulate at the expense of (optically inactive) meso-d2-molecules.

We have also observed a definite preponderance of (S)deuterated succinic acids when succinate-d4 was incubated

				Table :	1			
	Reaction							Per cent
Exp.	time	Per	cent of	deuterat	ed speci	es	[a] 20°	of opt.
No.	(h)	ď.	$\mathbf{d_1}$	\mathbf{d}_2	d _a	\mathbf{d}_{4}	1^{α_1} 244 m μ	act.*
1F	0	94	4	2	-	-		
2F	1	85	9	5	1	0	1·96°	8.6
3F	2	60	21.8	14.2	3	1	4·65°	28
4F	4	38	28.8	23.2	7.2	2.8	6·98°	84
5F	8.25	14.2	28.6	32.5	18.3	6.4	16·3°	81
6F	12	5	22.5	37	27	8.5	22·6°	108

The reaction mixture contained 300 moles disodium succinate, 100 moles disodium fumerate, 270 moles disodium hydrogen phosphate, 30 moles potassium hydrogen phosphate, 5 mg succinic dehydrogenase.

Volume 3.2 ml. in deuterium oxide; temperature 25° C, pD = 7.8; anaerobic conditions.

* Mean of the values at eight-fourteen different wavelengths as compared with the ORD curve of optically pure (-)-(R) succinic acid-d₁.

anaerobically with the enzyme in water; though here the isotopic species which increase most rapidly at first are succinate-d2 and succinate-d0.

Regardless of the precise mechanism of the reaction, it is clear from our results that the enantioscopic H-atoms of the substrate must have different fates and that the HRatoms are more prone to exchange with the medium than their Hs counterparts. This finding contrasts with the claim of Hüfner et al.12 to have demonstrated that all four hydrogen atoms of succinate are equivalent in the exchange

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Bioassay of Captan by Zebrafish Larvae

CAPTAN (N-trichloromethylthio-4-cyclohexene-1,2-dicarboxyimide) is an agricultural fungicide of low mammalian toxicity, and its residues* in agricultural products are considered to offer less risk than most pesticides. A recent report¹ draws attention to the mutagenic and teratogenic properties of captan, which has been observed to increase the mutation rate in bacteria (Escherichia coli), to inhibit mitotic division in the cell cultures and to induce physical abnormalities in chicks treated in the embryonic stage.

We are developing a general bioassay method for pesticides using zebrafish larvae. During this investigation we noted a particularly unusual response by captan. Although captan can be detected either chemically or biologically against fungal conidia2, the zebrafish larvae test shows promise in its convenience, rapid reaction, sensitivity and reproducibility.

The zebrafish, Brachydanio rerio (Hamilton-Buchanan), is an ornamental aquaria fish, 1.5 to 2 in. long, with length-

wise zebra stripes. This oviparous species can be bred in the laboratory throughout the year, providing large numbers of eggs which at room temperature require only 3 days to hatch. An individual fish produces 150-600 eggs at an interval of 10-15 days and a stock of 150 mature fish has been found sufficient to obtain enough eggs and larvae for tests with pesticides. The stock is kept in two aquaria with a capacity of 15 gallons, which are maintained at 26° C±1° C and with a filter outside the aquaria for bubbling clean air through the water. The fish are fed on dry commercial fish food and live mosquito larvae. One feeding in the morning is usually sufficient, but three feedings a day may be required if the fish are being used to produce large numbers of eggs.

Eggs are collected in a separate aquarium which is maintained in the same conditions of temperature and aeration as the stock aquaria are. As the species breeds at daybreak, the breeding arrangements are made the preceding evening. After the last feeding of the day, a number of gravid (plump) female fish with twice as many males are isolated in special breeding traps (with 10-mesh screen floor) which are hooked on the walls of the breeding aquarium. Underneath the traps, on the aquarium floor, finger bowls are provided for the collection of eggs. Early in the morning the fish drop their eggs within approximately 30 min. The bowls are taken out. the eggs are separated from faecal matter and debris. placed in Petri dishes with distilled water and allowed to develop. The embryos develop rapidly and larvae are produced in 3 days. The larvae hatch out with considerable embryonic yolk and need no feeding or special care before being used when they are 4 days old.

The larvae were unaffected by ethanol or acctone at concentrations up to 1 per cent (v/v). Standard solutions of recrystallized captan (m.p. 173° C) were prepared in acetone and 0.2 ml. of these solutions were diluted to give a final volume of 50 ml. The tests were made in glass dishes 1.5 in. high and 3 in. in diameter with 50-70 four day old larvae. There was no mortality in the 0.4 per cent acetone control used for the tests. Captan exerted a rapid and drastic effect on the larvae. At a concentration of 1 p.p.m. it strongly excited the larvae after exposure for 5 min and this excitation continued for another 30 min: the larvae became severely affected, lost the power of movement and went into a state of coma. After a further 10 min the larvae started dying and were all dead in the following 50 min (Fig. 1). The death of

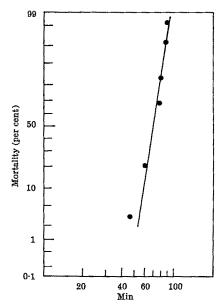


Fig. 1. Response of *Brachydanio rerio* larvae to 1 p.p.m. captan within 90 min.

^{*} Captan residue levels permissible in seventy-three fruits and vegetables in Canada, 40 p.p.m.

the larvae from captan poisoning was invariably associated with an observable head injury (Fig. 2) in which the eye balls, while still retaining connexions with the optic tissues, were blown out of the sockets and the head was ruptured into lateral halves to give a bicephalous appearance. This dramatic head injury resulted in appearance. immediate death and was used as an index in mortality

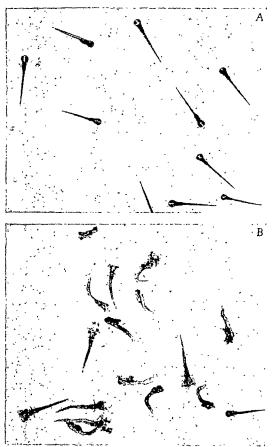


Fig. 2. Larvae of *Brachydanio rerio* before (A) and after (B) exposure to 1 p.p.m. captan for 75 min.

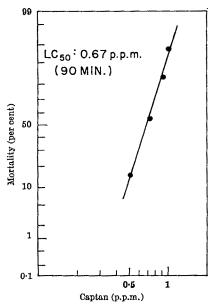


Fig. 3. Dosage-mortality response of *Brachydanio rerio* larvae to captan (mortality counts after exposure for 90 min).

counts for developing the larval response curves shown in Figs. 1 and 3.

Ninety-eight per cent of larvae died in 90 min at a concentration of 1 p.p.m. of captan, and a standard dosage-mortality curve (Fig. 3) can be prepared in this period of time for a rapid assay of microgram quantities of captan. Both the rapid kill and the head injury seem to be specific to captan, because these effects were not observed in similar tests with other pesticides including DDT, dieldrin, malathion, parathion, carbaryl, 2,4-D and warfarin.

Captan is not reported to be toxic to fish (personal communication, J. R. Thomas), and the unusual sensitivity of zebrafish larvae is probably a case of selective toxicity of captan.

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Hydroxyproline-O-glycosidic Linkage of the Plant Cell Wall Glycoprotein Extensin

PRIMARY cell walls contain a protein, extensin, which is rich in trans 4-L-hydroxyproline¹⁻³. I suggested earlier that this protein could cross link wall polysaccharides4; if some of these cross linkages were labile, it would provide a chemical basis for changes in cell wall plasticity which are necessary for extension of plant cells. The recent isolation of several glycopeptides, rich in hydroxyproline from enzymatic digests of tomato cell walls, confirmed the inference of a covalent carbohydrate-protein linkage in The chemical composition and properties of these glycopeptides also led me to the preliminary conclusion that attachment of the carbohydrate is by a glycosidic link through the hydroxyl group of hydroxy-This rather unexpected possibility receives additional support from results presented in this communication, describing the isolation of hydroxyproline-Oglycosides from partial alkaline hydrolysates of tomato cell walls. These cell wall preparations account for more than 95 per cent of the hydroxyproline of the cells³. Most of this hydroxyproline (about 70 per cent) can be released as hydroxyproline glycosides. The method is based on the fact that glycosidic linkages are usually stable to those alkaline conditions which lead to rapid hydrolysis of peptide bonds.

Suspension cultures of the species shown in Table 1 were grown and walls prepared as previously described3,7. Dry walls from cell suspensions of tomato (Lycopersicon esculentum Mill.) were mixed (10 mg/ml.) with a saturated solution of barium hydroxide (0.43 normal) and were heated for 8 h at 90° C in a stirred stoppered flask. The contents of the flask were cooled, neutralized with 1 normal sulphuric acid, and centrifuged for 15 min (or until clear) at 1,000 r.p.m. in a bench centrifuge. The supernatant fraction was evaporated to dryness, taken up in 5 ml. of 0.1 molar acetic acid and fractionated on a $120 \text{ cm} \times 3.8 \text{ cm}$ 'Sephadex G-25' column (void volume 360 ml.). Samples from each tube were assayed for free hydroxyproline by oxidation with sodium hypobromite and subsequent reaction with Ehrlich's reagent8. Peptide-bound hydroxy-

proline does not react.

Fig. 1 shows two peaks of non-peptide bound hydroxyproline. Peak II has an elution volume (762 ml.) similar to that of other monomers and is free hydroxyproline.

Table 1. Electrophoretic mobilities of hydroxyproline glycosides from gell wall preparations of gell suspension gultures of tomato and other plants

			RHypro		
Species	1	2	3	4	5
Lycopersicon esculentum, Mill. Acer pseudoplatanus, L. Nicotiana tabacum, L. Apium graveolens, L. Çentaurea cyanus, L.	0·47 0·47 0·48 0·50 0·51	0.52 0.52 0.53 0.54 0.55 0.55	0.57 0.57 0.58 0.60 0.60 0.61	0.66 0.66 0.67 0.68 0.67 0.68	0.70 0.70 0.71 0.72 0.71 0.71
Rosa sp. Lactuca sativa, L. Linum perenne, L. Oryza sativa, L. Ginkgo biloba, L.	0·51 0·52 0·50 0·48	0.55 0.56 0.55 0.54 0.52	0-62 0-60 0-61 0-57	0.68 0.68 0.65	0·71 0·73 0·72 0·72
Solanum tuberosum, L.* Pisum sativum, L. (root) Pisum sativum, L. (epicotyl)	0.48 0.50 0.49	0·52 0·55 0·55	0.58 0.61 0.60	0-66 0-68 0-68	0·71 0·73 0·78

Cell walls were isolated from cultured material unless otherwise indicated, After hydrolysis for 8 h in 0.43 normal barium hydroxide at 90° C, portions of the neutralized hydrolysate were directly subjected to paper electrophoresis at pH 1.9 for 2 h at 155 V/cm. In these conditions free hydroxyproline migrated 40-45 cm towards the cathode. Detection of the spots was by the method described. The fastest moving compounds (columns 4 and 5) are minor commonate. * Solanum tuberosum also gave a large spot with Rhypro = 0.44.

Peak I contains hydroxyproline which is not peptide bound because it reacts with Ehrlich's reagent as free hydroxyproline, while its elution volume (650 ml.) indicates a molecular weight considerably greater than that of free hydroxyproline. Peak I subjected to paper electrophoresis at pH 1.9, followed by the usual test for hydroxyproline (isatin followed by Ehrlich's reagent9), gave three main and two minor spots. Other species gave similar compounds (Table 1). Chromatography of the G-25 peak I on the cation exchange resin 'Aminex' (Fig. 2) separated the three main hydroxyproline compounds. Their relative migration rates through the 'Aminex' column were inversely related to their relative migration rates on electrophoresis at pH 1.9. For convenience, we designate the most slowly moving electrophoretic component of the series as hypro-X1 (peak I on the 'Aminex' column shown in Fig. 2). Further analysis by acid hydrolysis and paper chromatography showed that the hypro-X compounds also contain arabinose (compare Fig. 2). No other sugars or amino-acids were detected. Hydroxyproline and arabinose estimations (Table 2) indicate an empirical formula for hypro-X1 as hypro₁ arabinose₄, and hypro-X3 as hypro₁ arabinose₃. Hypro-X2 seems to be a mixture (but not of hypro-X1 and X2): thus, first, it contains roughly equal amounts of cis and trans 4-hydroxyproline;

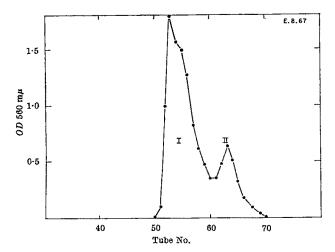


Fig. 1. Fractionation of the partial alkaline hydrolysate of primary cell walls of tomato cell suspension cultures. Dry tomato cell walls (2·3 g) obtained from a suspension culture were treated with 230 ml. of 0·43 normal barlum hydroxide for 8 h at 90° C. After neutralization with sulphuric acid, centrifugation and evaporation as described in the text, the soluble material was fractionated on a 'Sephadex C-25' column. Fractions of 12 ml. were collected of which 0·1 ml. samples were assayed for hydroxyproline. These hydrolysis conditions release all the wall hydroxyproline, 80-90 per cent of which then reacts as non-peptide bound hydroxyproline.

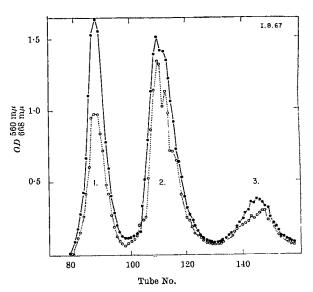


Fig. 2. Further 'Aminex' fractionation of a partial alkaline hydrolysate from tomato cell walls. The 'Sephadex G-25' peak I was pooled, evaporated, taken up in 2 ml. of buffer, pH 1·9 (8·7 per cent acetic, 2·5 per cent formic acid v/v), and placed on a 25 × 1 cm 'Aminex' (200-325 mesh) column in the H + form. The column was eluted with buffer pH 1·9 and 4 ml. fractions were collected. These were assayed for hydroxyproline (○ · · · · ○, method in text) and for arabinose (◆ — ◆) by heating the sample for 90 min at 90° C with 8 normal hydrochloric acid containing 0·1 per cent orcinol and 0·1 per cent hydrated ferric chloride, and then reading the OD at 688 mµ.

second, the hydroxyproline arabinose molar ratio falls between that of hypro-X1 and hypro-X3; and third, the 'Aminex' separation shows a consistently skewed hypro-X2 peak (Fig. 2). (The cis 4-hydroxyproline arises by racemization during the alkaline hydrolysis, as this isomer cannot be detected in acid hydrolysates of cell walls.)

Table 2. HYDROXYPROLINE GLYCOSIDES OBTAINED FROM TOMATO CELL WALLS: MOLAR RATIOS OF ARABINOSE/HYDROXYPROLINE

	Found	Theoretical	Hypro epimer
Hypro-X1	3.7	4	Trans
Hypro-X2	3.4	?	Cis and trans
Hypro-X3	2.7	3	Mainly cis

The following data indicate the most likely point of attachment between the arabinose and hydroxyproline: (1) Alkali stability rules out an ester linkage and acid lability makes an ether linkage unlikely. (2) After paper electrophoresis the hypro-X series did not react with an alkaline silver nitrate reagent¹⁰. Thus the reducing group of arabinose is blocked, indicating a glycosidic link between arabinose and hydroxyproline. (3) The secondary amino group of the hydroxyproline is free (for example, it can be cyanoethylated by the method of Fletcher¹¹ and therefore cannot be involved in the glycosidic linkage. (4) The hydroxyproline carboxyl group is free, as estimated from the neutral behaviour of hypro-X on electrophoresis at pH 6.5, and from the observation that hydroxyproline in peptide linkage (including dipeptides such as glycyl hydroxyproline and hydroxyprolyl glycine), unlike free hydroxyproline, cannot be oxidized to products which torm chromogens with Ehrlich's reagent.

In sum total, these data indicate that in the higher plants so far examined (Table 1) an arabinose oligosaccharide is attached as a substituent on the hydroxyl group of hydroxyproline, that is, carbon 1 of arabinose is linked glycosidically to carbon 4 of hydroxyproline. Thus a picture of extensin emerges in the form of a polypeptide backbone where most of, if not all, the numerous hydroxyproline residues (about 30 per cent of all the amino-acid residues in tomato cell walls) are involved in a new and hitherto unsuspected carbohydrate-protein linkage. The short arabinose oligosaccharides may serve as attachment regions for other wall polysaccharides. A short sequence of extensin, for example, consisting of two hydroxyproline residues and two or three other amino-acid residues, could be regarded as the cross link between two polysaccharide chains. Thus a small amount of extensin is potentially capable of cross linking a hugely disproportionate amount of wall polysaccharide. In this way even the minute amounts of wall hydroxyproline, characteristic of some species3, could play an important part in determining the properties of the primary cell wall.

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Activation of Glucose-6-phosphatase of Rat Liver at High pH: Differential Response of Basal and Induced Activities

THE significance of increases in levels of glucose-6-phosphatase following induction of diabetes or treatment with glucocorticoids has been a matter of some controversy1,2. A contributory factor to this controversy has been that the actual level of enzyme activity determined in a particular sample is very dependent on the chosen conditions of assay. Thus, citrate, a buffer commonly used in earlier studies, is now known to be an inhibitor of the enzyme, the degree of inhibition depending markedly on the pH of the assay3. Pretreatment of the enzyme with detergents activates the enzyme but also labilizes it, while treatment of the enzyme at high pH has been shown to result in a similar activation without parallel labilization4. Activation by either treatment, however, has been shown to alter both the pH optimum of the enzyme and the Kmfor substrate4,6. A further complication has been that some workers have studied the enzyme activity in well washed microsomal preparations while others have used whole tissue homogenates or crude particulate fractions. Moreover, even when using such crude preparations some workers have made no attempt to correct data thus obtained for the activity of non-specific acid phosphatases of lysosomal origin.

In connexion with a study on possible changes in the localization of certain enzymes in response to nutritional or hormonal treatments we have been concerned to monitor glucose-6-phosphatase activity in rat liver. In view of the above considerations it was decided to assay this enzyme activity in washed microsomal preparations and also to correct the observed phosphate release for the residual activity of non-specific acid phosphatases. Activity was measured both before and after activation by incubation in ammonium hydroxide. In order to avoid ambiguities, resulting from possible changes in Km, the assays were performed at a relatively high concentration of substrate (80 mmolar). Activities were

determined in cacodylate buffer at pH 6.1. As shown by Stetten and Burnetts, this pH is on the alkaline end of the plateau of optimum pH for the unstimulated enzyme while being close to the peak in the pH-activity curve of the ammonia-activated enzyme. We have confirmed this observation for microsomal preparations from livers of rats of all groups included in the present study. In some experiments, citrate buffer at $p\bar{\rm H}$ 6·1 was also used in parallel assays. Activity was linear with time under all assay conditions employed.

Briefly the experimental procedure was as follows. Microsomes were isolated from homogenates of livers from Wistar rats, washed once and resuspended in 0.25 molar sucrose containing I mmolar EDTA (pH 7.2). The volume of the suspending medium was such that the preparations contained the equivalent of 0.5 g of liver per ml. The protein content of such suspensions did not vary significantly with age or pretreatment of experimental animals. Glucose-6-phosphatase activity of freshly prepared microsomal fractions was determined essentially as described by Stetten and Burnett⁵ except that the buffer used was 0·1 molar cacodylate, pH 6·1. Suitable corrections were made for the small content of contaminant inorganic phosphate of the substrate and for the activity of non-specific acid phosphatases?. Activation by ammonium hydroxide was carried out as described by Stetten and Burnett⁴. Activities are expressed as µmoles of inorganic phosphate released in 10 min at 30° by 0·1 ml. of microsomal suspension (corresponding to 50 mg liver). The diabetic rats, used in this study, had been treated by intraperitoneal injection of alloxan (15 mg/100 g body weight) and were sacrificed when glycosuric.

Initially, we compared activities of preparations from young adult rats (about 150 g) which had been well fed or subjected to mild fasting (up to 24 h). As expected, it was observed consistently that fasting tended to increase the glucose-6-phosphatase activity of the liver microsomal preparations. Two other observations, however, were also consistently made. One was independent of the pretreatment of the animals. This was that when enzymatic activity was measured in citrate buffer, not only was there inhibition of activity (by comparison with assays carried out in cacodylate buffer), but activity was further decreased following pre-incubation of microsomal pre-parations at high pH (see Table 1). This was in marked contrast to the findings of Stetten and Burnett, and in contradistinction to the stimulatory effect of such pretreatment on activity as assayed in cacodylate (see Table 1 and below). This unexpected finding could be interpreted in terms of an increased exposure of the active site to the incubation medium following treatment at high pH. Such an exposure might render the enzyme more susceptible to inhibition by citrate.

The second observation which was consistently made was that the degree of stimulation of glucose-6-phosphatase activity (measured in cacodylate buffer), after treatment of microsomal preparations, at high pH, was greater in the case of the fasted rats than in the case of the animals fed ad libitum. This observation was confirmed when further studies were made with a group of adult rats (200 g to 300 g). This distinction between fed and fasted rats was almost invariably maintained for given matched pairs of animals (of either sex). For an evaluation of the data, however, it was not found prac-

Table 1. Effect of pretreatment at high pH on the gluoose-6-phosphatase activity of rat liver miorosomal preparations assayed in cacodylate and citrate buffers

Activity in cacodylate buffer
Untreated Microsomes
microsomes treated at
high pH Activity in citrate buffer
Untreated Microsomes
microsomes treated at
high pH microsomes microsomes 1.54 ± 0.26 2.36 ± 0.48 1.03 ± 0.19

Experimental details and definition of unit of activity are given in the text. The data presented are for eleven rats in the weight range 130 g to 160 g. Data are given as mean values \pm standard errors of means.

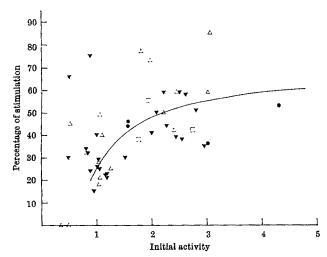


Fig. 1. The relationship between initial activity (units defined in text) and degree of stimulation by treatment at high pH of rat liver microsomal preparations. Φ , Rats 30 to 40 g; Δ , rats 100 to 200 g; Ψ , rats 200 to 300 g; Π , diabetic rats. The line is an arbitrary "theoretical" curve for two co-existent forms of enzyme (see text).

ticable to divide animals into fed and fasted groups because the extent of the fasting in some cases was only a few hours and the variability in enzyme activity among control animals was quite wide. There was thus some overlap in enzyme activity among the groups of animals. It was therefore decided to analyse the data in terms of initial glucose-6-phosphatase activity of the microsomal preparation (measured in cacodylate buffer) and degree of stimulation of this activity following pretreatment at high pH in the conditions described.

The results are plotted in Fig. 1, activity being expressed per 50 mg wet weight of liver. (Relative activities were shown to be essentially similar when activity was expressed in terms of microsomal protein.) It can be seen that, at least over a range of initial activity up to 2.0, the degree of stimulation by pretreatment at high pH seems to increase with initial activity. Thereafter the degree of stimulation seems to remain relatively constant. (The derivation of the line drawn in Fig. 1 will be discussed.)

The relationship between initial activity and degree of stimulation is highly significant for the entire group of animals tested (including some younger rats, weight 30 to 40 g, but not including the diabetic animals) (see Table 2). The relationship is also significant when young adult rats or older animals are treated as separate groups. Although not included in the statistical analysis, it can be seen from Fig. 1 that the data for adult rats rendered diabetic with alloxan fit well with the corresponding data for animals with high initial activities induced by fasting.

These conclusions are not likely to be caused by any false assumptions regarding the relatively minor correction for the activity of non-specific acid phosphatases (which were not significantly activated by the high pH treatment). Essentially the same pattern would have emerged had no correction at all been made for this activity. Moreover, the conditions of pre-incubation at high pHwere not critical for this demonstration. Indeed, subsequent studies revealed that microsomal preparations of

Table 2. Significance of difference in degree of stimulation of microsomal preparations of initially low and high activity respectively

Weight range (g)	th	activity less an 1.56 Stimulation by treatment at high pH (per cent)		activity greater han 1.56 Stimulation by treatment at high pH (per cent)	Significance of difference in mean stimulation
100-200 200-300	10 15	25.8 ± 5.1 32.8 ± 4.1	7 10	63.6 ± 5.4 47.4 ± 2.8	$P \le 0.001$ P < 0.02
30-300	25	30.0 + 3.3	21	52.3 ± 2.9	$P \ll 0.001$

The initial activity limit of 1.56 set for dividing the animals into groups of low and high initial activity for statistical analysis was chosen arbitrarily by inspection of Fig. 1. Data are given as mean values \pm standard errors of means.

low initial activity were fully activated by incubation for 10 min in the conditions referred to, while preparations of high initial activity (from fasted or diabetic animals) only reached their maximal activity after incubation for about 1 h. The distinction between preparations of low and high initial activity would therefore have been even more marked had a longer period of preincubation been routinely employed.

These findings indicate that, in the well-fed animal. an important proportion of the microsomal glucose-6phosphatase is in a form which is not greatly stimulated by treatment at high pH. When enzyme activity is increased following fasting or by diabetes, the "new" enzyme is in a form which is more susceptible to stimulation. Whether the "new" enzyme derives from the basal enzyme, or from an inactive precursor or by de novo protein synthesis is at present obscure. Assuming that the activities are quite separate, however (as suggested by Freedland and Harper⁸), it is possible to derive theoretical curves for the relationship between initial activity and degree of stimulation by selecting some quite arbitrary values for three parameters. These are the basal activity for all animals, the degree of stimulation of this basal activity by treatment at high pH and the degree of stimulation of all "new" enzyme following this treatment. Making the assumptions that the basal activity is 0.9 units (see here), and that the degrees of stimulation of basal and "new" enzyme are 20 per cent and 70 per cent. respectively, the line in Fig. 1 is obtained.

The satisfactory fit of the arbitrary curve to the experimental points is, of course, very tenuous evidence for the hypothesis of two, co-existent forms of the enzyme. It is hoped to resolve this question by direct fractionation procedures, taking advantage of the difference in properties between "basal" and "induced" enzyme activities revealed in this study.

In confirmation of our observations, it has recently been reported that microsomal glucose-6-phosphatase activity from livers of alloxan-diabetic rats is stimulated by detergent treatment to a greater extent than is the corresponding activity of normal rats.

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BIOPHYSICS

Potassium Ion Activity in the Cytoplasm and the Vacuole of Cells of Chara and Griffithsia

A CHARACTERISTIC of plant cells is that they have adjoining phases of different physico-chemical properties. These are the cell wall, the cytoplasm and the vacuole. Unequal distributions of ionic activities between these phases are expected, as are electrical potential differences. Investigations of the ion-exchange processes in algal cells of the Characeae1, and analyses of the cytoplasmic and vacuolar composition of centrifuged cells2, confirm the existence of separate intracellular phases. Although an electrical potential difference between the cell vacuole and the medium bathing the cell has long been established, confirmation of potential steps between the remaining phases of plant cells was only found later.

During the past few years it has been possible to observe potential differences at the junctions between the cell wall and medium^{3,4}, and between the cytoplasm and the vacuole^{5,6}. Although our knowledge of the distribution of electrical potentials in the adjoining phases of the plant cell has substantially increased, the details of the distribution of ionic activity between the same phases have remained obscure. First attempts have, however, been made to determine the ionic activities of potassium ions and chloride ions in the vacuolar sap^{7,8} and chloride ions in the cytoplasm⁹ of Characeae cells.

The present work is concerned with determining the potassium ion activity (a_{K+}) in the vacuole, cytoplasm and the surrounding medium, with concurrent measurements of the potential differences between these phases. The investigation was made with cells of the green, freshwater alga Chara australis R.Br. var nobilis A.Br. and the red marine alga Griffithsia sp. The cells of C. australis were cultivated in artificial conditions, and before the experiments were kept for at least 2 days in an artificial pond water (1.0 mmolar sodium chloride+ 0.1 mmolar potassium chloride+0.1 mmolar calcium chloride). The cells were dissected from the whorls at the nodes; whorl cells are sufficiently transparent to enable the phases of cytoplasm and vacuole to be distinguished. The cells used were 2-5 mm long and 0.5-0.8 mm in diameter. Collected Griffithsia cells with dimensions of approximately 1.5×1.7 mm were kept for several days in an artificial sea water (potassium chloride, 10 mmolar; sodium chloride, 470 mmolar; magnesium chloride, 25 mmolar; magnesium sulphate, 25 mmolar; sodium bromide, I mmolar; sodium bicarbonate, 2.5 mmolar; calcium chloride, 11.5 mmolar).

For the intracellular measurement of $a_{\mathbb{R}^+}$ and of potential differences, two microelectrodes (one filled with 3 molar potassium chloride and the other a potassium ionsensitive microelectrode) both with tip diameters of about 1μ were inserted while being viewed through a microscope (magnification \times 180). Simultaneous records were obtained of two potential differences, one between the microelectrode and potassium ion-sensitive microelectrode both maintained in the same cell phase, and the other between the intracellular and a reference microelectrode. The distance between the two intracellular electrodes was $40-80\mu$, the angle between them being 30° .

Microelectrodes sensitive to potassium were designed to measure a_{K+}. The tips contained a precipitate of either sodium cobaltinitrite or dipicrylamine, and the microelectrodes functioned as potassium ion electrodes in the concentration range 10-3-1 molar potassium chloride. The selectivity coefficient was usually 0.2, the best value achieved was 0.12 and in the experiments with Griffithsia cells the value was 0.46. The microelectrodes sensitive to potassium were calibrated in solutions of potassium chloride and sodium chloride before, and on conclusion of, the experiment. The accuracy of measurement of a_{K+} in the presence of sodium ions in the ratio [K+]: [Na+]= 2.5:1 was in the vicinity of 10-15 per cent. The half-time for the response of the sensitive microelectrodes, when the concentration was changed ten-fold, was about 3-4 sec. The resistance of these microelectrodes was in the range $10^{10}\text{--}3 \times 10^{10}~\Omega$. The measurements of a_{K^+} and of potentials were carried out in August and September 1967 with the temperature of the surrounding medium 20°-22° C.

The first potential step of -60 mV was registered when the microelectrode was inserted into the cell wall of *Chara*. Immediately after the cell wall was penetrated a second potential step of about -100 mV was observed which corresponded to the cytoplasmic phase. The

typical thickness of the flowing cytoplasm was 15 to 20μ and the entrance of the microelectrode into the cytoplasm induced local cessation of cytoplasmic flow and the initiation of a large cytoplasmic clot (cloud) about 40μ thick. A few minutes later this cloud began to disappear. To measure a_{K^+} in the vacuolar sap, and the vacuole potential, the microelectrodes were inserted to a depth of $150-200\mu$.

Measurements could be made in the cytoplasm for as long as 20 min, then as the electrode became sealed off from the cytoplasm the potential dropped to that of the cell wall. When recording the two potential differences between the three electrodes as described, the beginning of the microelectrode transition into another phase could be established with great accuracy. It was thus possible to be sure that both microelectrodes were in the same phase, which served as a control in measuring a_{K^+} and the potential differences. In the *Griffithsia* cells the cytoplasm adhered to the microelectrode tip and followed it like a trail, and the cytoplasmic potential could be measured for a period of many hours.

The activity of potassium ions in the cytoplasm of C. australis cells was found to be $2\cdot 3$ times greater than that of the vacuolar sap (Table 1). In the Griffithsia cells, however, the reverse was observed, and the cytoplasmic $a_{\mathbb{K}^+}$ was $2\cdot 2$ times less than that of the vacuole. It is interesting to note that $a_{\mathbb{K}^+}$ in the cytoplasm of the marine and the freshwater algae were in the ratio of only $1\cdot 4$ to 1, while in the vacuolar sap the ratio was $7\cdot 3$. When microelectrodes became sealed off after insertion in the cytoplasm, the potential difference corresponded to $a_{\mathbb{K}^+}=7-10$ mmolar.

For comparison with the results obtained for ion activity, analyses were made of the vacuolar sap, using a flame photometer. It was found that the *Griffithsia* cells had an average value (unpublished results of G. P. Findlay and A. B. Hope) of 546 mmolar for [K+] vac and 63 mmolar for [Na+] vac. It we assume that the activity coefficient, γ , in the vacuolar sap of the marine algae is equal to γ of artificial sea water, that is, approximately 0.68, then $a_{\mathbb{K}^+}$ calculated from results for the potassium ion concentration would be equal to 370 mmolar. This value corresponds to the mean intracellular $a_{\mathbb{K}^+}$ measured in living cells (Table 1).

The activity coefficient in the vacuolar sap of the C. australis cells was established by determining the chloride ion activity, $a_{\rm Cl}$ -, using the method of microsample extraction. The mean value of $a_{\rm Cl}$ - was equal to 84 mmolar. The activity coefficient in the solution of potassium chloride, the concentration of which corresponds to the observed $a_{\rm Cl}$ -, is equal to 0.75. The potassium concentration in the vacuolar sap of C. australis (5 cells) was found to have a mean value of 60 ± 3 mmolar which corresponds to $a_{\rm K}=45$ mmolar. This is closely related to the activity measured with potassium ion-sensitive microelectrodes.

Comparisons can now be made between the equilibrium potassium ion-potential, $E_{\mathbb{K}^+}$ (Table 1), for the cytoplasmic and vacuolar phases, calculated on the basis of experimental results for $a_{\mathbb{K}^+}$, and the potentials measured in the corresponding phases, using the Nernst equation. It is clear that the equilibrium potentials of potassium ions between artificial pond water and the cytoplasm or the vacuole of the C. australis cells, and between artificial

Table 1. Potassium ion activities compared with measured potential differences (Eoi, mv) and estimated nernst potentials ($E_{\rm K}+$, mv) in cells of Chara australis and Griffithsia

Cells	Phase	Potassium ion (mmola	ır)	E K +(m∇)	Eci (mV)
		Intracellular	External		, ,
Chara australis Griffithsia	Cytoplasm Vacuole Cytoplasm Vacuole	115±10 (9) 48±3 (6) 153±6 (6) 343±10 (7)	0·096* 0·096 6·8* 6·8	-178 -156 -78 -99	-173 ± 1.5 -155 ± 2 -80 ± 1 -50 ± 3

* Activity coefficients in artificial pond water and artificial sea water are taken to be 0.96 and 0.68, which are the coefficients for sodium chloride solutions of concentrations 1.3 and 553 mmolar, respectively.

sea water and the cytoplasm of Griffithsia cells, are nearly equal to the observed potential differences. This suggests that potassium ions are in electrochemical equilibrium in these phases.

On the other hand, the electrochemical potentials of potassium ions in the vacuole and the cytoplasm of Griffithsia cells differ significantly. The difference, $\Delta\mu_{K^+}=\mu_{K^+\nu ac}-\mu_{K^+cyt}$, is equal to 1,100 cal. mole-1 with the gradient directed from the vacuole to the cytoplasm. It is therefore suggested that there is an active transport of potassium ions from the cytoplasm to the vacuole.

This work was carried out in the Department of Physiology, Australian National University, and in the School of Biological Sciences, Flinders University, while I held an exchange fellowship between Moscow State University and the Australian National University.

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PHYSIOLOGY

Effects of Dehydration on Blood Viscosity and on Distribution of Plasma Proteins in Experimental Macroglobulinaemia

A GREAT variety of plasma cell tumours has been induced in mice and transplanted in successive generations of these animals^{1,2}. The tumours secrete large amounts of myeloma proteins and make it possible to examine various problems concerning the myelomas and dysproteinaemias. Usually, as the concentration of myeloma protein rises in the plasma of affected animals, the concentration of normal protein diminishes2. A similar reduction of normal plasma proteins in humans with dysproteinaemias has been related either to decreased synthesis or to increased catabolism of normal protein fractions. As yet, no data have appeared concerning the plasma concentrations of abnormal and normal proteins in experimental macroglobulinaemia. The plasma cell tumour designated MOPC-104E secretes large amounts of macroglobulin (IgM). Mice bearing this tumour resemble humans with macroglobulinaemia, in that both display an elevated blood viscosity which is related to the plasma concentration of IgM and modified by the haematocrit⁵⁻⁷. It was of additional interest to determine whether these mice also resembled human macroglobulinaemics3 with respect to the relative intravascular concentrations of IgM and non-macroglobulin (non-IgM). This investigation examines this question. In addition, an attempt was made to alter the intravascular distribution of proteins by changing the osmotic balance of the animals through dehydration.

Plasma cell tumour MOPC-104E was transplanted to female Balb/c mice by subcutaneous injection into the lower abdominal wall. Four to six weeks later the tumours had grown to large solid masses, and animals were selected for dehydration, which was accomplished by depriving them of drinking water for 24 h. Dehydrated and nondeprived mice bore tumours of approximately the same size and had carried them for the same length of time. Animals were anaesthetized with sodium pentobarbital and blood was obtained by cardiac puncture. Blood was drawn through a 23 gauge needle into a syringe containing sufficient heparin to yield a final concentration of 4-8 U, ml. blood.

Microhaematocrit determinations were performed on all blood samples. The apparent relative viscosity (hereafter abbreviated as "viscosity") of blood was measured at 37°C with a capillary viscosimeter operating at a shear rate in excess of 1,000 reciprocal seconds. (at a pressure of -100 mm of mercury, 0.1 ml. water took 1 sec to pass through the capillary, which was 306µ wide and 36 mm long). At such high rates of shear, blood viscosity is no longer affected by shear rate; thus values in the present study represent the asymptotic portion of the curve relating shear rate to shear stress10.

A portion of each blood sample was centrifuged in 'Wintrobe' tubes and the plasma was decanted. plasma protein was determined with the biuret procedure¹¹ and IgM concentrations were measured by radial immunodiffusion¹². Subtraction of the IgM concentration from the total protein gave the concentration of non-IgM.

The effects of dehydration are indicated in Table 1. Blood viscosity doubled, as did the concentration of IgM, results which are consonant with earlier data suggesting a linear relationship between these variables. The increases in haematocrit and total protein concentration also contributed to the rise in blood viscosity 6.10, and shifts in the relative concentration of individual, unmeasured protein fractions may have influenced the magnitude of the viscosity elevation6.

Although dehydration increased the IgM level by 100 per cent, the increase in total protein was only 30 per cent (Table 1). Thus, while the IgM concentration rose during dehydration, the intravascular concentration of non-IgM (total protein minus IgM) decreased (Fig. 1).

Study of the non-deprived or control mice (Fig. 1) indicates that, in these animals also, the IgM level was inversely related to the concentration of non-IgM, a relationship also observed in humans with macroglobulinaemia3. The data in Fig. 1 suggest that similar factors may control the intravascular distribution of proteins in both the dehydrated and the control macroglobulinaemic animals, because the points representing the two groups of mice overlap and appear to be distributed along a common curve.

Alterations in the rates of synthesis or catabolism of IgM and non-IgM may have affected the distribution of intravascular proteins in these mice^{3,13}. The present data do not cast light on the question. The data do suggest, however, that osmotic factors played a part in adjusting the relative concentrations of IgM and non-IgM, at least during dehydration. Water deprivation increased the haematocrit by 25 per cent, but a parallel and equal reduction in total body water would have resulted in death¹⁴. Thus water must have been lost in disproportionate amounts from the vascular compartment, a phenomenon known to occur during mild to moderate dehydration¹⁴. This preferential loss of intravascular water was accompanied by an almost equivalent (25 per cent) decrease of intravascular non-IgM, which suggests that normal proteins followed water in its passage out of On the other hand, the IgM level rose the vessels. markedly and, in fact, the percentage increase in IgM concentration was four times greater than the increment The basis for the greater increase in in haematocrit.

Table 1. MACROGLOBULINAEMIC MICE

	Haematocrit	Blood viscosity	IgM (mg/ml.)	Total protein (mg,ml.)
Not dehydrated (11 mice)	$32 \pm 4*$	4.3 ± 1.0	32 ± 11	69 ± 6
Dehydrated	40 ± 4	$8 \cdot 0 \pm 2 \cdot 7$	63 ± 29	91 ± 20

^{*} Mean \pm S.D. All differences between the two groups of macroglobulinaemic mice are significant at 0.01 level (Mann-Whitney test).

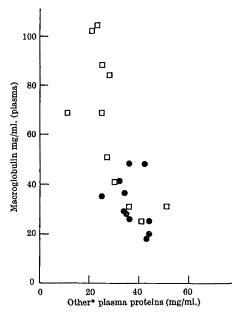


Fig. 1. Macroglobulinaemic mice. The greater the IgM level, the lower the concentration of non-IgM (Spearman rank correlation coefficient = -0.81, P < 0.01). * Total protein minus IgM. •, Control; __, dehydrated.

plasma IgM is not understood, but the increase itself suggests that IgM molecules could not readily follow the outward movement of water. Data from hydrated humans with macroglobulinaemia also indicate an inability of IgM to leave the vascular compartment¹³. Thus 80 per cent of the body's IgM is located intravascularly in macroglobulinaemia while only 40 per cent of the body's albumin, 6.6S gamma globulin, or transferrin are similarly distributed^{3,13}. In the present experiments the intravascular concentration of IgM apparently could not be readily altered to meet the requirements of the new osmotic situation engendered by the loss of intravascular water. The decreased level of non-IgM thereby takes on even more importance as a homeostatic mechanism for keeping intravascular osmotic pressure within tolerable bounds.

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Disodium Cromoglycate (FPL 670) ('Intal'*): a Specific Inhibitor of Reaginic Antibody-Antigen Mechanisms

CHROMONE derivatives exhibiting unusual biological activity have been studied in these laboratories for several years. One of these, disodium cromoglycate (FPL 670), has been found to inhibit specifically the liberation of the mediators of anaphylaxis initiated by the interaction of antigen with reagin type antibodies. It is neither a bronchodilator nor an anti-inflammatory agent and its action is distinct from that of corticosteroids.

In experiments on an asthmatic volunteer, inhaling the compound inhibited the acute effect of subsequent antigen inhalation¹. In patients suffering from allergic asthma, inhalation of this compound was followed by clinical improvement2.

Disodium cromoglycate is the sodium salt of 1,3-bis-(2-earboxychromon-5-yloxy)-2-hydroxypropane. It is an odourless, white, hydrated crystalline powder moderately soluble in water but practically insoluble in alcohol. Physical investigations (elementary analysis, infrared, ultraviolet, nuclear magnetic resonance and mass spectra) were consistent with the assigned structure

The compound inhibited the passive cutaneous anaphylactic (PCA) reactions in monkeys, sensitized with human reaginic serum, when the compound was given intradermally with the antigen, but did not affect the skin reactions to intradermal histamine, 5-hydroxytryptamine or bradykinin. Antigen induced bronchoconstriction in anaesthetized marmosets, sensitized intravenously with human reaginic serum, was substantially reduced by

disodium cromoglycate.

Homologous PCA reactions with reagin-like antibody in rats, using both the egg albumen/B. pertussis³ and Nippostrongylus brasiliensis⁴ systems, showed substantial inhibition in the presence of the compound. Although the compound inhibited the PCA reaction it failed to affect the skin lesions induced by compound 48/80.

In contrast, in guinea-pigs, homologous PCA reactions with precipitating antibody were unaffected, as were aerosol or intravenous antigen induced bronchospasm and the release of histamine and SRS-A (slow reacting substance-anaphylaxis) from actively or passively sensitized lung in vitro.

The release of histamine and SRS-A from portions of chopped human lung, passively sensitized with human reaginic serum, has been measured after exposure to specific antigen(s) in vitro5. Inhibition with disodium cromoglycate was found over a narrow range of concentrations.

In an in vitro system in which contractions of human bronchial chain, exposed to passively sensitized and shocked human lung, were used to simulate the supposed events in an attack of allergic asthma, FPL 670 at 10 µg/ml. caused a significant reduction (40 per cent) in the contractile response.

It was considered important to study the effect of the compound on those antibody systems concerned with immunity. No adverse effect was found on several in vitro virus/antibody neutralizing systems including influenza A, polio virus type II, vaccinia and herpes simplex with human and rabbit antisera.

Likewise no effect was found on the LD₅₀ in mice of mouse adapted polio virus, nor on their protection by

* Trade mark-Fisons Pharmaceuticals, Ltd.

Salk vaccine. No effect was observed on the neutralization of Clostridium welchii type A α -toxin by specific antiserum nor on the cytotoxic behaviour of rabbit anti-HeLa on HeLa cells in vitro. Nor did it interfere with any of the several bacterial agglutinating systems tested.

Further in vivo work was carried out on the effect of the compound on the passive protection of guinea-pigs with diphtheria antitoxin, on the development of immunity and response of immune mice to pneumococcus and on the development of experimental tuberculous lesions in guinea-pigs. In addition, an examination was made of the rate of production of antibody and the titre achieved in monkeys to T₁ coliphage. In all these in vivo systems no change in the normal pattern was observed over the range of doses administered.

Disodium cromoglycate has few general pharmacological effects. Relatively large doses (up to 100 mg/kg) given intravenously, intraperitoneally or to the cerebral ventricles in conscious animals from a wide range of species produced negative or only weak non-specific effects in various behavioural studies. Only in the dog were marked effects observed, intravenous injection of moderate doses (for example, 8 mg/kg) causing immediate collapse and transient apnoea with rapid recovery.

In studies of anaesthetized animals, large doses of disodium cromoglycate had only weak inconsistent effects on the cardiovascular and respiratory systems of the cat, pig, monkey, guinea-pig and rat. In anaesthetized dogs, doses as small as $10~\mu g/kg$ elicited reflex mechanisms originating in the pulmonary and coronary circulations producing bradycardia, hypotension and in some cases apnoea. In marmosets, however, the compound caused a rise in blood pressure and heart rate with little effect on respiration.

Studies with the new compound on bronchial tone in anaesthetized guinea-pigs demonstrated little activity. It had no direct action on human bronchial chain nor did it antagonize the responses to histamine, SRS-A or acetylcholine. The compound showed no significant effect in several anti-inflammatory tests nor did it have any consistent influence on the anti-inflammatory activity of hydrocortisone.

Disodium cromoglycate had no significant effect on several other functions studied including steroid metabolism, gastrointestinal mobility and urine and bile flow. It had little effect on the ciliary activity of either isolated frog oesophagus or human bronchial epithelium. The compound had no direct action on Trendelenburg's reflex of isolated guinea-pig ileum, neither did it antagonize the response of the ileum to histamine, 5-hydroxytryptamine, acetylcholine, nicotine, substance P, bradykinin or SRS-A.

The absorption, distribution and excretion of disodium cromoglycate have been studied in a variety of laboratory animals. Following oral administration, only a very small proportion (less than 0.5 per cent) of the dose was absorbed. After inhalation of the powder aerosol, only a small proportion of the dose reached the peripheral lungs, a major part being trapped in the upper respiratory tract. Plasma and lung tissue levels indicated that the powder reaching the peripheral lungs was rapidly and completely absorbed. The compound was rapidly eliminated, the major portion unchanged, via the urine and bile; no accumulation could be detected in any tissue even after repeated daily intramuscular injections. Studies of the urinary excretion and plasma levels in human volunteers have suggested that the same general pattern applies in man.

Disodium eromoglycate has been shown to be relatively non-toxic. Thus in *in vitro* tests a concentration of at least 5 mg/ml. was required to produce effects on the morphology of HEp2 cells and chick embryo fibroblast cells, and on the migration of guinea-pig macrophages. In acute toxicity tests in small laboratory animals, the LD_{50} on parenteral administration was usually between 2,000 and 4,000 mg/kg, while in a prolonged test in rats

no toxic effect resulted from ninety daily subcutaneous injections, except at doses greater than 30 mg/kg. The only pathological lesion produced in any of these tests was an inflammation and degeneration of the renal tubules. Neither this nor any other toxic effect was found in 90-day inhalation studies.

No teratogenic effects were seen in rabbits in which the compound was given intravenously daily throughout

pregnancy in doses up to 250 mg/kg.

Disodium eromoglycate had few general pharmacological effects, was rapidly excreted and seemed to have a low order of toxicity. The compound appeared to inhibit specifically the anaphylactic process initiated by reaginic antibody—antigen interactions. This novel property may permit a more specific treatment of allergic disease, especially of the lung, and will undoubtedly contribute to basic knowledge in these areas.

The results given here represent the combined efforts of the staff of the Research Departments at Holmes Chapel

too numerous to name individually.

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ATPase Activity at the Functional Contacts between Retinal Cells which produce S-potential

According to Cajal1, the horizontal cells of the teleost retina are arranged in three superimposed tangential layers formed by the external, medial and internal horizontal cells. Cajal classified the horizontal cells as short axon (Golgi II) cells and showed that radially oriented apical processes make contact with the receptor Golgi studies and ultrastructural studies on the teleost retina (refs. 2 and 3 and unpublished results of V. Parthe and myself) confirm Cajal's description of these cells which in certain teleosts are enormous compared with the bipolar cells in the same retinal layer. tangentially oriented extensions were not found in the external horizontal cells in Parthe's Golgi material. Such extensions of the medial and internal horizontal cells do not exceed in length the diameter of the cell body. Axon-like structures were not seen by Parthe or myself in any of the horizontal cell layers. In ultrastructural work4 each row of horizontal cells appears as a reticulum in which the cells are interconnected by membrane to membrane apposition of their lateral processes. Yamada and Ishikawa³ described in the carp, shark and ray retinae "fused membrane structures" at the apposition areas between the short lateral processes of the horizontal cells. But specialized junctions were not seen between horizontal cells belonging to different layers. These findings have been confirmed (Fig. 1) and similar fused membrane structures have been seen between stellate amacrine cells and between interstitial amacrine cells, in the regions where membrane to membrane contacts are formed between adjacent cells (my unpublished work).

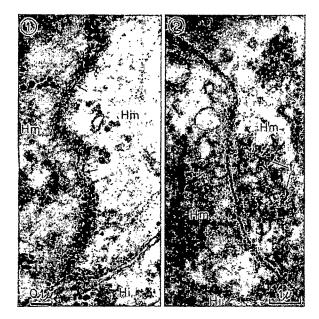


Fig. 1. Section of a Mugil brasiliensis retina showing partially two medial horizontal cells (Hm) and one internal horizontal cell (Hi). Zones of fused membranes alternate with zones of apposed membranes (arrows) at the lateral contacts between the two medial horizontal cells; no synaptic vesicles are visible. There are no fused membrane structures at the apposed membranes of the medial and internal horizontal cells. Glutaraldehyde-osmium post-fixed material. (\times c. 58,900.)

Fig. 2. Formalin fixed material incubated in Wachstein-Meisel medium and post-fixed in esmium. The ATPase reaction product is seen as lead precipitate at the fused membrane structures. Precipitate is less abundant at the apposed membranes of the medial and internal horizontal cells. f_* Fibrils (\times c_* 8,180).

Light and electron microscopy shows that all the cells, which are of a giant size in certain teleost retinae, form tangential networks which are continuous throughout the retina (ref. 5 and unpublished results of V. Parthe and myself). Synaptic vesicles have not been observed in the region of the fused membrane structures (Fig. 1).

Torack⁶ demonstrated high ATPase activity in the vicinity of the plasma membrane of oligodendroglial cells, while in the astroglia the ATPase was confined to processes in contact with neurones. Marchesi et al.⁷ found, in the rat retina, ATPase activity at the cell borders between glia and glia, and glia and neurones, but not between neurones. Novikoff et al.⁸ demonstrated the presence of an ATPase, which apparently is not of the transport type, at the interface between the Schwann cell and the non-myelinated axon in the peripheral nerve of the rat, and the interface between the ganglion cell and its satellite cells in the dorsal root ganglion.

The present attempts to demonstrate ATPase activity were carried out on teleost (Mugil brasiliensis) retinae incubated in a solution containing lead salt by the method of Wachstein and Meisel modified by Torack⁶. Retinae fixed in glutaraldehyde or hydroxyadipaldehyde gave negative results. In formalin-fixed material, ATPase reaction products were constantly found at the fused membrane structures at the lateral contact region between neighbouring cells of different horizontal cell networks. Fig. 2 shows the fused membrane structure of a lateral contact area between two medial horizontal cells; fused membrane zones alternate with apposed membrane zones. Fig. 3 shows that a positive reaction for ATPase activity is also found at the fibrillar structures, which are seen perpendicular to the fused plasma membranes of the horizontal cells. ATPase activity, in the form of electron dense granules, is more abundant at the fused areas. ATPase reaction products were scarcely apparent along the plasma membrane of the horizontal cells (Fig. 2), and scattered granules were occasionally observed in the cytoplasm. Mitochondria appear free of the reaction product.

A total inhibition of the ATPase reaction (Fig. 4) was achieved by adding nickel nitrate $(1\times10^{-2}\ \mathrm{molar})$ or L-cysteine $(5\times10^{-3}\ \mathrm{molar})$ to the final concentration of the Wachstein–Meisel medium. An almost complete inhibition was obtained with sodium fluoride $(6\times10^{-2}\ \mathrm{molar})$, whereas ouabain $(5\times10^{-4}\ \mathrm{molar})$ had no effect.

The ATPase stimulated by sodium and potassium⁹, which seems to be implicated in ion transport across cell membranes, is inhibited by the lead salt contained in the Wachstein-Meisel incubation medium¹⁰. Thus the ATPase positive reaction shown at the fused plasma membrane structures in this work seems not to be the result of the presence of the cation transport ATPase⁸. This ATPase shows similar characteristics to that observed by Torack⁶ in oligodendroglial processes; both are resistant to formalin but not to glutaraldehyde and hydroxyadipaldehyde.

The S-potentials of the luminosity (L) type originate in the large horizontal cells, whereas the chromatic (C) responses are recorded from the stellate and interstitial amacrine cells11. These potentials, evoked by light stimulation, are membrane potential changes, which show a lateral spread several mm away from the illuminated area. The spreading velocity of the L-response and of the hyperpolarizing C-response has been shown to be about 0.35 msec12. The calculated conduction time across one external or one medial horizontal cell is of the order of 0·1 msec and 0·2 msec, respectively, which is too short a time to account for any synaptic delay. The tight junctions found between glial cells in the optic nerve of Necturus and the frog are assumed to represent low resistance connexions for electrical interactions between neighbouring cells¹⁵. The "tight junctions" between adjacent supramedullary neurones in fish have been shown to operate as electrotonic junctions which permit electrical transmission between adjacent neurones16. The fused membrane structures described here are structurally and functionally different from tight junctions. The horizontal cell membrane is electrically inexcitable¹³, which excludes the possibility of an electrical synapse and of a spread of excitation by means of local ionic current flow. Negishi (unpublished results) has

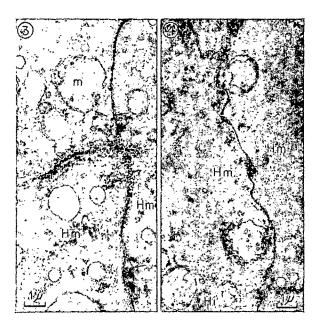


Fig. 3. Formalin fixed material incubated in Wachstein-Meisel medium and post-fixed in osmium. The ATPase reaction product is seen at fibrils (f), which are perpendicularly attached to the fused membrane structures of two medial horizontal cells (Hm). Precipitate is less abundant at the apposed, non-fused area (arrows). m, Mitochondria $(\times c. 16,360)$.

Fig. 4. Formalin fixed material incubated in Wachstein-Meisel medium containing nickel nitrate $(1\times 10^{-2}$ molar), and post-fixed in osmium. Absence of reaction product is evident. m, Mitochondria (\times c. 10,470).

recently confirmed that the behaviour of the S-potential producing membrane is not influenced by electrical ionic current flow and that S-potential spread from cell to cell in a given network is unrelated to ionic current flow.

It has been demonstrated that the S-potential function is strictly dependent on aerobic metabolism and that the membrane potential of S-cells is strongly influenced by temperature changes, which indicates a dependence on enzymatic systems¹¹. But the high membrane potential level (70 mV) of the S-cells produced by anoxia is a diffusion potential. It was suggested that the S-potentials depend on a plasma membrane respiration and that the spread of excitation in a given network is related to the function of an ATP-producing electron transfer system^{6,11}. The intercellular transfer of excitation within the S-cell networks "occurs only at specialized regions ('transferapse') where the apposed plasma membranes are partly fused'".

Most of the ATP formed by glucose oxidation is produced within the mitochondria, and because the mitochondrial membrane is relatively impermeable to ATP, a special system exists for transferring energy to the extramitochondrial space¹⁴. ATPase is a specific enzyme for the liberation of energy from ATP, so it is reasonable to assume that both ATP and ATPase are present at the same cellular locations. The question of the origin and function of the ATP hydrolysed by the non-transport ATPase at the fused plasma membrane structures in the retina arises. The respiratory chains are constituents of the mitochondrial membrane14, and therefore it is suggested that this ATP is formed by respiratory chains located in the fused plasma membrane structures, probably for the transfer of excitation from cell to cell within the S-cell networks. The ATPase found close to the cell surface is probably related to a mechanism for the spread of the S-potential along the plasma membrane.

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Unusual Endplate Potentials which reflect the Complexity of Muscle Structure

Falk and Fatt1,2 recently proposed a new equivalent circuit to account for the electrical impedance of frog sartorius and crayfish muscle fibres. In this new model (Fig. 1) the transverse impedance of the resting membrane

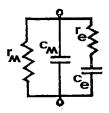


Fig. 1. Drawing of electrically equivalent model for the membrane impedance of frog striated muscle fibre, based on the model suggested by Falk and Fatt¹. If the total transmembrane resistance of the model is to be 500 k Ω (the normal d.c. input resistance of extensor digitorum longus IV fibres), $\tau_m = 500$ k Ω , $\tau_c = 50$ k Ω , $\tau_m = 0.015$ F, $\tau_c = 0.033$ F. The older model³ could result if τ_c were shorted.

is represented by a network of several elements rather than by a resistance and a capacitance in parallel as in the simple rc core conductor cable model3. Falk and Fatt4 have suggested that variations which have been reported in some neuromuscular junctional potentials of crustacean muscle can be attributed to the impedance properties of this more complicated circuit model. The model for frog sartorius muscle is in good agreement with other studies of the structural and electrical properties of the fibres⁵⁻⁸. (A model which differs somewhat from that of Falk and Fatt but is similarly complicated has been proposed for crayfish muscle9.)

While studying frog skeletal muscles which have been treated with certain drugs, I have observed that an endplate current consisting of an early transient component and a continuously declining prolonged component can produce a complex endplate potential which exhibits a minimum between an early transient component and a prolonged component of greater or equal amplitude. Injected currents of similar configuration produce similar complex potentials in the absence of drugs. The correlation of potential with current provides a qualitative demonstration of the complicated nature of the muscle impedance.

 $ar{ ext{S}}$ ingle muscle fibres of extensor digitorum longus $ext{IV}$ (toe muscles) of Rana pipiens were impaled with two micropipettes which were inserted into the endplate region10. Conventional recording equipment was used to detect intracellular endplate potentials (epps) or potential changes which were evoked by injecting current through the second electrode. The injected currents were produced by using a small analogue computer as a voltage source. The motor nerve was electrically stimulated to evoke epps which were reduced in amplitude below the muscle action potential threshold by including in the bath 0.0005 to 0.005 mmolar d-tubocurarine (d-TC) or 0.1 to 2.0 mmolar chemical QX-314 or QX-222. The latter two drugs are, respectively, the triethyl and the trimethyl quaternary ammonium derivatives of the local anaesthetic lidocaine (lignocaine, Xylocaine, Fig. 2). These particular lidocaine derivatives modify the time course and the amplitude of the epp without having any detectable effect on the passive electrical properties of nerve or muscle; they do not act as local anaesthetics at concentrations below 5 mmolar, although lidocaine itself does so at concentrations below 1 mmolar11,12.

In comparison with an epp of like amplitude which was recorded during treatment with d-TC, epps recorded during treatment with drugs QX-314 or QX-222 are of altered waveform. The epp always has a time of rise

to an early peak which is briefer than usual, and a rapid early fall. During treatment with drug QX-222, there is also a prolonged component which typically exceeds the early peak in amplitude, and the resulting epp shows a minimum (Fig. 2B). During treatment with drug QX-314, the prolonged component is not present, and the entire epp is briefer than usual (Fig. 2C).

The time course of the endplate current (epc) which is associated with endplate potentials such as those in Fig. 2 was determined by a modification of the method of voltage clamping used by Takeuchi and Takeuchi¹³. The epc during treatment with drug QX-314 was of brief duration (rise time 0·3 to 0·6 msec, half time of fall 0.2 to 0.5 msec). The epc during treatment with drug QX-222 had a similar earlier transient, but also had a prolonged monotonically decaying residual component of maximum amplitude 0.1 to 0.25 of the brief transient, and half time of decay 20 to 50 msec. The epcs never showed a minimum between early transient and prolonged component, even in cases where the epc did show a minimum (for example, Fig. 2B).

Monophasic currents of form similar to those recorded during treatment with drugs, when injected into a muscle fibre through a second micropipette, could produce the full range of potential changes which are observed during

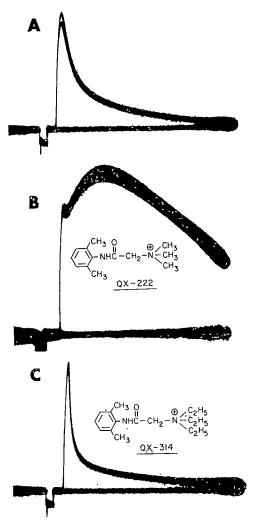
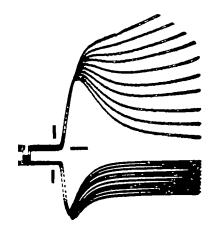


Fig. 2. Endplate potentials recorded from a single fibre of extensor digitorum longus IV during treatment (A) with 0.004 mmolar d-TC; (B) with 0.8 mmolar QX-222; (C) with 0.34 mmolar QX-314. Each trace is a photographic composite of at least three successive epps. The changes in waveform produced by the drugs were completely reversible. Resting potential: -86 mV. Temperature, about 24° C. Ringer solution: 111-3 mmolar sodium chloride, 2·1 mmolar potassium chloride, 2·0 mmolar patassium chloride, 2·0 mmolar calcium chloride, buffered to pH 6·8 with 10 mmolar glycylglycine-piperazine. Calibration pulses (on traces): 1 mV, 1 msec.



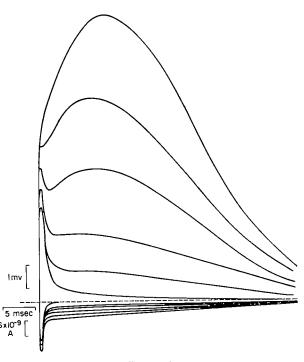


Fig. 3. Response of extensor digitorum longus IV fibre to a transmembrane current. Upper: the waveforms of potential (upper traces) were produced by the transmembrane currents shown in the lower traces. To produce the series shown, the amplitude of the prolonged component of current was gradually increased. Lower: tracings from a similar experiment, showing the range of potentials produced by the currents below the dotted line baseline. The rising phase of the waveforms is not accurately drawn. It is clear that currents which decline monotonically after an early translent can produce potentials that have aminimum. Both series were produced in the absence of nerve stimulation or chemical treatment; similar results could be obtained with pipettes inserted anywhere in the musclefbre. Resting potential: about -84mV in both preparations. Calibrations: upper series, 2 mV, 1 msec, 5 × 10-6 amp. Lower traces as shown.

drug treatment (Fig. 3). The presence or absence of drugs during such experiments with injected currents did not change the potential which was recorded, showing that the relevant effect of the drugs is to change the time course of the epc.

The observation that currents which decline monotonically after an early peak can produce potential changes which have two maxima of roughly equal amplitude can be understood in terms of the model of muscle membrane impedance which was proposed by Falk and Fatt^{1,2}. The rapid decay of potential which can be observed after a current lasting less than 2 msec (Figs. 2C and 3) primarily reflects the re-distribution of charge from c_m to c_e through r_e (Fig. 1). The continued rise of potential during the falling phase of a prolonged current from 2 up to 20 msec after its beginning (Fig. 3) reflects the continued charging of c_m and c_e effectively in parallel with r_m . Qualitatively similar phenomena do occur in a simple rc cable (with transverse elements resulting from r_e in Fig. 1 being shortcircuited13,14), for the capacity of parts of the membrane distant from the point where current is injected has to be charged up through the longitudinal conductance of the core. This effect is, however, much less pronounced than that which is caused by the presence of a resistance as represented by r_o in Fig. 1, and calculations have shown that currents similar to those shown in Fig. 3, when injected into a simple rc cable, give rise to potential changes which do not show a minimum. For the normal epp (including that recorded during treatment with d-TC) which is generated by a current of 2 to 4 msec duration, the complicated nature of the muscle impedance is not evident when potential change is correlated with current. During treatment with drugs QX-314 or QX-222, the endplate current has an early brief component, and, in the latter case, a prolonged component. In such cases it becomes necessary to adopt a more realistic model of the muscle impedance to explain the endplate potentials which were observed.

The relations noted in this study between current. both physiological and artificially produced, and the potential change produced across the muscle fibre membrane do not extend the elegant experiments of Falk and Fatt, but do provide a simple qualitative demonstration of the complexity of muscle impedance. indicate that it is necessary to adopt a realistic view of the impedance of the muscle fibre membrane in any studies of drugs which change the time course of the epc (refs. 16 and 17).

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Depressing Effect of Methamphetamine on Self-stimulation in the Cat

It has been shown that the lateral hypothalamus is involved in the feeding response and also in the rewarding reaction to certain stimuli¹. This suggests that amphetamine, an anorexic agent, should depress the self-stimulation of the lateral hypothalamus. Several investigators, using the variable interval as a reinforcement schedule. have, however, reported that the amphetamines, at relatively small doses, had facilitating effects—that is, they caused an increase in response and a lowering of the threshold². In contrast to these observations, the present study shows that methamphetamine, in appropriate doses which depress food and water intake, decreased the rate of response and elevated the threshold for self-stimulation of the lateral hypothalamus.

Bipolar, enamelled stainless-steel wire electrodes were implanted in the lateral hypothalamus in cats (two pairs in each animal). Two weeks after the operation, each animal was stimulated through the electrodes in a behaviour-observation cage. Stimulus-bound feeding responses3 were obtained in seven out of twenty-eight cats. These seven animals were tested for self-stimulation in a modified Skinner box. In this experiment, every bar-pressing was reinforced by intracranial stimulation; a 0.2 sec train of 100 p.p.s., 1 msec pulse from a square wave generator.

Table 1. THRESHOLDS FOR FEEDING BEHAVIOUR AND REWARD, AND RATES OF SELF-STIMULATION BY THE OPTIMAL CURRENTS

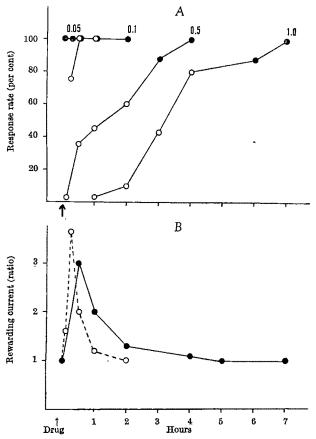
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High rates of self-stimulation were obtained in all seven cats when they were deprived of food for 24 h. Table 1 shows the threshold currents for reward, rates of barpressing by the optimal currents and the thresholds for feeding responses. In six out of seven cats the optimal currents for self-stimulation were lower than the threshold currents for feeding responses. In the self-stimulation experiment, the currents which were strong enough to elicit feeding responses usually induced forced motor movements such as "sniffing" or "searching", but this behaviour did not prevent the cat from continuous bar-pressing.

Small doses of methamphetamine (0.01-0.05 mg/kg, intravenously) had no apparent effects on normal feeding behaviour, while doses larger than 0.1 mg/kg temporarily inhibited the food intake of the animal. Doses of more than 2.0 mg/kg were apparently an overdose for this experiment because the animals showed immobility accompanied by piloerection and pupil dilatation. These results show that the dose should be lower than 2.0 mg/kg to observe the intrinsic effect of methamphetamine. Accordingly, methamphetamine hydrochloride in doses of 0.05-1.0 mg/kg was twice administered intravenously in random order and at least I week was allowed before another injection.

Fig. 1A shows dose-response relation in seven animals. Methamphetamine of 0.05 mg/kg had no effect and 0.1 mg/kg decreased the rate of response to 76 per cent of the control during 5-15 min after injection, while doses of 0.5-1.0 mg/kg markedly depressed the rate of barpressing. It was repeatedly observed that some animals kept the foreleg on the lever for a few seconds while others reached for the lever with apparent hesitation. As mentioned before, these depressing effects were not caused by overdose or overstimulation, because neither increase in locomotive activity nor ataxia with pupil dilatation was observed.

The effects of the drug on the threshold for self-stimulation were observed by adjusting the stimulus current to a level which barely maintained a stable rate of barpressing response (compare Table 1). Soon after injection of methamphetamine 0.5-1.0 mg/kg, bar-pressing became



irregular and current was manually adjusted to maintain a stable response rate. These experiments showed that methamphetamine elevated the threshold for self-stimulation by two or three times during 15-75 min after injection. This depressing effect was further confirmed by results obtained by the administration of L-DOPA. It has been reported that the central action of the amphetamines is mediated through the catecholamines4. This suggests that the catecholamines should have a similar effect to methamphetamine on self-stimulation. To explore this possibility, L-DOPA, which crosses the blood-brain barrier readily, has been used intravenously at doses of 5-10 mg/kg, which were able to antagonize the effects of reserpine, 0.1 mg/kg, subcutaneously, that is, cats which had been sedated by reserpine were awakened by injection of L-DOPA. At these doses the effect of L-DOPA was to raise the threshold for self-stimulation which was similar to the effect produced by methamphetamine. The duration of the effect was, however, relatively short, probably because of rapid metabolism by monoamine oxidase. A typical result is shown in Fig. 1B and it supports the conclusion on the action of methamphetamine.

Why then was facilitation observed in the experiments of Stein and other investigators? The different results obtained in this and earlier studies seem to be the result of the reinforcement schedule. Dews and Morse⁵ have pointed out that the effect of amphetamine on behaviour is influenced by the control or the predrug rate of response. As mentioned before, Stein and others² have used the variable or fixed interval schedules which strongly inhibited the output of behaviour. Using these schedules the amphetamines could have acted to increase the rate of response even when reinforcement was food or water. The facilitating effects observed in earlier studies could therefore have been the result of depression by the experimental schedules, that is, amphetamine merely released the animal from the inhibition which was imposed by the reinforcement schedule. If this is so, the facilitating effects of amphetamine on behaviour were not necessarily the result of the effects of the agent on the lateral hypothalamus. It should be pointed out that in the earlier experiments² drugs were effective at relatively small doses which were not enough to inhibit the normal feeding behaviour.

The present study shows that, under the continuous reinforcement schedule, methamphetamine in doses which depress normal feeding responses also depresses the selfstimulation of the lateral hypothalamus. This leads to the conclusion that methamphetamine has depressing effects on the mechanism which is involved in the feeding and rewarding reaction in the lateral hypothalamus.

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Binding of Cortisol to Human Albumin

An investigation of the binding of cortisol to human plasma proteins led us to re-examine the interaction between cortisol and pure human albumin. Previous workers1-3 have obtained widely differing results for the percentage of cortisol which was bound by a given concentration of albumin, and Brunkhorst and Hess4 have suggested that the number of available binding sites/mole of albumin increases as the albumin solution is diluted. Our results confirm the quantitative findings of Chen et al.3 and suggest that some of the discrepancies in this field may be the result of experimental artefact.

Albumin was obtained from Calbiochem as recrystallized human albumin grade B. It was seen to consist of only one immunological component when reacted in immunoelectrophoresis against anti-human serum (Burroughs Wellcome). Albumin solutions were prepared in 0.07 molar phosphate buffer, pH 7.4, and were then standardized by micro-Kjeldahl estimations using a factor for protein nitrogen of 6.4 (ref. 5). Paper chromatography of 1,3-3H-cortisol obtained from Amersham showed that it was 98 per cent pure.

Samples of cortisol which had been treated with ethanol were taken to dryness under nitrogen; albumin solution was added and the mixture was incubated for 30 min at 37° C. Complete solution was shown by radioactive counting. The concentrations of cortisol which were employed ranged from 0-4 to 400 μ g/100 ml. Ultrafiltration was carried out through 8/32 'Visking' tubing under 4 atm. pressure at 37° C (ref. 6). Within 30 min, five sequential samples of ultrafiltrate of 0.5 ml. volume were obtained from each 5 ml. of albumin solution. percentage of ultrafiltered cortisol was derived from liquid scintillation counting of the original albumin solution and the ultrafiltrate. Loss of cortisol by adsorption onto the sac was noted with aqueous solutions and to a lesser extent with albumin solutions. A plateau value for ultrafiltered cortisol was always reached by the fourth 0.5 ml. sample and this value was accepted as representative of the true level of cortisol in free solution within the sac. We were unable to eliminate the adsorptive loss by any prewashing of the 'Visking' tubing in water or ethanol at room temperature or at 60° C.

Contrary to the expectation of some workers1,7 have found that ultrafiltration of up to 70 per cent of the sac volume does not disturb the initial equilibrium position between bound and free cortisol either in albumin solutions or in plasma, and this finding is in accord with mathematical considerations based on mass action theory8.

A 3.2 per cent solution of albumin which was loaded with cortisol at concentrations of 0.4, 20 and 400 μg/100 ml. gave plateau values for percentage of free cortisol of 38, 39 and 39, respectively. The apparently enhanced binding of cortisol to albumin at low concentrations of cortisol which was noted by some workers4 was not found. This might be the result of adsorptive loss or contamination of albumin with transcortin. The fixed percentage of binding over a wide range of concentrations of cortisol conforms to the hypothesis that albumin has a very large binding capacity for cortisol. In these circumstances, it can be shown1 that the ratio of bound to free cortisol will vary linearly with the albumin concentration, provided that the number of binding sites/mole of albumin remains constant. A relationship of this type has been shown for salicylate binding to albumin. Results obtained with a cortisol concentration of 375 µg/100 ml. and albumin concentrations ranging from 0.01 per cent to 3.2 per cent are shown in Fig. 1 (line A). Line B represents the result which is obtained if the first aliquots of ultrafiltrate are used. The upward displacement of the line could represent increased avidity for cortisol at low albumin con-centrations but is in fact entirely the result of adsorptive

Keller et al.7 noted a decrease in binding of cortisol to rat plasma in the presence of ethanol, and we have confirmed this observation using albumin solutions and human plasma. Additions of 1 per cent and 3.5 per cent ethanol to 3.2 per cent albumin increased the percentage of free

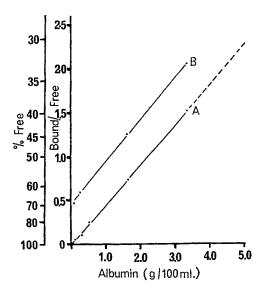


Fig. 1. Relationship between concentration of albumin and binding of cortisol at a total cortisol concentration of 375 μ g/100 ml.

cortisol from 39 per cent to 41 per cent and 50 per cent, respectively.

Extrapolation of line A to 5 per cent albumin suggests that 30 per cent of cortisol would be free and this agrees closely with the value of 31 per cent free found by Chen et al. at this albumin concentration. We have failed to demonstrate enhanced binding by low concentrations of albumin. The variety of results that have been obtained for cortisol-albumin interaction may be a consequence of albumin impurity, use of ethanol as a vehicle for cortisol or adsorptive loss onto semipermeable membrane.

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Estimation of the Respiratory Rate of Young Plaice (Pleuronectes' platessa L.) in Natural Conditions using Zinc-65

THE dynamics of a population of 0 group plaice (that is, fish less than 1 year old) which live near the low water mark is being studied as part of an investigation of food chains on a sandy beach in Loch Ewe, Wester Ross. In this work, it is of prime importance that the respiratory rate of the fish in the sea be estimated, because the energy used for metabolism cannot be calculated unless this rate is known. Until recently it has been impossible to measure the respiratory rate of a fish in its natural environment. Mishima and Odum¹ have suggested, however, that the isotope zinc-65 may be used to measure this rate. These workers found that the elimination rate of zinc-65 from the body of the molluse Littorina irrorata was related to the air temperature and to the body size of the animals, and they suggested that the isotope might be used to study the respiratory rate of the animal in its natural environment.

In the present study, plaice varying in length from 2 to 10 cm were used. Experiments were performed to measure simultaneously the respiratory rate and the elimination rate of zine-65 by plaice which were kept at different temperatures and different feeding levels. The fish were given tracer doses of isotope by introduction of zinc-65 chloride into the water containing the fish. After 48 h the fish were removed and washed, and were then placed in fresh sea water. Whole body counts were made in a Panax scintillation counter, type ZM 28, containing a 1 in. × 1.5 in. sodium iodide crystal, situated 0.25 in. away from the counting tray. The respiratory rate of the fish was measured in a closed system respirometer incorporating a Beckman oxygen electrode, model 777.

The pattern of elimination of the isotope from a group of six plaice held at 16° C is shown in Fig. 1, where all counts have been corrected for decay of the isotope. The fish

Table 1. ELIMINATION RATE OF ZINC-65 IN THE PERIOD DAY 7-DAY 17 IN 0 GROUP PLAIGE IN RELATION TO RESPIRATORY RATE

Temperature (° C)	Measured oxygen consumption cc/g/h	Percentage of zinc-65 excreted in the period day 7-day 17	Feeding level of plaice
8.5	0.07	1.0	Starved
16·0	0.25	2.75	,,
20.0	0.41	6.0	12
22.0	0.44	9-0	"
10.0	0.20	2.5	Excess
10.0	0.44	9-0	***
16.0	0.37	4.75	Maintenance
17.0	0.62	12.5	Excess
17.0	0.62	12.5	,,
18.0	0.75	26.5	"
20.0	0.72	23.5	10
20.0	0.50	12.5	"
	7.00		,,

were fed at maintenance level during the experiment, and had a mean respiratory rate of 0.37 cc of oxygen/g live weight/h. There was an initial rapid loss up to the fifth day, followed by a slower loss from day 5 to day 43, after which time the isotope became stabilized within the body. Within the limits of accuracy of these results, a linear relation for the period day 5 to day 43 seems adequate. Similar observations on the elimination rate of isotopes from the body have been observed by Hoss² using cerium-144 in flounders and by Graham and Telle³ using zinc-65 in rabbits.

The mechanism by which the zinc-65 is eliminated from the body has not been determined in the present case. Estimates were made of the amount of activity in different tissues in the body, 5 days after removal from the isotope. It was then observed that the stomach and intestine had the highest activities, followed by skin, skeleton, muscle, liver, kidney and blood in approximately that order. Certain enzymes, for example, carbonic anhydrase, glutamic dehydrogenase, and the carboxypeptidase enzymes of the intestine, are known to contain zinc.

To obtain a range of respiratory and zinc-65 elimination rates, groups of five-ten plaice of similar sizes were kept at different temperatures: some groups were starved and others were fed. The elimination rate of the isotope was measured from day 7 onwards, and some of the graphs thus obtained are shown in Fig. 2. It will be seen that as the respiratory rate increases, so also does the elimination rate of the isotope. The number of counts/min of each fish during day 7 was considered to be 100 per cent, and further measurements were expressed as a percentage of this. Because in most cases the results for the period day 7-day 17 appeared linear, they were used as the standard for comparison with respiration. A summary of the results so obtained is given in Table 1. In Fig. 3, the percentage of the isotope eliminated in this period has

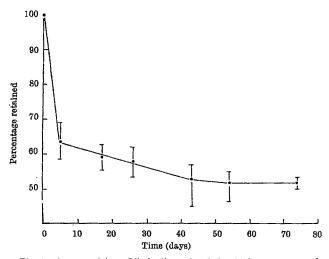


Fig. 1. 0 group plate. Elimination rate of zinc-65 from six fish at 16° C. Vertical ranges indicate extremes of individual variation.

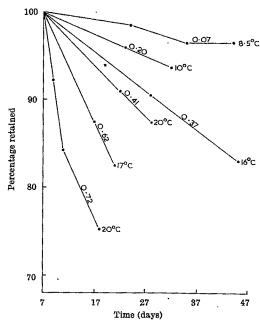


Fig. 2. 0 group plaice. Elimination rate of zinc-65 from 7 days onwards at different respiratory rates. Temperature and oxygen consumption as cc/g/h are given on graphs.

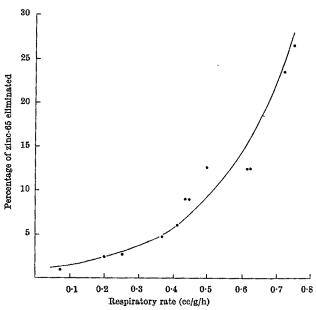


Fig. 3. 0 group plaice. Relationship of zinc-65 eliminated in the period day 7-day 17 with respiratory rate. $Y=0.9337e^{4-8563}X$; standard error of exponent= ± 0.1258 .

been plotted against the respiratory rate; this gives a relation which seems to be independent of temperature.

After this relationship had been established for fish which were confined in containers in the laboratory, the elimination rate of the isotope in place held in a large outdoor tank and in netting tanks on the sea bed was measured. The fish which were used in these experiments were individually marked by subcutaneous injections of coloured liquid latex as described by Riley⁴.

In the first experiment a concrete tank measuring 9 ft. \times 3 ft. \times 3 ft. was connected to a pumped sea water supply and the bottom was covered with sand from the spring tide low water mark. This sand was obtained from the natural habitat of the 0 group plaice, and the fish were fed on animals which were in and above the sand. Twelve 0 group plaice varying in length from 7 to 10 cm were put

into the tank and were fed daily on chopped mussels. The mean quantity of isotope eliminated in the period day 7-day 17 was 8.0 per cent, the pattern of elimination being similar to that in Fig. 1. The mean water temperature was 12.5° C. In the second experiment, sixty plaice varying in length from 3.2 to 5.9 cm were placed in two netting tanks on the sea bed in Loch Ewe, just below the low water spring tide mark. These tanks, which were 12 ft. \times 6 ft. \times 1 ft. high, were made of nylon mesh 5 mm thick, held in a metal frame as described by Steele⁵. The tanks had previously been filled to a depth of 3 in. with sand from the spring tide low water mark. The mean quantity of isotope eliminated in the period day 7-day 17 was 18.25 per cent, the mean water temperature being 14° C. In Fig. 3, a respiratory rate of 0.47 cc/g/h (95 per cent limits 0.45-0.50) is indicated for the first experiment and of 0.65 cc/g/h (95 per cent limits 0.62-0.70) is indicated for the second experiment.

A "standard" respiratory rate for fish has been defined (Fry and Hart⁶) as that obtained from fish which had been fed up until 24 h before measurement and at rest during the period of measurement. In the first experiment, resting plaice of this size had a "standard" respiratory rate of 0.20 cc/g/h at 12.5° C and in the second experiment 0.32 cc/g/h at 14° C. Thus the fish in the first experiment had a respiratory rate of 2.35 times, and the fish in the second experiment a rate of 2.03 times the "standard" rate.

This extra metabolic activity must be attributed to movement in these tanks. In the laboratory experiments where fish were kept in small containers, it was not possible to obtain comparisons of the rate of elimination of zinc-65 and the respiratory rate of actively moving fish over long periods. In short term respiration experiments, however, rates of three-four times the "standard" rate were obtained for actively swimming fish. Thus the respiratory rates of the fish in the tanks lie within the scope of activity measured for 0 group plaice in the laboratory.

The value of 2.03 times the "standard" rate which was obtained in fairly natural conditions is very close to the value of two proposed by Winberg' and provides a basis for calculating the metabolic requirements of the natural population of 0 group plaice in Loch Ewe. Variations in the environment, however, particularly in quantity and quality of food, could affect this value, and it is hoped to undertake more detailed studies to test this method.

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Effect on Brain Enzyme and Behaviour in the Rat of Visual Pattern Restriction in Early Life

SEVERAL studies have shown that certain behavioural and biochemical changes can be produced in rats if they are raised in an "enriched environment" or subjected to stress or handling during infancy. Typically, an enriched environment is created by placing some small objects or mates in the cage of the animal or by handling the animal every day for a specified period of time. All these manipulations require an extended period of physical contact either with other animals, with the experimenter or with

both. When animals are handled or permitted to live together, the resulting temperature differential alone can produce physiological changes2, so it is not clear whether some behavioural or physiological changes can be produced in an adult animal by manipulating just the visual or auditory environment in infancy. By eliminating group living and handling, and manipulating only the visual complexity of the environment in which rats were raised, we found significant differences in body weight, brain cholinesterase (ChE) activity and sensory conditioning.

Nineteen litter-mate female Holtzman albino rats, weaned at 30 days of age and matched for body weight, were randomly assigned to either a visual pattern (VP) or a restricted visual pattern (RVP) group. The visual environment of both groups was restricted by a wooden enclosure extending 30.48 cm beyond the front of the cage. The enclosure of the VP group had alternate black and white stripes painted on it, while the enclosure of the RVP group was painted flat white. Each animal was placed in an individual wire mesh cage and remained in its experimental environment, and was supplied with unlimited food and water until it was 90 days of age. The overhead fluorescent illumination diffused light evenly over all cages and a 12 h light-dark cycle was maintained. The animals were never touched nor were their cages removed throughout the experimental period except when the animals were weighed and rated for emotionality3 at 60 days of age. The original experiment which contained only seven rats (four in the VP and three in the RVP group) was replicated with twelve rats (six in each group). Because the trend of behavioural tests and weight gain was identical for original and replicated experiments, all the data have been pooled.

At 90 days of age all animals were weighed and rated for emotionality a second time. The animals did not differ in emotionality rating either when 60 or 90 days old. The VP group, however, gained significantly more body weight than did the RVP group at both 60 and 90 days of age. As evident from Table 1, the VP group also maintained its higher weight gains than the RVP group at 101 days of age when final body weights were obtained.

		ľ	able 1		
Rearing condition	Body weight (g)	30	60 Age	(days) 90	101
VP	$_{M}^{\mathrm{Mean}}$	$92.3 \\ 2.76$	206·8 3·43	251·3 5·85	273·2 9·82
RVP	Mean M	92·2 2·82	199.9 3.26	237·8 2·49	248·5 4·35
Difference	27.4	0.1	6.5*	13.5*	23.7*

* Significant at 0.05 level.

Mean body weights for restricted visual pattern group (RVP) and visual pattern group (VP) for the four weighing periods.

We also tested all animals for spontaneous activity in a photoelectric activity cage for 12 h each day for three consecutive days, but within-group variability was excessive and the differences in activity were not significant. Finally, we tested all animals for sensory reinforcement for 7 days, at the conclusion of which the animals were killed and their brains were removed for measurement of ChE activity.

The sensory reinforcement test consists of conditioning an animal to perform a specific task, usually touching a lever, when the only reward is a flash of light in a lightproof chamber in which the animal is working4. We used a sound- and light-proof chamber (28.57 cm × 19.68 cm × 23.49 cm) which contained a stationary lever on one side, 8.89 cm above the floor. The lever was connected to a contact relay which, when touched, could activate a flash of light (6 W) for 0.75 sec in the box and a counter. Each animal was placed for 25 min daily for 7 consecutive days and the number of times the lever was touched was recorded. For the first 3 (adaptation) and last 3 (extinction) days, touching the lever did not activate the light in the box, but the number of touches was recorded. On the 2 days of conditioning, however, the flash of light accompanied each touch of the lever. There were no

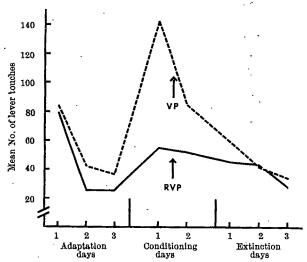


Fig. 1. Mean number of touches by visual pattern (VP) and restricted visual pattern (RVP) groups on sensory reinforcement test. The light flashes were contingent on the lever touching only on conditioning days.

significant differences between the groups on adaptation and extinction days, but the VP group made significantly more responses than the RVP group (P=0.05) on the

days of conditioning (Fig. 1).

Measures of brain ChE activity were obtained for only the twelve animals which were used in the replication. Animals were decapitated and their brains were removed. The brains were weighed and cut into equal anterior and posterior halves. For each half, the cortical (lateral, ventral and dorsal) and subcortical tissues were divided. The four samples thus obtained for each rat (anterior cortex, posterior cortex, anterior subcortex and posterior subcortex) were individually weighed and frozen in dry All samples were then coded so that the analyst could determine whether the tissue was cortical or subcortical, but he was unaware from which group the tissue was taken. The ChE activity was determined by the rate of hydrolysis of acetylcholine perchlorate (ACh). The procedure employed was identical to that of Rosenzweig, Krech and Bennett⁵.

	•	Table 2		
Rearing condition	ChE activity*	Anterior cortex	Posterior cortex	Total cortex
VP	$_{M}^{\mathrm{Mean}}$	55·00† 7·90	55·80 ‡ 4·55	53·09 ‡ 4·45
RVP	Mean M	38·67† 5·11	27·33 ‡ 6·06	33·00 ‡ 4·02
Difference		16.33 †	28.47	20·00 ±

All ChE values are in moles ACh × 10¹⁰ hydrolysed/min/mg of tissue.
 † Significant at 0.05 level.
 † Significant at 0.01 level.

The ChE activity for restricted visual pattern group (RVP) and visual pattern group (VP).

Because the subcortical ChE activity between VP and RVP groups was not significantly different, Table 2 presents the ChE activity for the cortical tissue samples only. The posterior cortex sample, which contained the visual cortex, showed significantly greater ChE activity for the VP group than for the RVP group. The ChE activity between anterior and posterior cortical samples within a group, either VP or RVP, did not show significant differences. When the total cortex between the groups was compared, however, the ChE activity for the VP group was much higher than that for the RVP group. Our findings are not in agreement with those of Rosenzweig et .al.1; they found decreased ChE activity in the cortex and increased activity in the subcortex of those rats which were raised in an enriched environment. Our brain samples are not strictly comparable with those in their study, however, because our cortical samples contained lateral, .dorsal and ventral portions of cortex, while in their study only the lateral portion of the cortex was used1,

The results of our study strongly suggest that visual pattern restriction or enrichment in early life may be a critical factor in those studies which have shown behavioural, physiological and biochemical changes in the adult animals raised in groups or handled during infancy. The fact that greater body weight and increased ChE activity of brain can be induced by manipulating visual patterns indicates that the nature of visual input in infancy may cause enduring alteration in some basic physiological mechanisms.

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Cortical Activity preceding Speech: Semantic Specificity

Reliable, non-random changes in the electrical activity of the human brain, designated "cortical command potentials", have been observed preceding voluntary hand and foot movements1, finger pressing2 and speech3. Command potentials which have been detected to date have shown relatively undifferentiated uniphasic or biphasic waveforms over the contralateral motor area; no specificity with respect to the voluntary activity time-locked to these potentials has been reported.

This communication shows that average command potentials from the human scalp, preceding and timelocked to speech onset, are characteristically different for the spoken letters "T", "O" and "P". This specificity of command potentials prevails over both the right sensori-motor area and the left temporal "speech association" area; characteristic differences have also been noted between the areas.

The EEG of one young adult male (myself) was measured with bipolar scalp contact electrodes over the right sensorimotor area (C4 to A2 with ground to A1 in the 10-20 system) and the left temporal "speech association" area (T5 to A1 with ground to A2) and recorded on multichannel magnetic tape in a bandwidth 3 dB down at 3 and 100 c/s. A microphone pickup of spoken words triggered a voice-activated switch* which produced a pulse at the audible onset of each spoken word. This pulse was shaped, delayed by 100 msec and recorded on FM tape together with the amplified, non-filtered microphone voice pickup. In any given experiment, I spoke the designated words fifty times each at a self-paced rate of about once every 2 sec. Fifteen experiments were conducted in all, each on separate days over a 2 month period, using the spoken letters "T", "O" and "P", the numbers "2" and "10" and the words "yes" and "no".

Tape recorded EEG data were filtered 6 dB down at

3 and 20 c/s to eliminate possible movement and muscle

^{*} Exploratory studies with all letters in the alphabet indicated that the spoken letters "T", "O" and "P" showed greatest temporal stability in triggering the Mousseau Scientific Instruments AT-1 voice activated switch.

artefact. The tape was then played backwards into the ND-801 'Enhancetron' digital computer which extracted cortical activity time-locked to the trigger pulse representing the onset of each spoken word. The delay which was introduced when recording permitted, on playback, demonstration of summated EEG and voice activity 525 msec before and 100 msec after the onset of a given word.

before and 100 msec after the onset of a given word.

Results for the spoken letters "T", "O" and "P" on different occasions and from two cortical areas are presented in Fig. 1. Command potentials preceding these spoken letters were stable during several experiments (traces 1-2; 3-4; 5-6; 8-9; 10-11; 12-13); they were different for different spoken letters when recording from the same cortical area (traces 1, 2-3, 4-5, 6; 8, 9-10, 11-12, 13), and were unique in the two cortical areas preceding the same spoken letter (traces 1, 2-8, 9; 3, 4-10, 11; 5, 6-12, 13). The non-filtered microphone pickup of the spoken letters which was averaged by computer indicates the stability of the trigger point (traces 7 and 14). Reliable command potential components occurred as early as 310 msec before voice onset. Command potentials preceding the spoken numbers "2" and "10" and the words "yes" and "no" were not as reliable as those preceding the letters because of less stable triggering of the voice activated switch. These numbers and words, however, also seem to show specificity in their respective command potentials.

Previous work with sub-dural electrodes¹ and low frequency filtering to selectively average high frequency muscle artefact activity³ indicates that command potentials preceding voluntary movement and speech are of cortical origin. Speculation regarding the origin and

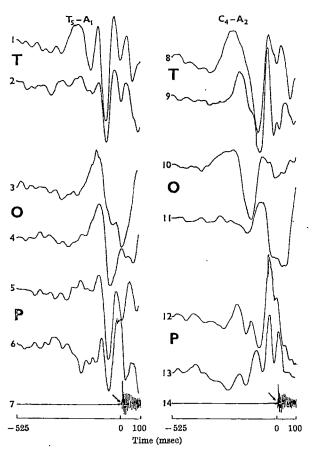


Fig. 1. Average cortical potentials preceding and 100 msec following the spoken onset of the letters "T", "O" and "P" as recorded from the left temporal "speech association" area (75-41) and the right sensori-motor area (C4-A2). Each trace is the average of fifty EEG samples obtained on separate experimental occasions. Arrow indicates point of voice onset.

significance of the specificity of cortical command potentials is undoubtedly premature. Tentatively, it could be suggested that the later pre-voice onset components (latencies up to 200 msec) might correspond to the initiation of pyramidal tract neuronal firing which controls the vocal musculature specific to a particular word while the early pre-voice components (latencies over 200 msec) might be thought of as the electrophysiological signs, associated with the act of selecting and deciding to speak a given word.

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Rheology of Microspheres injected into Circulation of Rabbits

A RECENT innovation in the measurement of regional blood flow in animals is the use of radioactively labelled, rigid, inert microspheres of 50μ ($\pm 20\mu$) diameter which are injected into the circulation. The amount of radioactivity in an organ is taken as an index of the number of spheres trapped by the microcirculation of the organ and, thus, of the relative blood flow to that organ.

Determination of the distribution of these spheres in the blood flowing through the larger arteries is useful not only as a check of the validity of the method but also as a source of information about the rheology of mixed suspensions of particles of different characteristics. We have studied the distribution of these microspheres within flowing blood by ultra-rapid freezing of perfused medium-sized arteries in living rabbits. The method for ultra-rapid freezing of the femoral artery of a rabbit in vivo and for subsequent histological processing has been described^{2,3}. With this technique, blood flowing through the segment of artery is immobilized in less than 0.05 sec (ref. 2) and the cross-sections of the artery show an end-on view of the blood as it was when flow was stopped.

In each of two anaesthetized adult rabbits in which the medial branch of the femoral artery (internal diameters 1.0 and 1.1 mm) was exposed and prepared for freezing, approximately 5×10^4 plastic microspheres suspended in 1 ml. of saline containing 'Tween-80' (1:2,000) were injected into the left ventricle of the heart through a catheter passed through the right carotid artery. injection took 5 sec and the femoral arteries were frozen immediately afterwards. In the light microscope, microspheres appeared as semi-transparent amber-coloured disks with no underlying erythrocytes (Fig. 1). (The amber colour may have been the result of the iodine-125 with which this batch of spheres was labelled.) There was seldom more than one sphere in each 8-15µ thick section. Each sphere was surrounded by a layer, two to three cells thick, of closely packed erythrocytes, each with its long axis at a tangent to the curve of the sphere. (This clustering of erythrocytes around the microspheres was not present in a mixture of whole blood and microspheres on a microscopic slide, where there was no flow.) Otherwise, the erythrocyte orientation was the same as in other arteries which were frozen by this technique³. Fig. 2 shows the location of all spheres found in serial sections of one segment of a frozen artery 6 mm long.

The distance from the centre of each sphere to the centre of the vessel was measured with an eyepiece micrometer. This distance was expressed as a fraction of the internal radius of the artery; the artery radius being divided arbitrarily into ten units. A histogram (Fig. 3) was made of the number of microspheres in each of five designated intervals of radial distance. The intervals increased from centre to edge of the artery as a function of the square root so that the specific area of artery described by each interval was equal to each of the others. Thus the number of spheres found in each interval could be compared with the number in any other interval. There was a marked change in microsphere concentration, with a peak concentration at about half the radial distance from the centre of the arteries and a progressive decrease peripherally. These differences in concentration are statistically significant when evaluated by the chi square test (P < 0.001).

The axial accumulation of the microspheres (Fig. 3) was similar to that observed in suspensions of neutrally buoyant spheres flowing through rigid tubes4,5. It resembles more closely the pattern which is observed when the spheres are not prevented from spinning as they flow⁵. The radial variation in the concentration of microspheres extends much further in from the periphery than would be expected from wall exclusion or entrance effect. This suggests that the spheres have been acted on by a force which operates over at least the peripheral half of the vessel radius.

It is likely that the microspheres in the arteries spin in the flowing blood. The sheer across such a relatively large particle in laminar flow would tend to make it spin about an axis parallel to the plane of sheer in laminar flow. Usually, in such arteries, erythrocytes would be oriented with the long axis parallel to the plane of sheer

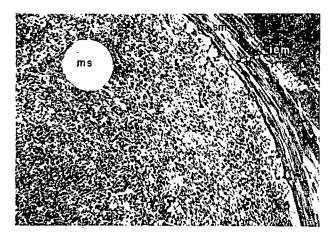


Fig. 1. Photomicrograph of cross-section of ultra-rapidly frozen rabbit femoral artery. Note orientation of erythrocytes around the microsphere. sm, Smooth muscle of arterial wall; iem, internal elastic membrane; ms, microsphere.

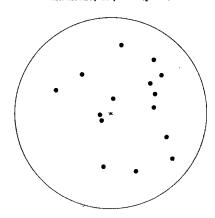


Fig. 2. Map of all microspheres in one segment of frozen artery 6 mm long. * Centre of vessel.

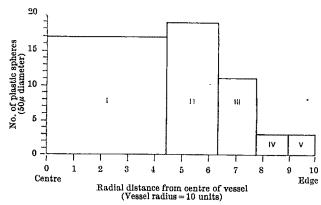


Fig. 3. Itadial distribution of all microspheres found in all segments of both frozen arteries. Cross-sectional area defined by interval I=II=III=IV=V. All three microspheres in area V were touching the edge of the artery.

of laminar flow3. The re-orientation of the erythrocytes immediately surrounding the microspheres suggests a change in the normal pattern of flow, such as would be expected if the microspheres were spinning.

There is no generalized axial accumulation of blood cells in normal blood flowing through such arteries. The only change in the concentration of erythrocytes is a slight decrease at the periphery, which can be attributed to wall effect (entrance effect)3. Concentration of leucocytes tends to increase peripherally, with a peak concentration at one-tenth to two-tenths the radial distance in from the periphery². The distribution of microspheres, which are both larger and more rigid than leucocytes and erythrocytes, shows that axial accumulation of particles can occur in these arteries even though the microspheres must move inward through the crowded mass of erythro-

There was no evident sedimentation of the microspheres, despite the fact that their specific gravity (1.1 to 1.6) was somewhat greater than that of whole blood (1.05). The distribution shown in Fig. 3 suggests that the flow of spheres from such a medium-sized artery into smaller branch arteries is likely to be proportional to the flow of blood into each branch artery.

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RADIOBIOLOGY

Body Burdens of Caesium-137 in Toronto in 1964-1967

SINCE 1958 the caesium-137 body burden of persons living in Toronto who have no history of radioactive contamination apart from that present in the normal population has been measured regularly. When the last report was made the burdens of caesium-137 caused by

fall-out appeared to have passed a maximum—the highest average figure recorded was 23.7 nc. in the winter of 1963-64.

Subjects were tested in the University of Toronto steel room². Two groups of between twenty and twenty-five male subjects were the chief source of all data. As far as possible caesium-137 was counted in the same subjects each year.

Table 1. RECENT WHOLE-BODY MEASUREMENTS OF CAESIUM-137

Period of measurements	Average caesium-137 (nc)	Average body potassium (g)	Ratio of caesium-1 potassium (pc./g
Winter 1964-5	21.2	147	144
Summer 1965	18-1	146	125
Winter 1965-6	13.3	143	93
Summer 1966	9.8	137	71
Winter 1966-7	7.3	147	50
Summer 1967	6.4	143	45

The most recent figure (summer 1967) for the average body burdens of caesium-137 in the sample subjects is 6.7 nc. Results for the past 3 yr are given in Table 1. Table 1 also gives the average body potassium measured in grams, and the resultant ratio of caesium-137: potassium (pc./g). The present results, expressed as a ratio of caesium: potassium, are plotted in Fig. 1, together with the corresponding results from previous years¹.

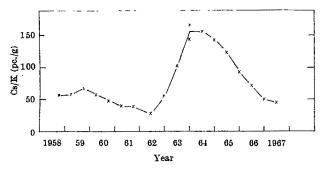


Fig. 1. Average caesium burden of the human body for male adults living in the Toronto area, expressed in relation to the potassium content in the body, as a function of time. The units of measurement are picocuries of caesium-137/g of potassium.

The figure shows that the data for 1963-64 represented a maximum, and that the current levels are approximately those that obtained before the results of the bomb tests of 1961 were noticed. If the data for the period from the winter of 1964-65 until today are approximated by an exponential leading to a constant value of about 30 units, it takes about a year for the caesium burden to be halved, and this is presumably related to the time of residence of fission products in the stratosphere.

Table 2. Comparable figures for body burden for males and females

Period of measurements	Average caesium-137 (nc.)	Average body potassium (g)	Ratio of caesium- 137 : potassium (pc./g)	Range of ratios
Summer 1966				
Males	9.8	137	71	41-148
Females	5.4	97	56	23-98
Autumn 1966				
Males	8.0	146	54	29-99
Females	4.3	94	48	21-80

As part of an epidemiological study of the caesium-137 burden of persons living near sites of future nuclear power stations, measurements were carried out on male and female subjects from one area. Each group consisted of between twenty and thirty subjects. The average caesium-137, potassium and caesium: potassium levels for the men and women are shown in Table 2. These results help to confirm the observed differences in the ratios of caesium: potassium between the sexes.

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BIOLOGY

Effect of High Concentration of Carbon Dioxide on the Ionic Composition of Rainbow Trout Blood

Previous work has demonstrated that although moderately high concentrations of free carbon dioxide in the water decrease the ability of fish to extract oxygen from the water 1.2 and to withstand low oxygen concentrations 2. this effect is diminished if the fish become acclimatized to the level of free carbon dioxide which is present 2.4. It is known that acclimatization involves an increase in the alkali reserve of the blood to maintain the pH value at the normal venous level of 7.2 (ref. 5). The experiment reported here was made to determine changes in other ionic components of the blood, as part of a wider programme to investigate the reactions of fish to physical and chemical changes in their environment.

Tests were made with yearling rainbow trout (Salmo giardnerii Richardson) held in aquaria of 40 l. capacity and supplied with a hard de-chlorinated tapwater and aerated with controlled mixtures of air and carbon Concentrations of free carbon dioxide in the water were calculated from the pH value, bicarbonate alkalinity, temperature and total solids, using the monograms of Dye. Temperatures were not controlled and ranged from 12.5° to 16.0° C, with variations of not more than ±1.5° C for any single experiment. Fish were transferred from normal aerated water to the test aquaria and, after a fixed interval, were anaesthetized with MS 222; they were then removed and a sample of cardiac blood was Control fish were used with each experiment. Blood samples from groups of at least three fish were pooled, allowed to clot, centrifuged and the serum was analysed for sodium, bicarbonate (Conway microdiffusion technique?), chloride (EEl chloride meter) and phosphate*. At least two replicates were made for each test condition run with high levels of free carbon dioxide.

Results of the experiments are shown in Fig. 1. With 35 p.p.m. of free carbon dioxide in the water, the bicarbonate in the serum rose from the control level of 6.75 mequiv./l. to 32.2 mequiv./l. within 24 h, and to 39.5 mequiv./l. with 65 p.p.m. of free carbon dioxide; somewhat higher than that given for whole rainbow trout blood by Lloyd and Jordan⁵ of 30.1 mequiv./l. after exposure of trout for 6 days to 50 p.p.m. of free carbon dioxide. The increase in bicarbonate concentration was fairly slow, being only half complete at 6 h, and a roughly corresponding decrease occurred in serum chloride levels, which fell from 152.5 mequiv./l. to 114.6 mequiv./l. after 24 h at a concentration of 35 p.p.m. of free carbon dioxide and 111.0 mequiv./l. with 65 p.p.m. of free carbon dioxide.

Sodium concentrations in the serum did not differ significantly from the controls, neither did the levels of inorganic phosphate (average 12.7 mequiv./l.). change in serum chloride content of fish which were exposed to 35 p.p.m. of free carbon dioxide for 24 h was, however, 13 mequiv./l. greater than the corresponding change in bicarbonate level; the nature of the extra anionic component to compensate for this is not known.

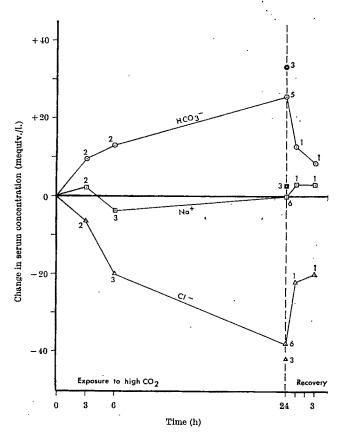


Fig. 1. Change in serum sodium, chloride and bicarbonate concentrations of rainbow trout exposed to high levels of free carbon dioxide. Open symbols: 35 p.p.m. of free CO₂; closed symbols: 65 p.p.m. of free CO₂. Figures against each point are the number of replicate tests made. Control values (mequiv./l.) with standard deviations: sodium 170 ± 5.7 , chloride 152.5 ± 6.8 , bicarbonate 6.75 ± 2.8 .

On removal of the high level of free carbon dioxide from the water, the serum bicarbonate and chloride levels returned towards the normal levels, but the curves tend to flatten out after 1 h. Even after 3 h, the chloride level was still 20.5 mequiv./l. lower, and the bicarbonate 8.7 mequiv./l. greater, than the controls, indicating that some other anionic component or components totalling about 12 mequiv./l. was still present.

A decrease in the concentration of serum chloride has been observed by several authors in salmon (Salmo salar) during the transition from the parr to the smolt stage and has been thought to be an adaptive stage towards a seawards migration because it is accompanied by changes in the activity of the chloride secretory cells of the gills. A decrease in the serum chloride : sodium ratio has been found for adult salmon in sea water10, and the lower chloride levels were thought to be balanced by the formation of an unknown anionic component. The data presented here show that a decrease in the serum chloride: sodium ratio, accompanied by an increase in bicarbonate level and an unknown anionic component, can also result from an environmental stress which is completely unrelated to problems of osmotic adaptation.

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Gibberellin from Barley Embryos

In recent years, considerable interest has been shown in the potentialities of the aleurone layer of barley as a site for synthesis of α-amylase in response to added gibberellins^{1,2}. It now seems probable³ that, when barley germinates without additives, an endogenous gibberellin is concerned in the formation of many of the hydrolytic enzymes which degrade the reserves of the endosperm. It was therefore of interest to study the embryo with a view to determining which tissues may be involved in the production of the postulated enzyme-inducing hormone.

On the basis of results of experiments involving excision of various organs from barley embryos, followed by measurement of the rates of cell wall degradation and formation of α -amylase in the endosperm, MacLeod and Palmer4 reported that during germination the axis of the seedling is responsible for the production of a gibberellin-like hormone which controls the synthesis of hydrolytic enzymes in the aleurone. Recently, Radley⁵ has claimed that the scutellum is the organ involved in the production of gibberellins; this claim was based on estimations and tentative identifications of gibberellins which were actually present in the scutellum and the axis of the embryo or secreted from them into agar.

There need be no real conflict, however, between the two sets of observations because, whereas MacLeod and Palmer performed their dissections on grain which had been allowed to imbibe water for 2 h at 25° C (the minimum time of hydration which allows the tissues of the embryo to be manoeuvred), Radley worked with grain which had been previously supplied with water for 18 h. Many events of metabolic importance take place during the first 18 h of imbibition: for example, in Proctor barley, the root has emerged from the coleorhiza (that is, the grain has germinated) and degradation of endospermic reserves has begun4. This observation suggests that a gibberellinlike material had migrated from the embryo to the aleurone layer within 18 h of introducing water to the grain.

With a view to re-examining interactions between axis and scutellum during this 18-h period, either the seedling axis (coleoptile, coleorhiza and the enclosed seedling leaf, node and rootlets) or else the entire embryo (the axis+ scutellum) was removed from six dehusked grains at intervals after these grains had been set to germinate at 25° C

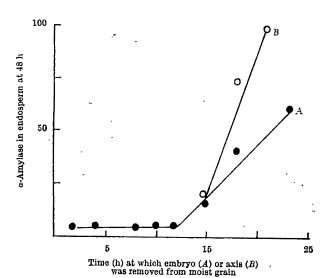


Fig. 1. Development of a-amylase in endosperm of mutilated barley. A, Embryo removed at times shown on the abscissa; B, axis removed, and scutclium left in position, at times shown on the abscissa. Results for A and B were identical up to 12 h. a-Amylase units are according to Briggs*, and are for six grains. Each point is the mean of results of two separate experiments.

on irrigated filter paper, and the mutilated grains were returned to the germination chamber for the remainder of a 48 h period. The endosperms were then assayed for α-amylase activity by the method of Briggs⁶; results of these analyses are shown in Fig. 1.

It is clear that, during the first 12 h of imbibition, no material capable of stimulating formation of α-amylase was released from embryo to aleurone because the separated endosperms (Fig. 1A) showed no evidence of enzyme synthesis during the subsequent incubations of between 36 and 46 h. Nor was there any response to the presence of the scutellum alone, for, when the axis was removed between 2 and 12 h leaving the scutellum to exert its influence independently for the remainder of the incubation period (Fig. 1B), once again no formation of α-amylase could be observed. It thus seems that, for the period covered by the experiment, the scutellum in isolation is incapable of secreting gibberellin-like material to the endosperm.

When the embryo was left in situ for 15 h or longer, subsequent formation of α-amylase in the endosperm took place successfully, and the longer the preliminary association between embryo and endosperm, the greater was the amount of a-amylase formed at 48 h. This is consistent with the observation of Kirsop and Pollock' that a 3 day association of the embryo with its endosperm is needed for maximum development of extract in barley during malting; it is also consistent with the recent demonstration by Chrispeels and Varners that isolated aleurone requires a continuous supply of gibberellic acid to allow continued production of α -amylase.

The amount of a-amylase formed in grains in which the scutellum was left in position was approximately twice as great as in those from which the whole embryo had been removed. This need not imply that the scutellum synthesizing additional gibberellin; because the scutellum functions as an organ of translocation, the extra activity could have resulted from a transient accumulation in the scutellum of gibberellin which was originally derived from the axis.

Although it is clear from the results presented here that the axis of the embryo is intimately involved in initiating the metabolic sequence which culminates in synthesis of α-amylase in the aleurone, it must be emphasized that this does not constitute proof that the axis is the site of gibberellin synthesis, though this is possibly the simplest explanation of the findings. Nor do these results disprove Radley's contention that the scutellum is the site of synthesis of gibberellins after growth of the grain for 18 h. If the scutellum is the organ involved, however, then it would seem that it is initially dependent on the axis for some factor needed for gibberellin production.

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Conflicting Measurements of Sodium and Potassium Uptake by Barley Roots

Barley roots have been widely used in studies of ion uptake, but there are conflicting reports about the uptake of sodium and potassium which need some reconciliation.

Some published data show that in 10 mmolar chloride solutions there is little or no selectivity between sodium and potassium. Jackson and Stieff¹ found that uptake from (5 mmolar potassium chloride + 5 mmolar sodium chloride) favoured sodium, but Epstein^{2,3}, using solution containing additional calcium sulphate, found that uptake of tracer from 10 mmolar solutions showed a small preference for potassium (about two-fold). The difference between these two sets of results seems to have been caused by the presence of calcium in Epstein's experiments3.

I have found4 the uptake of potassium to be about six times faster than that of sodium from solutions which contained calcium, and in which the ratio of potassium to sodium was 0.33. This is an eighteen-fold selectivity of potassium; selectivity is defined as:

potassium uptake sodium concentration outside sodium uptake potassium concentration outside

The data in Table I also show this high preference for potassium against sodium because rubidium-86 acts as a tracer for potassium in barley roots2.

Table 1. UPTAKE OF RUBIDIUM-86 AND SODIUM-22 FROM SALT SOLUTIONS*

	Cor	atent ·	Uptake (μ equiv./g/h)		
Pretreatment:	(µeq	uiv./g	10 mmolar	2.5 mmolar	+ 7.5 mmolar
24 h in `	fresh	weight)	KCl	KCl	NaCl
•	K.	Na	86Rb	$^{88}\mathrm{Rb}$	**Na
0.5 mmolar CaSO4	8	0.4	5.3 ± 0.3	3.1 ± 0.3	2.5 ±0.2
2·5 mmolar KCl+				•	
7.5 mmolar NaCl	50	46.5	4.8 ± 0.4	2.5 ± 0.1	0.19 ± 0.01
10 mmolar KCl	97	1-4	4.7 ± 0.1	2.6 ± 0.1	0.20 ± 0.01
* Solutions for pr	etreat	ment and	d uptake con	tained 0.5 m	molar calcium

sulphate.

There are similar differences in the amounts of sodium in the roots at salt saturation. Jackson and Stieff' found the ratio of potassium to sodium in the roots to be close to that in the solution; I have consistently found ratios in the root about six to ten times that in the solution.

One difference between these results is in the preparation of the tissue. Epstein, and Jackson and Stieff, used roots of seedlings grown on calcium sulphate solutions. Such plants have low contents of potassium and sodium (about 20 µequiv./g fresh weight and 2-4 µequiv./g fresh weight, respectively), because content is restricted to

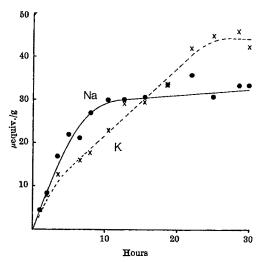


Fig. 1. Time course of sodium and potassium uptake (μ equiv./g) from a solution of 2.5 mmolar potassium chloride + 7.5 mmolar sodium chloride + 0.5 mmolar calcium sulphate. \bullet , Sodium; \times , potassium.

that available from the seed. The roots I used4,5 were from seedlings grown from germination on salt solution with added nutrients. When they were used, the roots seemed to have reached salt saturation for the particular solution in which they were grown, and contained about 70 μequiv./g fresh weight of potassium and 20 μequiv./g of sodium.

Fig. 1 shows the time course of salt uptake as "low roots reach salt saturation. Roots grown on 0.5 mmolar calcium sulphate were transferred to (2.5 mmolar potassium chloride + 7.5 mmolar sodium chloride + 0.5 mmolar calcium sulphate) at the start of the experiment and the salt content of the roots was measured during the next 30 h.

The principal point of interest is the different behaviour of sodium and potassium. Initially there was a rapid uptake of both ions, with a selectivity of about two to three-fold in favour of potassium. At about 10 h the rate of sodium uptake fell to a very low value, but potassium uptake continued and eventually the tissue had taken up more potassium than sodium. There are clearly large changes in selectivity between the early and later stages.

Table 1 compares tracer uptake from solutions labelled with rubidium-86 or sodium-22 by tissue in low and highsalt states, which shows that the decreased uptake of sodium is largely the result of a decrease in sodium influx-not of establishment of a state of flux equilibrium. The decreased influx of sodium occurs in tissue which has been in 10 mmolar potassium chloride as well as in tissue which has been in a solution containing sodium. The influx is therefore related not to the uptake of sodium as such, but to the total salt uptake irrespective of whether it is potassium or sodium (Table 1, lines 2 and 3). This change in selectivity may be related to the decrease in sugar content which takes place during salt uptake (from 60 µmoles/g in low salt to 15 µmoles/g after 24 h in salt solution).

The purpose of this communication is not to discuss the mechanisms involved in the uptake of sodium and potassium, but to make a point about the relation of this work to uptake by whole plants.

The rapid uptake of salt in the first 20 min or so after transfer from calcium sulphate to a salt solution has been shown by Epstein to be related to two different processes, with different selectivity for sodium and potassium. I believe there is a danger in assuming that these measurements represent the behaviour of plant roots growing in soil. The "low salt" roots grown on calcium sulphate solutions are in a state of mineral depletion and I suggest that this state leads to a "forced" uptake of salt in which some aspect of uptake is exaggerated, possibly Epstein's second component.

I do not mean to criticize the authors I have cited. Studies of the initial uptake have been most valuable, but their value will be increased when they can be related to uptake by roots which are nearer salt saturation. The establishment of this relationship is particularly important if attempts are made to interpret uptake of salt by plants growing in natural conditions. In these situations the continual exposure of roots to ions from the soil, even at a low level, may prevent the establishment of conditions during cell development which lead to the large initial uptake of these "low salt" roots.

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Effects of Kinetin and Actinomycin D on the Susceptibility of Nicotiana glutinosa L. to Infection by Tobacco Mosaic Virus

KINETIN (6-furfurylaminopurine) is known markedly to influence the rate of synthesis and degradation of plant protein and nucleic acid1. For this reason, its effect on virus infection has been of interest²⁻⁴. Although both increases and decreases in susceptibility have been noted2-4, Soans5 consistently found that when kinetin was applied to leaves of *Nicotiana tabacum* L. cv. "White Burley 'necrotic'", susceptibility to infection with tobacco mosaic virus (TMV) was reduced. Inoculation with TMV 4 days after treatment with kinetin showed reduced numbers, increased size and a delay in the appearance of the local lesions, while mixing kinetin with virus before inoculation produced no significant effects.

We studied the effect of varying the concentration and number of treatments with kinetin on the susceptibility of detached N. glutinosa L. leaves to TMV. Eight leaves from the four mid-positions of 10 week old N. glutinosa seedlings were used as the minimum sample in these experiments. Petioles were dipped through holes in 0.25 in. 'Styrofoam' sheets floated on tap water in an enamelled pan which was almost completely covered with a glass plate. The pan was kept continuously under 'Daylight' fluorescent tubes (700 ft.-candles at leaf level) at 24° C. Kinetin was brought into solution by autoclaving in distilled water for I h at 15 lb./in. and was applied by spreading the solution on the right or left half of the leaf. After 4 days, the entire leaf surface was inoculated with purified TMV (U-1) at 40 µg/ml. to which was added 50 mg of 'Celite'/ml. of inoculum. The half leaf which did not receive kinetin served as a control. Local lesions were counted 4 days after inoculation. 30 p.p.m. of kinetin was the optimum concentration for inhibition by kinetin of susceptibility to infection when applied 4 days before inoculation. This concentration was used in sub-We also demonstrated that insequent experiments. hibition increased with up to three daily applications of kinetin.

Inhibition of virus infection induced by kinetin may be related to its stimulation of host RNA and protein synthesis3. Conversely, inhibitors of host-RNA and protein synthesis might be expected at sufficiently low concentrations to favour infection with RNA virus. Actinomycin D inhibits DNA-dependent RNA synthesis in cells6, so we were interested in whether it could act in this way.

Soans⁵, in some preliminary experiments, had demonstrated that while 25 µg/ml. of actinomycin D when fed through the petioles of detached N. glutinosa leaves was extremely toxic, the leaves tolerated a concentration of 5 μg/ml. Exposures to the latter concentration for 3 h increased susceptibility to infection when leaf blades were then rubbed with TMV, but pretreatment for 8 and 12 h resulted in fewer lesions as compared with the controls.

Actinomycin D was dissolved in distilled water, and the petioles of detached leaves of N. glutinosa were dipped into vials for 2 h in different concentrations of actinomycin. The control leaves were cultured in distilled water. After the appropriate treatment with actinomycin D, the leaves were rinsed and transferred to vials containing distilled water and inoculated with TMV as before. Concentrations of actinomycin D of 2.5, 5.0, 10.0 and 15.0 μ g/ml. produced 148, 134, 92 and 88 per cent, respectively, of lesions in treated leaves as compared with controls. The experiment was repeated three times with similar results. The optimum concentration of 2.5 µg/ml. of actinomycin D was used in subsequent experiments to determine the duration of exposure for maximum stimulatory effect. Pretreatment for 1, 2, 3, 4 and 5 h resulted in a percentage of lesions in treated leaves as compared with controls of 109, 160, 102, 89 and 77, respectively. The increase after exposure for 2 h was significant at the 5 per cent level.

Table 1. EFFECT OF ACTINOMYCIN D ON THE SUSCEPTIBILITY OF Nicotiana

		9700000	W XU XMI		
No	actinomycir	ı D	A	ctinomycin .	D
Kinetin- treated half-leaves	Control (C) halves	Kn/C × 100%	Kinetin- treated half-leaves	Control (C) halves	Kn/C × 100%
1,344	2,498	53	1,416	1,758	81
Each figure	represents l	local lesions	for thirty-two l	alf-leaves.	

To study a possible combined effect of the two substances on susceptibility, 30 p.p.m. of kinetin was applied twice at intervals of 24 h by spreading it on an equal number of right and left half-leaves of thirty-two detached N. glutinosa leaves with their petioles dipping in distilled water in vials. Three days after treatment with kinetin, actinomycin at 2.5 $\mu g/ml$. was applied for 2 h by the method previously described. Sixteen of the leaves used in each experiment received water instead of actinomycin D. The leaves were then inoculated with TMV, and kept under the lights for 4 days, with their petioles dipping in distilled water. Had the effects of actinomycin D and kinetin been independent of one another, then one would expect the ratio of lesions in kinetin-treated half-leaves to those in untreated halves to be unaffected by treatment with actinomycin D. Table 1 shows, however, that the percentage of lesions in half-leaves treated with kinetin as compared with untreated halves was 81 with actinomycin D treatment and 53 without, indicating an interaction between the two substances.

Results similar to ours, showing an inhibitory effect of kinetin on susceptibility to plant viruses, have been reported3,4, although a stimulation has also been described². Actinomycin D has been found to be inhibitory to cellular RNA without reducing TMV-RNA synthesis7,8. This is the first report, however, of the stimulation of susceptibility to infection by actinomycin D (ref. 9).

We measured the initial establishment of infection in these experiments. Establishment of the virus and subsequent replication are distinct phases of the infection process, and are affected in different ways by different treatments10. It is therefore of interest that the compounds we used in this work should affect establishment as one might expect them to influence replication. Our experiments with kinetin and actinomycin D combined suggest some kind of interaction between the two compounds.

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Free Dissolved Enzymes in Lake Waters

THERE has been much discussion about the release of enzymes from living or dead organisms in lake and sca water, and their subsequent participation in chemical transformations¹⁻⁵. There is, however, little and only incomplete evidence of detectable amounts of free enzymes which are still active in bodies of water. In particular, some authors attribute a catalytic role to free dissolved phosphatases in the hydrolysis of phosphate esters by natural waters⁶⁻¹⁰. This communication reports investigations of free phosphatases in the waters of eutrophic lakes, including Pluss-See near Ploen, with special reference to their ecological importance.

The activity of free dissolved alkaline phosphomonoesterases (EC.3.1.3) was directly determined in each sample by a slight modification of an earlier method11, using p-nitrophenylphosphate as the substrate. As soon as possible after collection, 10 ml. of lake water was mixed with an equal volume of 1 molar tris-hydroxymethylaminomethan-hydrochloric acid-buffer, pH 8.4, containing 5×10^{-3} molar magnesium chloride and immediately filtered through a membrane filter ('Göttingen', MF 50, with a maximum pore size of 0.6μ). A sample of the filtrate (2 ml.) was thoroughly mixed in sterile 15 ml. tubes closed by silicon stoppers, and containing 1 ml. of 10-3 molar p-nitrophenylphosphate substrate. After 96 h incubation at 25° C the extinction of p-nitrophenol hydrolysate was measured with a Zeiss 'Elko II' photometer (filter S 42, 418 mu) against distilled water. Each determination required one substrate-buffer blank (1 ml. of substrate solution and 2 ml. of 0.5 molar buffer solution) and one sample-buffer blank (1 ml. of 1 molar buffer solution and 2 ml. of the mixture of lake water and buffer). The mean values of the blanks were subtracted from the standard All determinations were repeated three times. The sterility of the experiments was tested by plating out 0.1 ml. of test solution on 10 ml. of 1.5 per cent Difco nutrient agar in Petri dishes. The amount of p-nitrophenol hydrolysate formed in a minute in 1 ml. of water sample (nmole/ml./min) was taken as the total enzyme activity. Specific enzyme activity was obtained by referring the total activity to the content of protein dissolved in the lake water, which was estimated by the Lowry-Folin reagent method after Povoledo and Gerletti12: 1 ml. of a solution of 10 per cent sodium carbonate in 5 normal sodium hydroxide and 0.3 ml. of a 0.5 per cent aqueous solution of CuSO₄.5H₂O were made up to 15 ml. with filtered lake water. After addition of 1 ml. of Folin's

phenol reagent (Merck) the mixture was kept at 55° C for 45 min and then quickly chilled. Extinction was measured with a Zeiss 'Elko II' photometer using filter S 75. The extent of microbial phosphatase activity was shown by assaying unfiltered water by the same procedure. Other pH dependent phosphomonoesterases, apart from the mentioned phosphomonoesterases, were estimated by use of sodium citrate buffer, pH 3·2-6·4, and glycine-sodium hydroxide buffer, pH 9·0-11·5. Dissolved inorganic o-phosphate was determined by reducing the phosphomolybdate complex with stannochloride. The total dissolved phosphorus was found by treating the water with 1/50 volume of 20 per cent sulphuric acid. The difference between total dissolved phosphorus and inorganic o-phosphate was termed substrate phosphorus.

In the eutrophic lakes which we have studied the maximum activity of free dissolved alkaline phosphomonoesterases amounted to 15 nmoles/ml./min and 2×10^{-6} nmoles/ml./min/mg of protein for the maximum specific value. Considering the complexity of such biotopes this activity is obviously caused by a mixture of diverse isodynamic enzymes of the same or different origin in extremely low concentrations. Analysis of free lake isoenzymes at different pHs, however, showed only two very marked maxima of activity at pH 5.6 and pH 8.7-9.1, respectively (Fig. 1). This means that acid phosphatases had their maximum activity in lake water at pH 5.6, whereas the pH optimum for alkaline phosphatases ranges from pH 8.7 to pH 9.1. In the slightly alkaline lake water, therefore, the complex of free alkaline phosphomonoesterases can probably be considered to be catalytically efficient and therefore of ecological importance. obtain some idea of the enzyme activity of these alkaline phosphomonoesterases we considered that it would be interesting to investigate certain characteristics of these lake water enzymes. About 75 per cent of the enzyme activity was inhibited by treatment for 10 min at 100° C. Temperature investigations indicated optimum activity at 27° C. Examination of the activation energy using the Arrhenius equation in the form $\ln K = (E/R \times T) + \text{constant}$ revealed an activation energy of 16.5 kcal/mole (Fig. 2). The effect of substrate concentration $(X)_0$ on the activity (v) of alkaline lake phosphomonoesterases at pH 9.0 was

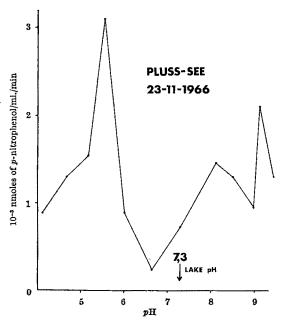


Fig. 1. Effect of $p{\rm H}$ on the activity (10^{-3} nmoles of $p{\rm -nitrophenol/ml/min}$) of free alkaline lake phosphomonoesterases from Pluss-See. The momentary value for the $p{\rm H}$ of the water sample was 7·3. The solutions containing $3\cdot3\times10^{-4}$ moles/l. of $p{\rm -nitrophenylphosphate}$ were buffered with $3\cdot3\times10^{-4}$ moles/l. of sodium citrate ($p{\rm H}$ 3·2-6·4), trishydrochloric, acid ($p{\rm H}$ 7·2-8·5) and glycine-sodium hydroxide ($p{\rm H}$ 9·0-11·5) and were incubated at 27° C.

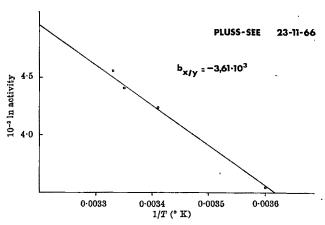


Fig. 2. Estimation of the activation energy of free alkaline lake phosphatases at pH 8·8 buffered with 6·7 × 10⁻⁴ moles/l. of glycine-sodium hydroxide. The logarithm of the activity (10⁻³ moles/ml./min) is plotted against the reciprocals of temperature (°K). Substrate concentration was 3·3 × 10⁻⁴ moles/l. of p-nitrophenylphosphate.

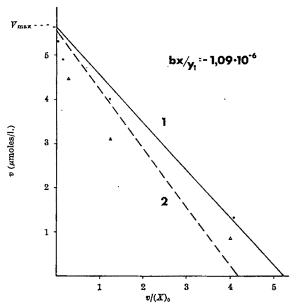


Fig. 3. Relationship between reaction velocity v (μmoles/l. of hydrolysate) and substrate concentration (X)₀ (μmoles/l.) for alkaline lake phosphatases plotted in the graphical transformation of Eadie¹³. Slope 1 (Φ), without inhibitors; slope 2 (Δ), with addition of 3·4 μmoles/l. of sodium orthophosphate. The mixture was incubated in 0·17 moles/l. of glycine-sodium hydroxide buffer, μH 8·9, at 27° C.

investigated using p-nitrophenylphosphate ranging at a concentration of from 3.3×10^{-7} to 3.3×10^{-4} moles/l. Michaelis-Menten constants (Km) were estimated by a graphical transformation after Eadie¹³, using the equation V_{max} V_{o} To correspond with the negative \overline{Km} \overline{Km} $(X)_0$ value of the slope of the best fitted curve the value of Km was calculated as 1.09×10^{-6} moles/l. (Fig. 3). Enzyme activity was not significantly inhibited by orthophosphate up to the maximum concentration found in lake water $(3.4 \mu \text{moles/l.}; 319.6 \mu \text{g P(PO_4)/l.})$. Sodium sulphide (20 mg/l.), however, competitively inhibited enzyme activity significantly (t test) by increasing the value of Km approximately six-fold. Substrate constants of the lake enzymes were rather small compared with the Kmdata of alkaline phosphomonoesterases studied in some species of blue-green algae from the same lake. example, the Km of the alkaline phosphomonoesterases of Oscillatoria rubescens Gomont and Pseudanabaena galeata Böcher ranged from 5×10^{-4} moles/l. to $5\cdot 4\times 10^{-5}$ moles/l. The activity of the phosphatases of blue-green algae was competitively inhibited, in contrast to the lake

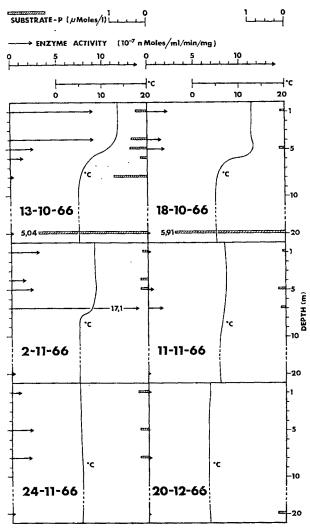


Fig. 4. Temporary variations in the specific activities of alkaline p-nitrophenyiphosphate phosphomonoesterases (10^{-7} nmoles/ml/min/mg of protein) in Pluss-See (strows) plotted against the thermcolines and the content of substrate phosphorus (μ moles/l. columns).

enzymes, by 3.1 µmoles/l. of orthophosphate. In lake water, however, optimal conditions for enzyme activity do not usually exist. Thus the alkaline phosphatases of the Pluss-See were shown to be less active (43 per cent) in the lake compared with the experimental data obtained for optimal pH and temperature conditions. The enzymes lost half of their activity after exposure for 3.2 days at 18° C.

The concentration of free enzymes in lake water is apparently correlated with the amounts of phytoplankton and bacteria. In Table 1 the activity (b) of dissolved phosphomonoesterases of filtered lake water is compared with the total activity of unfiltered water (a). In the chief zone of destruction of phytoplankton (4-5 m deep) mineralization is obviously coupled with a sudden release of enzymes, indicated by a striking decrease of the quotient a/b. At other depths this quotient had a medium value. Total and specific activities of lake phosphatases are almost exclusively restricted to the epilimnion rich in phytoplankton (chief phytoplankton of the Pluss-See are Oscillatoria agardhii Gomont, O. redekei van Goor and

Table 1. HYDROLYSIS OF p-NITROPHENYLPHOSPHATE AT DIFFERENT DEPTHS

Depth (m)	Blue-green algae (trichomes/ml. × 10 ³)	a Unfiltered	$_{ m Filtered}^{b}$
1	32.5	92-3	8-9
4	35.0	81.8	5.8
5	27.5	35.9	8.9
20	0.0	23.2	2.3

Hydrolysis of p-nitrophenylphosphate (nmoles/i.) after 4 days by samples of filtered and unfiltered lake water at several depths is compared with the momentary density of phytoplankton.

Aphanizomenon gracile Lemm). We detected very high activities of lake water phosphatases in the extremely stratified lake at the end of the summer stratification, as Fig. 4 shows. Further work is in progress.

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Polystyrene Replicas for Scanning Reflexion Electron Microscopy

THE preparation of biological material for examination in the scanning reflexion electron microscope frequently involves coating the specimen with evaporated metal. With a small, conventional vacuum coating unit it is often difficult to achieve an adequate coating without damaging the specimen by radiation from the metal source. The technique of Lingappa and Lockwood¹ which has been used in this laboratory for the observation, by light microscopy, of mycelia and stomata, has been modified and successfully applied to overcome this problem.

A solution was made of 10 per cent commercial, expanded polystyrene in a mixture of two parts benzene and one part toluene, and a small quantity was placed on the surface of a leaf. When the solvent had evaporated, the



Fig. 1. Scanning reflexion electron micrograph of a polystyrene replica of a salt gland of Limonium vulgare. (×1,180)

thin film of polystyrene was removed and placed under a microscope so that areas of interest could be selected. Pieces of the replica were fixed with 'Durofix' to a specimen holder and coated with gold-palladium in a vacuum coating unit before being examined in the 'Stereoscan' microscope.

A preliminary investigation shows that quite fine detail can be resolved from the replica, as seen in the accompanying micrograph of part of the leaf surface of *Limonium*

vulgare.

I thank the Cambridge Instrument Company Limited for the use of their 'Stereoscan' microscope, and Dr B. Shachar of this department for the use of the micrograph which forms part of a current project on *Limonium*.

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GENETICS

Genetic Load of an Evolutionary Change

According to Fisher's "Fundamental Theorem of Natural Selection", the rate of change of the mean fitness of a population equals the genetic variance in fitness. If w is the fitness and $V_{\rm Gw}$ the genetic variance in fitness, then by Fisher's theorem

$$\Delta \widetilde{w} = V_{GW}$$

Falconer² showed that if fitness varies in a way that is partially determined by variations in a character, x, then

$$\Delta \bar{x} = cov_{G}(x, w)$$

where $cov_G(x,w)$ is the genetic covariance of x and w. This equation Falconer calls the "Extension to Fisher's Fundamental Theorem". It will be true only if the regression of fitness and the character is linear. That is, we must have a relationship

w = a + bx

where

$$b = \frac{cov_{\rm G}(x,w)}{V_{\rm Gx}}$$

It then follows that the genetic load of any change $\Delta \bar{x}$ in the mean of the character can be calculated. We have by definition of the genetic load³

$$L = \frac{w_{\max} - \overline{w}}{w_{\max}}$$

where w_{max} is the fitness of the most fit genotype. Now we have

$$w_{\text{max}} = a + bx_{\text{max}}$$

and

$$\overline{w} = a + b\overline{x} = 1$$
 by convention

It then follows directly that

$$L = \frac{cov_{\rm G}(x_{\rm max} - \bar{x})}{V_{\rm Gx} + cov_{\rm G}(x_{\rm max} - \bar{x})}$$

If the change per generation in the mean of the character is known, then

$$L = \frac{\Delta \bar{x}(x_{\text{max}} - \bar{x})}{V_{\text{Gx}} + \Delta \bar{x}(x_{\text{max}} - \bar{x})}$$

The genetic load is an important quantity to know because it measures the intensity of natural selection. If fitness is the probability of survival, then the genetic load is the proportion of the deaths which arise from variations in fitness. Thus it L is large, a population may be in danger

of extinction. For example, suppose a continuous series of fossils shows a change to a new form. During a period of change, we can find $\Delta \bar{x}$ if we know the number of generations that has passed. x_{max} will be the value of the character when the new form has finally become stable. \tilde{x} will be the mean at some point in time in the evolutionary process. Thus we can find L at that time. Usually, evolutionary changes occur as populations adapt to new environments. In a new environment x_{max} will have a new value. The shift of \bar{x} away from x_{max} produces the genetic load which may be high if VGx is small. The formula for L shows why a large genetic variance helps a population to adapt to an environmental change: if V_{Gx} is low the genetic load may be high and the population may become extinct before it can adapt itself. It would therefore be interesting to compare the genetic loads in fossil populations which died out and in those that increased in numbers.

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Rare Pseudoallelic Crossover between Two Phenotypically Identical Alleles at a Restricted Sublocus of Dumpy in Drosophila melanogaster

The dumpy locus has been extensively mapped $^{1-4}$. Many of the sites within the dumpy gene have phenotypic specificity; that is, mutants of similar phenotypes map at the same restricted part of the region. A series of mutants which show an ov phenotype (oblique wings and thoracic vortices) were mapped to the right of lv^1 and to the left of v^2 (ref. 4). The ov mutants are viable as homozygotes and in combination with each other, except for ov^h , which is a facultative lethal in the homozygote but which is viable with the other ov alleles. The ov^h allele also shows a more extreme phenotype, and it is distinguishable in combination with other ov mutants by a more extreme phenotype. A crossover between ov^1 and ov^h was obtained by Southin. The work reported here was carried out using other ov alleles of indistinguishable phenotype (ov^1 and ov^{52}) in an attempt to establish critically the fine structure of the dumpy locus by obtaining crossovers between these alleles.

For the cross reported here, the virgin females used were 3-6 days old and the males were 1-7 days old. The flies were mated in groups of twenty males and twenty females and transferred to fresh medium every 3 days to establish four groups of bottles. The progeny were scored every other day, starting with the tenth day and continuing until the sixteenth day. The flies were cultured

at 27° C.

Virgin females of the genotype $ed\ ov^1\ cl/ov^{52b}$ were mated to males $ov^h/lv\ Cy$. The exceptional crossover type obtained was a female phenotypically wild type. On mating to an $ed\ ov^1\ cl$ male, the resulting progeny, excluding non-virginity, were $\sin x + cl/ed\ ov\ cl$, and five $ov^h/ed\ ov^1\ cl$. The progeny test indicates that the genotype of the exceptional female was $+ + cl/ov^h$. It is therefore unlikely that this exceptional female arose by mutation in either of the parental chromosomes, for this occurrence would require at least two simultaneous mutational events (the probability of such an occurrence would be

approximately 1 in 10°). It is also unlikely that the exception is caused by some conversion event, for the chromosome is recombinant for outside markers. It is reasonable to assume that this exception arose from a recombination event between the alleles ov^1 and ov^{52b} in a parental female. The resulting recombinant chromosome (++cl) establishes the position of ov^{52b} with respect to ov^1 ; ov^{52b} is to the right of ov^1 . A map of the "ov sublocus" may be drawn as follows, with three separate sites, because ov^h is known to be to the left of ov^1 .

 ov^h ov^1 ov^{52b}

The discovery of a crossover between phenotypically identical alleles critically establishes the fine structure of the dumpy locus; otherwise, it would be necessary to propose that the alleles of the dumpy region (even phenotypically identical alleles which map in contiguous parts of the map) each constitute a separate gene. This ad hoc hypothesis is rendered unlikely by the rare frequency of this recombinant event. The upper fiducial limit for this frequency (P=0.05) is $5.57/222,688=2.5 \times$ 10-3 map units⁶. The distance between these alleles is likely to be less than 0.0025 map units. If the dumpy gene has a map distance of 0.1 map units between the most distant markers in the gene, the entire map could accommodate at least forty sites. A more probable estimate would be several hundred sites; the lower fiducial limit (P = 0.05) would give as many as 10^4 sites². It is more reasonable to assume that these sites represent many mutable sites within one or a few genes rather than a large number of separate genes. Within the limits of such estimates, at least, these results argue for fine structure within the dumpy locus.

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CYTOLOGY

Transformation of Epithelial Cells in vitro

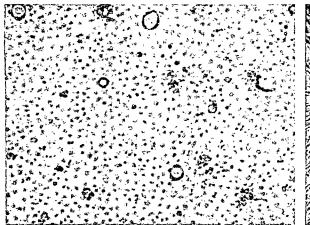
DURING attempts to demonstrate replication of herpes simplex virus in cultures of human leucocytes1, we observed that supernatants from uninoculated leucocyte cultures induced a morphological transformation when added to primary cultures of human amnion cells. When the leucocyte culture fluid was added, the human amnion cells became transformed from epithelial to fibroblast-like cells. This leucocyte factor was produced in cultures of human blood cells which contained the mitogenic agent phytohaemagglutinin (PHA; phytohaemagglutinin-M, Difco), and was found only in the extracellular medium. Neither extracts of leucocytes nor supernatants of leucocyte cultures prepared without PHA possessed this activity. We have called this factor which induces metamorphosis, "MF". Preliminary observations indicate that it is produced by lymphocytes.

Cultures of primary human amnion cells were prepared from human placentas according to generally accepted techniques². Maintenance medium consisted of 45 per cent Hanks salts, 45 per cent bovine amniotic fluid, 5 per cent beef embryo extract, 5 per cent inactivated horse serum and 100 units of penicillin and 100 µg of streptomycin/ml. Cells were maintained in stationary culture in 16 x 150 mm stoppered tubes and were regularly re-fed until a confluent sheet of epithelial cells was obtained. Only confluent cell sheets were used for assay. presence of transformation was determined by direct microscopic examination under low magnification. In several experiments, additional human epithelial cell cultures (HeLa, gingival and human kidney) were also tested for their response to this factor. These cultures were established and maintained by standard techniques2. Human leucocytes were obtained according to methods previously described3. The leucocyte cultures were established with 1×10^{6} lymphocytes/ml. and usually contained 60-80 per cent lymphocytes, 20-40 per cent neutrophils and 1-2 per cent monocytes. These cells were maintained in medium 199 (Earle's salts) and 25 per cent inactivated calf serum. Cells were stimulated with PHA, using a dose which had been previously determined to give maximum DNA synthesis in such cultures. Control leucocyte cultures without PHA were similarly prepared and maintained. Leucocyte cultures were usually killed after 4 days; the cells were spun at 300g for 5 min and the supernatant was separated. Serial dilutions, or undiluted samples of this supernatant of 0.2 ml. volume. were added to the 1 ml. of medium overlying the epithelial cell cultures, and daily observations were made. morphological appearance of the amnion cells before and after exposure to the leucocyte supernatant is shown in Fig. 1. The untreated cell sheet (left) consisted of large, round epithelial cells with dense nuclei and indistinct cytoplasmic boundaries. After addition of metamorphosing factor, the amnion cells sequentially became squared, then triangular, and finally spindle-shaped and tightly whorled. The fully transformed cells were characterized by distinct cytoplasm and cell boundaries. This sequence of transformation took about 5 days. Similar transformation was also observed in cultures of HeLa, gingival and human renal cells. Control preparations consisting of medium 199 with an appropriate amount of PHA did not induce morphological alterations when added to the epithelial cell cultures. In addition, transformation to fibroblast-like cells was not observed with the supernatant from lymphocyte cultures prepared without PHA, nor with extracts of such cultures. The quantity of metamorphosing factor was somewhat variable in different leucocyte cultures, ranging from 1:2 to 1:64. In experiments with varying percentages of lymphocytes and neutrophils, the titre of this material was proportional to the number of lymphocytes.

We have made some preliminary attempts to characterize the factor which was produced in the leucocyte cultures. The factor was retained in a dialysis bag, and there was no loss of activity with dialysis against fresh medium 199. The metamorphosing effect was therefore not the result of addition or removal of small sized metabolites by the leucocytes. The transforming activity was destroyed by boiling the leucocyte supernatant for 10 min.

Our data provide evidence that lymphocytes may influence other cells. The significance of our observations in vivo remains to be determined. It is possible, however, that the elaboration of this substance by lymphocytes which are present in areas of inflammation and wound repair could be responsible for initiating connective tissue proliferation in these conditions. A mechanism such as this was suggested by Virchow in 1871 (ref. 4).

Gresser⁵ observed that supernatants containing interferon, from human cell cultures infected with virus, produced morphological changes similar to those described in this communication. Although he suggested that the



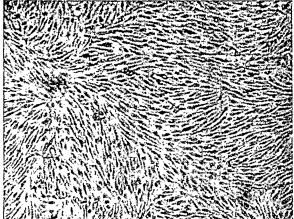


Fig. 1. Primary human amnion cell cultures before and after transformation; left, normal (epithelial morphology); right, transformed (fibroblast-like morphology).

material inducing these changes was interferon, his data indicated that the metamorphogenic and viral-inhibitory activities of his preparations might be dissociable.

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Calcium and the Control of Mitosis in the Mammal

INCREASING the calcium concentration in the environment of various types of isolated mammalian and insect cells hastens their progress through mitosis and also counteracts the transient mitotic inhibition following irradiation1-6. The same effects can be produced in the bone marrow and thymus of the whole animal by raising the blood calcium either directly, by injecting calcium salts, or indirectly through the action of parathyroid hormone7,8. Conversely, lowering the calcium level in the medium of isolated normal or irradiated cells reduces their rate of flow into mitosis. Reducing the total calcium level in the blood by injecting the chelating compound ethylene diamine tetra-acetic acid (EDTA) or inorganic phosphate should therefore reduce the mitotic activity in normal and irradiated marrow and thymus in vivo. Contrary to expectations, the present study shows that these compounds in fact stimulated mitosis.

At the start of any experiment, or immediately after irradiation (cobalt-60 γ-radiation), male Sprague-Dawley rats weighing 125 g were injected intraperitoneally with 0·1 mg of colchicine/100 g body weight, and were given a second injection of this same amount 2·5 h later. The progress of cells through mitosis is thus arrested at metaphase and their rate of entry into mitosis can be de-

termined. Either EDTA or disodium hydrogen phosphate was injected intraperitoneally immediately after the first colchicine injection. EDTA (30 mg/ml.) adjusted to pH 7·2 with sodium hydroxide, and disodium hydrogen phosphate solution (80 mmolar) buffered to pH 7·2 in 5 mmolar tris (hydroxymethyl) aminomethane (tris), were given as a dose of 1·0 ml./100 g. Neither the stress of injection nor the tris buffer affected mitotic activity.

At various times after the beginning of the experiment, marrow and thymus cell suspensions were prepared as described elsewhere. Samples of these suspensions were fixed in neutral formalin, stained in Delafield's haematoxylin and the percentage of cells in metaphase was then determined by examining at least a thousand cells.

Blood calcium levels were determined by removing blood samples from the abdominal aorta every 10 min after the injection of EDTA or phosphate; the concentration of total calcium was determined using the colorimetric method of Copp et al.¹⁰, and the ionized calcium concentration was determined with a specific calcium ion electrode (Orion Research, Ltd., Cambridge, Massachusetts).

In the normal animal, bone marrow cells progressed into mitosis in a regular fashion (Fig. 1A), and by 6 h,

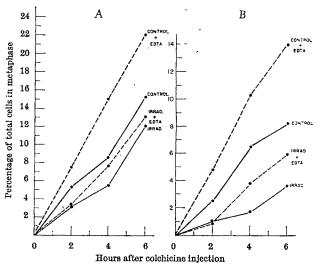


Fig. 1. Effect of EDTA on mitosis in bone marrow and thymus of normal and irradiated Sprague–Dawley rats. A, Bone marrow; B, thymus. In non-irradiated animals, the differences between the percentage of cells trapped at metaphase in normal and EDTA-treated animals were already significant by 2 h after the initial colchicine injection; P < 0.05 (bone marrow) and P < 0.01 (thymus). In Irradiated (100 r.) animals, EDTA caused a significant stimulation of mitosis in the thymus 4 h after the colchicine injection, P < 0.01. The consistent but slight stimulation in the bone marrow was not significant, P < 0.1. Each point is a mean value from at least four rats.

15.3 per cent of the cell population had collected at metaphase. EDTA stimulated mitosis; this stimulation became evident as early as 2 h after treatment, and by 6 h, 22·1 per cent of the cells had reached metaphase (Fig. 1A). Thymus tissue was less active mitotically than marrow and only 8.2 per cent of normal cells reached metaphase in 6 h, but EDTA treatment increased this fraction to 14.0 per cent (Fig. 1B). Phosphate solution also accelerated mitosis in thymus and marrow, but it was slightly less effective than EDTA (Fig. 2).

EDTA and phosphate also stimulated mitosis in the thymus and marrow of irradiated animals. During the first 2 h after irradiation and injection of either compound, there was no demonstrable effect. Between 2 and 6 h, however, a stimulation by these compounds became

evident (Figs. 1 and 2).

Despite the fact that both EDTA and phosphate stimulated mitosis, they also caused a decline in the level of both total and ionized calcium during the first 10 min after injection. The total calcium level subsequently returned to normal, but never exceeded this value. The majority of this restorative calcium surge, however, consisted of ionized calcium and its level rose well above the normal value. Thus 80 min after treatment with EDTA, when the total calcium had returned to normal, as much

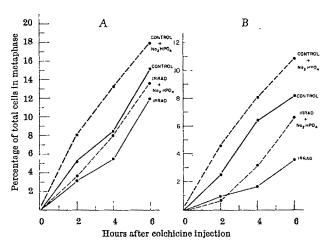


Fig. 2. Effect of disodium hydrogen phosphate on mitosis in bone marrow and thymus of normal and irradiated Sprague-Dawley rats. A, Bone marrow; B, thymus. In non-irradiated animals, the differences between the percentage of cells trapped at metaphase in normal and phosphate-treated animals were already significant by 2 h after the initial colchicine injection in both marrow and thymus (P < 0.01). In tradiated (100 r.) animals phosphate produced a stimulation of mitosis by 4 h in both tissues, P < 0.05 (marrow), P < 0.02 (thymus). Each point is a mean value from at least four rats.

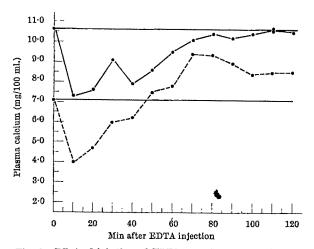


Fig. 3. Effect of injection of EDTA on total (——) and ionized (——) plasma calcium levels. Each point is a mean value from four rats.

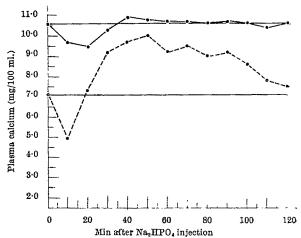


Fig. 4. Effect of 1. Effect of injection of disodium hydrogen phosphate on total and ionized (---) plasma calcium levels. Each point is a mean value from three rats.

as 93 per cent of it was ionized compared with 67 per cent in the normal animal (Figs. 3 and 4).

The unexpected stimulation of mitosis produced by EDTA and phosphate can now be attributed to the abnormally elevated ionized calcium levels which follow the initial lowering of this level induced by these compounds. Mitotic activity can obviously be affected by merely changing the level of ionized, in contrast to bound, calcium in the cellular environment. Plasma calcium levels are controlled by two antagonistic hormones: of these, parathyroid hormone causes a surge of ionized calcium which restores total calcium levels to normal after a sudden depression as in the present study, and calcitonin acts to depress abnormally high levels¹¹⁻¹³. The present results imply therefore that any changes in the balance of these hormones will probably be accompanied by changes in the mitotic activity of the lymphatic and haemopoietic tissues of the animal.

Regardless of the possible mechanisms by which calcium stimulates division in the mitotically competent cell1-3,5,14, in any future consideration of mitotic stimulation and control in the mammal, the role of a calcium and the interplay of the two principal hormones which affect its level cannot be neglected.

We thank P. K. Toelg and T. Youdale for their excellent, technical assistance.

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Mitotic Activity in the Chick Embryo Epidermis

AT the age of 9-10 days, the epidermis of the developing chick embryo is composed of two to three undifferentiated cell layers. In the next 10 days of embryonic life, proliferation and differentiation occur in the epidermis which results in the development of a layer of keratinized epidermis, six to ten cells deep. Interrelationship between proliferation and differentiation during the 10 day period has been reported, but the extent of the interreaction is not clear. An interrelationship has been suggested2-4, in which differentiation (keratinization) only begins when cell division has begun to decline. Tonofilaments and tonofibrils, the first indicators of differentiation, are detected2 for the first time in the basal cells of the embryonic epidermis on the thirteenth day. Also on the thirteenth day, labelling experiments with thymidine indicate a reduction in the total number of labelled nuclei3 and these are present only in the basal cell layer after that day.

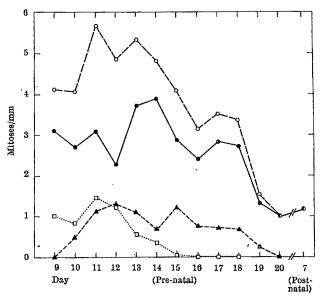
Before the beginning of differentiation on days 10-13, an increase in DNA polymerase activity suggests a possible increase in mitotic activity during that period. The decline in mitotic activity seems to relate to embryonic age. An inhibitor system, which is synthesized as a function of embryonic age, such as that of Bulloughe, could be responsible for the decline in mitotic activity.

To clarify further the state of mitotic activity before and after differentiation, the mitotic activity of the chick embryonic epidermis was examined on days 9 to 20, and was found to be maximum in all cell layers of the 11-13 day old epidermis. The maximal mitotic activity then decreased to the level which was found in the normally keratinizing post-natal epidermis. All stages of mitosis were counted in the epidermis of the chick embryos from day 9 to day 20. The length of the third toe was measured in order to relate the development of the embryo to the chronological age7. Skin specimens were obtained from The tissue was imthe metatarsal region of the leg. mediately fixed in 3 per cent acetic acid-Zenker's fluid for 30 min or in Cajals special for 12-18 h.

Serial paraffin sections, 7µ thick, were cut and the sections were overstained with Harris's haematoxylin. The sections were then decolourized in acid alcohol and were counterstained with eosin or FCF fast green. The number of mitoses in all cell layers were counted along the lengths of basement membrane, using an ocular micrometer. Mitotic counts were carried out on two complete sets of samples obtained from two chick embryos on days 9 to 20. An average length of 40 mm and 22 mm, respectively, was counted for samples of each set. By counting the number of nuclei per unit length, it was found that the width of basal cells increased from 5·6μ to 6·5μ between the ninth and seventeenth days.

In this communication, the basal cell layer is defined as the layer immediately adjacent to the basement membrane. The peridermal cell layer is the outermost layer composed of flattened cells, each containing a small The intermediate cell layers consist of cells nucleus. between the basal cell layer and the peridermal layer. In Fig. 1 the number of mitoses/mm of basement membrane for the different cell layers of the epidermis is plotted as a function of the foetal age of the chick skin. These plots indicate that in the early epidermis (age 9 days) mitotic activity is present chiefly in the basal cell layer. Mitotic activity in the intermediate and peridermal layers increases after the ninth day of embryonic life. The maximum mitotic activity of these cell layers is found on the eleventh day for peridermal, the twelfth to thirteenth day for intermediate cells, and the thirteenth to fourteenth day for the basal cell layer.

In the periderm, the maximum mitotic activity of the eleventh day decreases and at days 15-16 cell division ceases simultaneously with the shedding of the peri-



dermal layer. The decrease from peak mitotic activity of the basal and intermediate layers seems to be tentatively halted at days 17-18. The mitotic activity then continues to decrease to the post-natal level.

The differences in mitoses/mm between days 9-20 are much greater than can be explained by the small differences (10-20 per cent)⁸ in cell density occurring during this period. The results indicate that, before differentiation starts, there is an increase in mitotic activity in which all cell layers of the epidermis become germinative. The stimulation of mitotic activity decreases until days 17-18 when there is a weaker stimulation. This event coincides with the appearance of the stratum corneum, the end product of keratinization. As foetal life ends for the chick, the epidermis assumes its post-natal role which is the continuous production of stratum corneum, with the germinative layer supplying an adequate number of cells for maintenance of the epidermis.

The increase in mitotic events (days 11-13) is in agreement with the increase in DNA polymerase activity found on days 10-13 (ref. 5). The decline in mitotic activity could be the result of a factor or factors which reverse the apparent induction of DNA polymerase activity or the appearance of a mitotic inhibitor system. A mitotic inhibitor system, similar to that of Bullough's chalone-adrenaline epidermal mitotic inhibitor system, could be activated by increased cellular synthesis of the chalone and adrenaline after day 13.

The interrelationship, if any, between possible stimulants and inhibitors of mitosis during epidermal development and differentiation is being further investigated.

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BOOK REVIEWS

POPULAR BUT HARDLY POP

Automation: Its Anatomy and Physiology. By J. Rose. Pp. vii + 144. Chemical Exchanges in Man: A Physiological Essay. By B. F. Matthews. Pp. vii + 136. Molecular Biophysics: By D. Chapman and R. B. Leslie. Pp. vii + 151 + 9 plates. Electronic Computers: By J. C. Cluley. Pp. vii + 189. The Shapes of Molecules: Carbohydrate Polymers. By D. A. Rees. Pp. vii+141. The Basis of Modern Physics: By J. M. Irvine. Pp. vii + 124. The Evolution of the Galaxies: By V. C. Reddish. Pp. vii+119+16 plates. Attraction and Repulsion: Mechanical Applications of Permanent Magnets. By Malcolm McCraig. Pp. vii+119+9 plates. The Ideas of Physics: By Ernest H. Hutton. Pp. vii + 153. Automation: Its Uses and Consequences. By J. Rose. Pp. vii + 123. Reproduction and Man: By R. J. Harrison. Pp. vii + 134+16 plates. Noise and Society: By M. Rodda. Pp. vii+113. Life, Mind and Galaxies: By Axel V. Firsoff. Pp. vii+111+9 plates. Basic Astronomy: By Patrick Moore. Pp. vii + 120 + 8 plates. All: Contemporary Science Paperbacks. (Edinburgh and London: Oliver and Boyd, Ltd., 1967.) 7s. 6d. each.

In writing any scientific work the principal difficulty resides not so much in what to put in as in what to leave out, and the problem becomes the more acute the further one moves from the strictly professional towards the more popular. At the latter level it is not only a matter of what topics and concepts to discuss but also of how much general introductory explanation must be provided so that the intended reader is able to follow the arguments that are subsequently to be advanced. In so far as phenomena are merely being described all is well or nearly so, but if theoretical explanations of these phenomena are to be advanced, and also explanations of the patterns into which they fit, more and more props and aids must be provided if the reader is not to fall by the wayside.

The group of books under consideration, the first batch of a series of Contemporary Science Paperbacks, can be broadly described as being directed at the educated scientific layman, though some at least of them are perhaps a little more nearly professional than is commonly the case with such an aim. Thus The Shapes of Molecules, which deals with carbohydrate polymers, really requires some previous knowledge of chemistry, and thorough understanding of Molecular Biophysics would certainly be helped by it. By contrast, Chemical Exchanges in Man and Reproduction and Man, which are somewhat more descriptive, could be read and understood with little in the way of previous knowledge, provided always that the necessary degree of concentration was afforded by the

reader. One of the major dangers with short books such as these is that the author is tempted—and has not always here resisted the temptation—to get rather too much in his introduction dealing with the basic principles, situations and terminology of the subject.

On the physical sciences side, Basic Astronomy does not present any major problems, but The Basis of Modern Physics and still more The Ideas of Physics demand a good deal from the general reader because here it is concepts and more abstract considerations that have to be grappled with: a good deal more difficult to explain satisfactorily and requiring correspondingly greater effort on the part of the reader. By contrast, the demandmade by The Evolution of the Galaxies are less onerous: not that the concepts are significantly simpler, but the focus is somewhat more restricted and they therefore become at least relatively familiar the more rapidly. Life. Mind and Galaxies is interesting because it spans the whole range of physics, chemistry and biology: it is also rather more speculative than most of the other titles in the batch and, though this is not unreasonable in such a field, one rather gets the impression of a search for the spectacular, and the writing is less reasoned and convincing than in most of the other titles.

Attraction and Repulsion is scarcely surprisingly about magnets and this is one of the real successes of the batch. It is about something that is in essence familiar but the explanation and operation of which remain, for most people, mysterious. The author has provided a neat clear and stimulating account that should be of great use and interest to sixth form pupils as well as to a much wider public. The largest book in the group tells of a subject which is impinging increasingly on the lives of us all, Electronic Computers. This is described as being for the general reader, but I suspect that such a person is going to have some trouble in following the argument to the end, for there is quite a bit of electrical engineering theory in it and concepts and situations that are unfamiliar—this is scarcely the book for the entirely uninformed to start on.

Rather sensibly the author of the text on automation has split his work into two volumes, Automation: its Anatomy and Physiology and Automation: its Uses and Consequences. Such a division would scarcely work for all fields, but here it seems to have done very well, for the subject matter falls quite rationally into these divisions, and in the face of many people's fears of what automation may possibly do to their lives detailed and balanced consideration of its use and consequences is The first volume presents an arguvery welcome. ment that is not of itself simple but the treatment is lucid and forthright so that it should be followed fairly readily. The second volume deals essentially with the economic and social possibilities and problems that stem from automation and is the one more likely to be widely read: it should go some way to set at rest the more extreme fears about the widespread introduction of automation.

The last of the fourteen volumes could scarcely be more topical: noise is something that society is increasingly unable to escape from, and the introduction of civilian supersonic aircraft leaves one full of foreboding for the future. The author of Noise and Society makes plain the technical difficulties that are involved in measuring noise, and then, having done so, relates such scientific measurements to the question of general nuisance and, in the extreme, to actual damage to hearing. He leaves us with an impression of his own astonishment at how little has really been done legislatively to afford society any effective protection.

The general claim of the editors of the series is that their volumes "are lucid, compact and authoritative. Through them, scientific and technological ideas and achievements are made readily comprehensible". For the most part, I feel that their claim is justified; compact these volumes certainly are, sometimes just a little too compact for comfort where an author has been anxious to get that little extra bit in that might better have been left out. Lucid the majority of them are, and authoritative too with perhaps an odd exception. Probably more than half of them make scientific and technological ideas and achievements readily comprehensive, the rest make them comprehensible but not perhaps quite so readily as their authors imagined.

Production standards are generally good, line drawings being simple but effective and the black-and-white plates quite well done; the organic chemical formulae that appear in some volumes could be improved, however. All told, an excellent beginning that is to be highly commended, with volumes that are good value for money.

PETER SYKES

DETECTING ESP

Science, Philosophy and ESP By Charles McCreery. Pp. 199. (London: Faber and Faber, 1967.) 32s. 6d. net.

WE might expect ESP to be a proper study for experimental psychologists in the field of perception. But the failure of psychologists, in general, to take such phenomena seriously has led to the establishment of parapsychology, with its own journals and its own research institutions outside the universities. Mr McCreery is a research officer of the Psychophysical Research Unit in Oxford, and his book describes one aspect of the unit's work.

In the foreword by Professor H. H. Price, we are told that Part 1 could very well serve as an introduction to psychical research for readers who have little or no previous knowledge of the subject. Here examples are given of phenomena that have been considered by many people, including eminent scientists and philosophers, to defy explanation in terms of existing scientific knowledge. Its value must, however, be doubted. For, although the main evidence for ESP has been extensively criticized, Mr McCreery's method of dealing with criticism is to ignore it. Thus the remarkable feats of an Italian medium, Eusapia Palladino, are described in some detail. medium, while seated in semi-darkness at a table with her arms and legs held in the grip of members of various investigating committees, was able, according to the account, to cause remarkable manifestations of psychical The table rose from the floor; musical phenomena. instruments positioned on a table behind her emitted sounds; ghostly fingers pinched the learned investigators. But no mention is made of the fact that when she visited the United States her modus operandi was completely revealed. The reader who wishes to know more about this lady should consult a report made by Hugo Munsterburg concerning a sitting carried out in the psychology department at Harvard1; and a report, published in Science, submitted by a group of scientists at Columbia Uni-The latter report describes how investigators versity². concealed under the table saw it levitate on several They also observed Eusapia's footwork at occasions. close range, and the manner in which she assisted its movement. Experts in subterfuge sitting at the table were able to turn on or off other psychical phenomena by decreasing or increasing the effectiveness with which they controlled the movements of the medium's arms.

Again, Mr McCreery describes the well-known experiment in which it was claimed that Basil Shackleton could predict the identities of cards seen by a second person about two seconds after he had recorded his prediction, but no mention is made of criticisms of the experiment, nor of information that has come to light which reflects on its value.

Mr McCreery makes it clear that he is well aware of the necessity for checking the result of an experiment by repeating it and confirming the observations. The reader is, however, not only interested in cases where a series of observations have been confirmed by other investigators, but also with instances, like the investigation of Eusapia Palladino, where further investigations have not only failed to confirm the original reports but have also given clear indications that the original investigators were led

In general, this first part of the book must be regarded as an attempt to persuade the reader that the phenomena are genuine. It would have presented a far more impressive picture if Mr McCreery had acquainted his readers with the various criticisms together with his reasons for

disregarding them.

In the second part of his book Mr McCreery is concerned with methods by means of which the evidence for ESP and other psychical phenomena may be made more. convincing. His problem arises because ESP, if it exists, has quite unpredictable properties. For example, Basil Shackleton, according to Mr McCreery, only obtained high scores when a particular investigator was present, and, because he lost his ability to score at all shortly after the termination of the experiments, it was not possible to provide independent confirmation of his ESP ability. Other high-scoring subjects have also lost their ESP abilities shortly after the conclusion of an experiment. The problem is made even more difficult by the fact that individuals who can consistently obtain high scores seem to have disappeared altogether during the past few years. Some investigators claim that they can isolate individuals who have only slight ESP ability. It might therefore be expected, if a sufficiently large group of such people all guess the same cards, that a suitable statistical analysis would enable the cards to be identified with a high degree of success. But for some reason ESP does not operate in this fashion.

Mr McCreery suggests that it may be possible to improve the scoring rate by employing techniques which will enable the investigator to test subjects at times when their ESP abilities are at their peak. He writes, "To introduce an analogy: suppose that a cricketer found that he only scored more than fifty runs on days when his visual acuity was at its maximum. Then to improve his batting average he would only have to abstain from play on days when this condition did not obtain—if this were possible.

"To complete the analogy with the card-guesser, let us suppose that the batsman is not able to tell introspectively whether his acuity is at its maximum or not. Then it might be possible for him to employ a coach to apply an objective test, such as the letter-test used by opticians, whenever he wished to decide whether to volunteer for his team or not.'

If the subject in an ESP experiment knew when he got a hit and when he got a miss, it would be possible to select occasions when he thought he had got a hit and thus improve his score. ESP subjects, it seems, however, have no idea when they are successful or not.

Mr McCreery's solution is to use physiological concomitants of mental states which can be detected by means of suitable apparatus. These, he thinks, will enable him to monitor the subject for states conducive to ESP. After discussing a number of possibilities, he makes the follow-

ing predictions:
"The EEG record of the conscious ESP subject at the time of the occurrence of ESP will show the following

characteristics.

(1) Continuous alpha activity, which is

- (2) Slightly accelerated (or at the upper limit of its range of frequency for that particular subject).
- (3) No delta activity, and(4) No theta activity."

He continues:

"More tentatively, it is suggested that during the occurrence of ESP the conscious ESP subject may show:

(5) Decreased muscle-tone and

(6) Increased alveolar carbon dioxide pressure."

Whether we have much faith in his predictions or not, it must be agreed that Mr McCreery has faced up to a major problem facing parapsychologists. It remains for him to test his predictions and report the result. He must, however, pay more attention to those pitfalls encountered by previous ESP investigators than he has done in the first part of his book. C. E. M. HANSEL

⁴ Munsterburg, H., Metropolitan Magazine (February 1910).

² Miller, D. S., Science, LXXVII (1910).

MODEST PHYSICIST'S THOUGHTS

Symmetries and Relections

Scientific Essays. By Eugene P. Wigner. Pp. viii + 280. (Bloomington and London: Indiana University Press, \$7.50; 568.

This volume is a collection of addresses and essays by Professor Eugene P. Wigner, Nobel Laureate in physics. A wide range of topics is covered, including discussions on the meaning and interpretation of the basic concepts of physics, reminiscences of the first man-made nuclear chain reaction in Chicago in 1942, biographies of Enrico Fermi and John von Neumann, and evaluation of the problems facing humanity in a nuclear age. In all a fascinating collection, which will appeal to a much wider audience than merely physicists. On the whole the level is non-specialist, and these essays and talks are concerned more with aims, motives and meaning of science than with details of the author's own field, theoretical physics. Indeed, copies of this book could well be included in the libraries of high schools.

The reader may be interested in a quotation from Wigner's J. F. Carlson lecture, which forms the final "The Growth of Science-Its Promise and Its Dangers": "Already, we have more trouble in controlling the spread of dangerous and of habit-forming drugs than of nuclear weapons, and they have caused more unhappiness than the latter." GRAHAM DERRICK

PROSPECT OF EARTH

Viewing Weather from Space By E. C. Barrett. (Longmans' Geography Paperbacks.) Pp. xii+140+8 plates. (London: Longmans, Green and Co., Ltd., 1967.) 21s. net.

It is always useful to see another point of view and in certain fields of scientific endeavour it is extremely helpful to see from another viewing point. This is most true in the study of the Earth's atmosphere, the "ocean of air" drifting around and above us largely unseen if not entirely unperceived. How technology has aided the meteorologist, aeronomer and ionospheric physicist by providing the "topside soundings" is a fascinating story, largely told in press releases and popular articles, but a tale that loses nothing in retelling especially in such a concise and informative way as in Mr. Barrett's slim volume. Viewing Weather from Space is broad in its concept; its writer aims at a fairly general audience and seeks to show how the development of the meteorological satellite has stimulated new methods and revised concepts in the atmospheric sciences. Everyman is hard to please and it is possible that this book will find most favour with students, to whom its size, price and review nature will appeal.

It must be admitted that virtually all of the research in meteorological satellite development, data evaluation and utilization has been performed in the United States while the rest of the world has benefited in terms of improved forecasts. The book perforce is a summary of US experience, but this implies no geographical limitation in scope because most features revealed in satellite pictures

are the result of fundamental processes in the free atmo-This raises the question as to whether those chapters of the book which follow conventional climatological divisions of the atmosphere (the fourth, fifth and sixth chapters, "The General Circulation . . .", "The Tropical Atmosphere" and "Weather Systems in Temperate Latitudes") represent logical subdivisions or whether it might not have been better to present a framework based on the basic geometric structures which have been revealed by the pictures themselves, for example, vortices, linear features, cellular patterns and so on. Admittedly, it would be more difficult to insert the more basic clements of meteorological theory into this type of framework, but the approach could be considered more appropriate to a book dealing with satellite pictures and their features.

It is the photographs which are the basis for the work, so could not the publisher have avoided binding the plates in the centre of the book? No doubt this represents a saving in production costs, but it is a source of irritation to be faced with the choice of committing them to memory or turning back time after time to find the specific example. Economics probably prevented the author from including more examples, but could he not have substituted some sketch reproductions to illustrate his discussion? The quality of the illustrations is very high, but some of the diagrams are rather aged; thus the hurricane cross-section shows no primary and secondary cells or "hot tower" and the general circulation still has a driven meridional cell in middle latitudes. Forecasting in middle latitudes has been rendered a disservice when Mr Barrett states that in the middle troposphere isobars and isotherms tend to parallel each other, but then medium range forecasts are not mentioned as part of the forecaster's lot. What is important is that the operational value of photographs or nephanalyses is stressed. This brings us to the final point; at the moment the television camera satellite is an exciting new tool, but very soon it will be just another instrumental aid. Whether it will lead research workers to new conclusions about the atmosphere and its mechanisms is very much a matter for debate, but it certainly will excel at its designated task of surveillance and thue improve our synoptic appreciation. Viewing Weather from Space looks to the instrumental advances of the future and performs a service in putting into easily digested perspective the present role of the weather eye in the sky. P. Larsson

SULPHUR HETEROCYCLICS

Multi-Sulfur and Sulfur and Oxygen Five- and Sixmembered Heterocycles

By David S. Breslow and Herman Skolnik. Part 1: Pp. xxii+1-610. Part 2: Pp. xviii+611-1403. (The Chemistry of Heterocyclic Compounds: a Series of Mono. graphs, Vol. 21, Parts 1 and 2.) (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 600s.

THE literature of organic sulphur compounds has recently made some notable gains, and the addition of this volume to the well-known Interscience series on heterocyclic compounds provides useful coverage for a hitherto poorly documented area. More than two hundred parent ring systems are encompassed by the title, although many of these have only a small literature, having been only rarely studied for their intrinsic interest.

The two parts of the volume deal respectively with fiveand six-membered rings, and, as might be expected, the major sections in each concern systems containing two hetero-atoms. These include the more familiar 1,2- and 1,3-dithia- and oxathia-heterocycles, and the 1,4-dithianes; sizable chapters are also merited by cyclic sulphite, sulphate and sulphonate esters. A final chapter, curiously unadvertised in the title, is devoted to analogous systems containing selenium and tellurium; its comparative brevity reflects the meagre literature associated with these compounds.

Classification by ring size inevitably results in some duplication in the type of chemistry covered in the two parts. This is, however, a comprehensive reference work with the emphasis on preparative and descriptive detail rather than on mechanistic generalities. Thorough and painstaking reviews of the literature relating to each major parent compound are given, together with critical evaluation and reinterpretation where needed. As in other recent volumes in this series, comprehensive tables of individual compounds, with their associated physical data and literature references, accompany each section.

The authors have paid particular attention to nomenclature, often very confused in this field, and to the compilation of a systematic index. They have adopted the useful device of adding to each page citation pertaining to a listed compound a suffix indicating the type of information available on that page. While this undoubtedly aids the location of specific data concerning individual compounds, the index itself seems oriented almost entirely to this end, and contains few general entries relating to reaction type.

Nevertheless, the volume is an impressive contribution to the reference literature of organo-sulphur chemistry. It should be of value not only to the specialist in sulphur heterocyclics, but also to those interested in the uses and potentialities of many of the systems described, in general organic synthesis. J. D. Hobson

NEW JOURNAL

Journal of Molecular Structure

Vol. 1, No. 1 (October 1967). Published bimonthly. Pp. 1-116. Subscription price: 180s.; \$25; D.fl. 90 per volume (6 issues), plus postage. (Amsterdam: Elsevier Publishing Company, 1967.)

THE increasing application of a wide variety of physical techniques is leading to more and more structural information about molecules. The appearance of a new journal devoted entirely to molecular structure evidences the view that the field deserves a focal point of publication. The editors, Professors Orville-Thomas, Lecomte and Lippert, will cover English, French and German speaking territories. There is an equally distinguished editorial board whose members span North and South America and Europe. A team of this distinction augurs well for the success of the new journal.

One of the criteria of success must be whether the journal can become a real centre of publication of structural studies. It is clearly far too early to attempt to judge this at the present stage. The editors appear to be taking a very wide interpretation of the term "molecular structure". In the first issue of the journal one paper presents new structural information in the sense of internuclear distances and angles; it is a very interesting paper, combining, as it does, both microwave and electron diffraction data in a structural determination. Other papers provide evidence and arguments for certain atomic configurations or molecular conformations. Others provide infrared assignments in terms of assumed structures. Another is concerned with time-dependent interactions and yet another with molecular interactions in solution. Only with further issues will the range of the structural umbrella become clear.

There may well be some who, recognizing the need for journals to meet changing needs, nevertheless fear the contribution that such developments may make to further fragmentation in science. They may feel that these needs might be best met and the dangers averted by the appearance of some such journal as a "European Journal of Chemical Physics". However this may be, it is likely that many will watch with interest the development of this new journal. D. J. MILLEN

ENZYMES AND MITOCHONDRIA

Methods in Enzymology

Vol. 10: Oxidation and Phosphorylation. Edited by Ronald W. Estabrook and Maynard E. Pullman. Pp. xx+818. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 236s.

THE aim of this volume is, in the words of its editors. "to provide not only the specialist and the advanced student but also investigators from other areas of research with a single authoritative source for the vast and often difficult to retrieve and evaluate methodology associated with mitochondrial research". Contributions by nearly 150 experts in this field assure that the volume is both authoritative and topical. A particularly pleasing aspect of the contents is the inclusion of sections that are neither strictly mitochondrial nor enzymological, but are nevertheless highly relevant to mitochondrial research. For instance, the articles on microbial phosphorylating systems, electron paramagnetic resonance, the preparation of antisera to respiratory chain components, the preparation of respiratory chain mutants of yeast, and the biological applications of ion-specific glass electrodes should stimulate wider use of these potentially penetrating

Editorial interference is clearly minimal, and however convenient this may have been for the contributors it may lead to some confusion on the part of the reader, and particularly those from other areas of research. For instance, the five different preparations described for various types of NADH dehydrogenase from cardiac muscle could benefit from an independent critique. The reader is also likely to be confused by the section devoted to the preparation of inner and outer mitochondrial membranes, where the fractionation scheme of one article gives results that are diametrically opposed to those of another. Although it is impossible for a book such as this to be both up to date and also non-controversial, an appropriate but unbiased editorial note would assist the uninitiated reader in distinguishing between accepted and disputed methods.

A number of methods described in previous volumes of this series are not redescribed, presumably because the original accounts are still adequate. In at least one case of wide usage, that of optical techniques, the present ready availability and relatively low price of the com-ponents required for the assembly of fluorimeters and double-beam spectrophotometers suggests that an extensive article of "do-it-yourself" methods would be widely acceptable.

The volume goes far towards making a central point of reference in the methodology of mitochondrial research; a certain failure in the editors' achievement of their aim is both to be expected and desired from a subject that is still a field of active and novel experimentation. The success of this volume will be judged by the speed with which it renders itself obsolete or inadequate.
P. B. GARLAND

APPLIED ENZYME KINETICS

Design of Active-Site-Directed Irreversible Enzyme Inhibitors

The Organic Chemistry of the Enzymic Active-Site. By B. R. Baker. Pp. xiii + 325. (New York and London: John Wiley and Sons, Inc., 1967.) 108s.

STUDIES of enzyme inhibition have important implications in many areas of biochemistry and medicine, and are fundamental in chemotherapy and toxicology, as well as in investigations of enzyme structure, function and mechanism. Both the practical and academic aspects of the subject are well illustrated in this book, which should appeal equally to the enzymologist and the pharmacologist. From the results of kinetic measurements with large numbers of substrates and inhibitors of systematically varied structure, detailed conclusions are drawn about the modes of binding to several enzymes. The value of this type of comparative kinetic investigation of enzymes with wide specificity was shown by the work of Neurath, Niemann and others on chymotrypsin. work, and the results of similar studies of a number of enzymes concerned in nucleotide metabolism, largely from Professor Baker's own laboratory, are considered here as the first stage in a systematic approach to the design of "active-site-directed irreversible inhibitors" for these enzymes.

This term was coined by Professor Baker to describe a compound with the properties of both a reversible competitive inhibitor and an irreversible inhibitor. This can form a covalent bond with the enzyme by facile neighbouring-group reaction within the reversible complex, thereby making the inhibition irreversible and "labelling" a single group near the active centre. Such a bifunctional inhibitor is likely to be more specific and potent than either of its component types. Di-isopropyl phosphofluoridate was the first to be discovered, and its mechanism and that of azaserine, are considered in the introductory chapter, together with the general principles and potentialities of this kind of inhibition, and some general problems of chemotherapy illustrated by 6-mercaptopurine. In a later chapter, more recent work on other "affinity labelling" reagents for chymotrypsin is discussed, and the rate equations for enzyme inactivation by various mechanisms are derived.

The second chapter is a valuable survey of the types of bonds involved in reversible enzyme-substrate and inhibitor compounds, and the potentialities for such bond formation by protein groups and common functional groups of substrates and inhibitors. Kinetic "binding" studies with chymotrypsin, trypsin, thymidine kinase and phosphorylase, adenylosuccinate synthetase and guanine de-aminase are discussed in detail in succeeding chapters, with the objective of discovering which substrate or inhibitor groups are needed for strong binding, and where a bulky alkylating group, for example, can be tolerated

on a good reversible inhibitor.

The last stage in the design of an inhibitor of value in chemotherapy, which is Professor Baker's principal concern, is based on the idea that greater specificity will be achieved if the alkylating group can bridge to a nucleophilic enzyme group outside an active centre, where analogous enzymes from a different species or tissues may differ in structure. The last half of the book is devoted to systematic studies to this end with lactate and glutamate dehydrogenases, tetrahydrofolate dehydrogenase, adenosine de-aminase and thymidilate synthetase. For the first four of these, active-site-directed irreversible inhibitors were discovered; selective specificity for heart and skeletal muscle lactic dehydrogenase was also achieved, but not as yet the main objective of species specificity for the other enzymes. The book closes with a chapter on enzyme-specific columns.

In the face of such a formidable body of kinetic data and such fascinating and detailed rationalizations in terms of binding, it may seem carping to wish for some justification for the interpretation of Michaelis constants and inhibitor constants as dissociation constants, especially for the two-substrate and coenzyme-substrate enzymes studied. Some of the inconsistencies in the rationalizations may well be due to comparisons of K_m and K_i , and to an over-simplified treatment of inhibition. While it may be better to base the design of inhibitors for chemotherapy on doubtful generalizations than to proceed by random

synthesis, some of the conclusions about mechanisms may be seriously challenged, and the reader interested in this aspect must be aware of the assumptions underlying the whole approach. Nevertheless, this stimulating and timely book can be recommended to all concerned in any way with enzyme action. There are numerous references to the recent literature, the author's style and presentation are clear, and very few misprints have escaped the proof reader. Consequently, for a book in which some hundreds of compounds are listed in tables and discussed by number in the text, it is remarkably easy to read. K. DALZIEL

MARINE MICROBIOLOGY

Microbial Population of Oceans and Seas

By A. E. Kriss, I. E. Mishustina, I. N. Mitskevich and E. V. Zemtsova. Translated by K. Syers. Pp. 287. (London: Edward Arnold (Publishers), Ltd., 1967.) 90s. Microbial Population of Oceans and Seas by Kriss. Mishustina, Mitskevich and Zemtsova is similar in many respects to Professor Kriss's previous book, Marine Microbiology (Deep Sea), to which it is a supplement. The book itself is primarily an account of the authors' own work describing the species of micro-organisms they have isolated from the sea and the physiological and biochemical properties of their isolates.

The book is divided into three sections. section includes useful lists of species of micro-organisms isolated from the sea by various workers, an account of the salt requirement of the authors' isolates, the viability of these organisms, attacks on the work of Sorokin and Kuznetsov, as well as a chapter dealing with the distribution of organic matter in the sea. The latter chapter is in reality an account of distribution of bacterial numbers in the sea, presumed by the authors to be a measure of the amount of labile organic matter. This chapter contains some rather surprising statements about the distribution of organic material in the sea.

The next section is largely devoted to a description of the occurrence and features of some 200 species of bacteria and fungi isolated by the authors from various The bacteria, comprising the majority of the isolates, are classified by a Russian key. This section also contains a chapter devoted to the occurrence of bacteriocidal properties and bacteriophages among their isolates.

The final section contains short chapters on geographical distribution of bacterial numbers, a general discussion of the distribution of bacterial species and a detailed account of the occurrence of one particular species: Bacterium (probably Achromobacter) agile. In addition there is a chapter describing the variation in biochemical activities of species isolated from various localities. The authors place considerable stress on the results of this study, and in view of this it is unfortunate that the value of the tests employed is not discussed: for the ability to oxidize lactose or to produce hydrogen sulphide from proteins would not seem to be an important biochemical function of a marine micro-organism. The results of this chapter in conjunction with those of the distribution of organic matter lead the authors to a rather improbable conclusion regarding the cycle of organic material in the ocean and oceanic circulation in general.

The book suffers from a general shallowness of approach as well as containing some rather questionable assumptions and statements. Professor Fogg's editorial comments are in this respect invaluable, directing attention to contentious points. The text contains an enormous amount of information, much of which is unassimilated; consequently the book will be useful to the specialist primarily: it would leave the beginner floundering some-P. J. LE B. WILLIAMS

Announcements

A GRANT of £90,000 is to be awarded, over a period of five years, to the Institute of Operational Research by the Ministry of Health to enable the Institute to expand the work it does for hospital authorities. The purpose of the new institute is to undertake social research by applying the disciplines of operational research and the social sciences. In addition to tackling problems in planning which arise in the management of industrial organizations and public and social services, the institute also provides advisory and training services related to its field of research. At present the institute is undertaking studies commissioned by the governors of the teaching hospital in Birmingham, by the Birmingham, Wessex, and Manchester Regional Hospital Boards, and by the Ministry itself.

THE Messel Medal of the Society of Chemical Industry will be presented to Sir Paul Chambers during the annual general meeting of the society in July 1968.

Meetings

VALENCE and Reactivity, January 9-11, Oxford (Scientific Affairs Officer, The Chemical Society, Burlington House, Piccadilly, London, W1).

Low and Medium Energy Nuclear Physics, March 27–29, 1968, Atomic Energy Research Establishment, Harwell (The Meetings Officer, The Institute of Physics and The Physical Society, 47 Belgrave Square, London, SW1).

FAR Infrared Properties of Solids, August 5-23, 1968 Delft (Professor S. S. Mitra, University of Rhode Island, Kingston, Rhode Island).

MEDICAL Radioisotope Scintigraphy, August 6-15, 1968, Salzburg (Dr G. J. Hine, Section of Nuclear Medicine, IAEA, Vienna I).

SECOND International Liquid Crystal Conference, August 12–16, 1968, Kent State University (Dr Glenn H. Brown, Director, Liquid Crystal Institute, and Conference Chairman, Kent State University, Kent, Ohio 44240).

HISTOCHEMISTRY and Cytochemistry, August 18-22, 1968, New York City (Dr R. M. Rosenbaum, Secretary, Third International Congress of Histochemistry and Cytochemistry, Department of Pathology, Albert Einstein College of Medicine, New York, New York 10461).

LABORATORY Astrophysics, September 2-6, 1968, Lunteren (J. Rosenberg, Astronomical Observatory "Sonnenborgh", Servaasbolwerk 12, Utrecht, The Netherlands).

Solid State Devices, September 3-6, 1968, University of Manchester Institute of Science and Technology (The Meetings Officer, The Institute of Physics and The Physical Society, 47 Belgrave Square, London, SW1).

MOLECULAR Structure and Spectroscopy, September 3-7, 1968, Ohio State University (Professor K. Narahari Rao, Molecular Spectroscopy Symposium, Department of Physics, The Ohio State University, 174 West 18th Avenue, Columbus, Ohio 43210).

Physics in the Metal Forming Industries, November 19-21, 1968, Harrogate (The Meetings Officer, The Institute of Physics and The Physical Society, 47 Belgrave Square, London, SW1).

Physico-chemical Mechanisms of Carcinogenesis, October 21-25, 1968, Jerusalem (Professor E. D. Bergmann, Department of Chemistry, The Hebrew University, Jerusalem, Israel).

ERRATUM. In line 5 of the short note on "Protein from Petroleum" (*Nature*, 216, 843; 1967) we should, of course, have referred to the BP group, not the Shell BP group.

ERRATUM. On page 1124 of the article "Cerebral Potentials evoked by Pattern Reversal and their Suppression in Visual Rivalry," *Nature*, 216, 1123; 1967, lines 18 and 19 should read "... was 200 ft. lamberts" and not "... was 200 ft. lumens".

ERRATUM. The Symposium on Quantitative Thin Layer and Paper Chromatography on January 3 and 4, which was mentioned in the article "Thin-Layer Chromatography" by E. J. Shellard (Nature, 216, 1168; 1967), is organized by the Pharmaceutical Society and the Society for Analytical Chemistry, not by the Pharmacological Society, as was stated."

Erratum. In the communication "Factors influencing Lysis of Whole Blood Clots," by Fletcher B. Taylor and Hans J. Müller-Eberhard (*Nature*, 216, 1023; 1967) the second sentence of the legend to Fig. 1 should read: "0·25 ml. aliquots of partially purified rabbit antibodies to: (1) albumin; (2) C'4; (3) C'3; (4) γ M; (5) γ G; (6) α_2 -macroglobulin have been added."

CORRIGENDUM. In the article "Fitting Cosmological Models to the Radio Source Counts" by W. Davidson (Nature, 216, 1076; 1967) the second sentence of the legend to Fig. 2 should read: "The ordinate is normalized so that it gives the relative number of sources per unit of $l(l=\log_{10}P)$, that is, the ordinate is $\alpha\rho(l)P^{3/2}$ where $\rho_0(l)$ is number of sources $m^{-3}l^{-1}$, and $\int n_0(P) dl = 1$."

CORRESPONDENCE

Eradication of Culex pipiens fatigans through Cytoplasmic Incompatibility

SIR,-It would clearly be of the greatest importance to demonstrate that the utilization of cytoplasmic incompatibility can lead to the eradication of any species of mosquito from even the smallest island or "ecological island". In his recent communication under the above title, Laven (Nature, 216, 383; 1967) asserts that he has, in fact, eradicated an isolated population of the filariasis vector C.p. fatigans in the rice-field village of Okpo, Burma. His data undeniably indicate the achievement of a steadily increasing incidence of non-viable eggs, culminating in 100 per cent non-viability on May 9 and 10, 1967. Making due allowance for the duration of the larval and pupal stages, and the fact that a small percentage of the eggs examined on May 8 remained viable, he claimed that "no more adults were expected to emerge after about 10 days". His next sentence, however, reads "Unfortunately, the monsoons started on May 11 and the experiment had to stop." No evidence of any further relevant observations being presented, and larval, pupal and adult C.p. fatigans still being present in the study area at the moment when the coming of the rains profoundly altered local larval habitat availability, the old Scottish legal verdict of "Not proven" therefore applies. While it is much to be hoped that follow-up observations will justify Laven's claim, his data as presented are evidence of no more than population suppression to a low level.

Yours sincerely,

MARSHALL LAIRD, Professor and Head of Biology.

Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Tuesday, January 2

SOCIETY FOR EXPERIMENTAL BIOLOGY (at Birkbeck College, Malet Street, London, WCI), at 9.15 a.m.—Sessions of papers in the following fields: "Transport in Plants"; "Development of Chloroplasts"; "Photosynthesis"; "Nutrition of Plants" and "Techniques in Electrophoresis".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 2.30 p.m.—Colloquium in "The Economical Collection of Meteorological Data".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (in the Engineering Lecture Theatre, University College London, Gower Street, London, WC1), at 2.30 p.m.—Meeting on "Maintenance of Pure Culture Conditions in Industrial Fermentations".

Wednesday, January 3

SOCIETY FOR EXPERIMENTAL BIOLOGY (at Birkbeck College, Malet Street, London, WC1), at 9 a.m.—Sessions of papers in the following fields: "Nucleic Acids in Plants"; "Enzymes in Plants"; "Physiology of Insect Nerve and Muscle"; "General Histochemistry" and "Experimental Zoology".

COLOUR GROUP (Great Britain) (in the Department of Colour Chemistry, The University, Leeds), at 2 p.m.—Dr F. Jones: "Colour in Inorganic Compounds"; Dr G. Hallas: "Colour in Organic Compounds".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, WC2), at 2.30 p.m.—Dr S. Bradbury: "The Microscope" (Juvenile Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m .- Scientific Papers.

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP (at 14 Belgrave Square, London, SW1), at 6.15 p.m.—Dr A. Garton, FRS: "Factors Influencing the Composition of Adipose Tissue Triglycerides".

Wednesday, January 3-Thursday, January 4

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN, and the SOCIETY FOR ANALYTICAL CHEMISTRY, THIN LAYER CHROMATOGRAPHY GROUP (at Chelsea (College of Science and Technology, Manresa Road, London, SW3), at 2 p.m. on Wholesday and 9.45 a.m. on Thursday—Symposium on "Quantitative Thin Layer and Paper Chromatography".

Thursday, January 4

SOCIETY FOR EXPERIMENTAL BIOLOGY (In the Botany Theatre, University College London, Gower Street, London, WC1), at 9.15 a.m.—Symposium on "The Structure and Function of Mitochondria", and (at Birkbeck College, Malet Street, London, WC1), at 9.16 a.m.—Sessions of papers in the following fields: "Plant Development"; "Insect Hormones"; and "Invertebrate Physiology". Physiology".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr J. H. H. Merriman, OBE: "Changing Patterns in Communications" (Tenth Hunter Memorial Lecture).

Thursday, January 4-Friday, January 5

MINERALOGICAL SOCIETY (in the Department of Mineralogy and Petrology, The University, Downing Place, Cambridge)—Meeting on "The Mineralogy and Petrology of Glaucophane Schists and Associated Rocks".

Friday, January 5

BIOCHEMICAL SOCIETY (in the Department of Biochemistry and Chemistry, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1), at 9.30 a.m.—Colloquium on "Properties of Enzymes Attached to Solid Matrices", followed by an Ordinary Meeting in the after-

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (at the Chelsea College of chence and Technology, Manresa Road, London, SW3), at 10.15 a.m.— Science and Tec Winter Meeting.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr H. G. Hopkins and Mr W. A. S. Murray: "The Practical Use of Radar and D. F. Techniques in Locating Earth Satellites".

Monday, January 8

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Domain Originated Functional Integrated Circuits (DOFICS)" opened by Mr C. P. Sandbank.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Logarithmically Periodic Aerials" opened by Mr M. F. Radford.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, SW1), at 5.30 p.m.—Mr H. W. Vallender: "Whither Detergents?".

Monday, January 8-Tuesday, January 9

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP, and the PHYTOCHEMICAL SOCIETY (at the School of Pharmacy, Brunswick Square, London, WC1)—Symposium on "Plant Growth Regulators".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:
ASSISTANT (young graduate in chemistry or biochemistry, or a technician with appropriate experience) in the DEPARTMENT OF GENETICS to help with a project involving amino acid sequence determination in a protein)—Professor J. R. S. Fincham, The University, Leeds, 2, Yorkshire (January 5).
POSTDOCTORAL FELLOW OF POSTGRADUATE ASSISTANT (with a first-class or upper second-class honours degree or research experience in the field of electronmicroscopy or biochemistry) to study the mechanism of cold hardi-

ness in marine invertebrates—The Registrar, University College of North Wales, The Marine Science Laboratories, Menai Bridge, Anglesey (January 8).

CHAIR OF CANCER STUDIES and the DIRECTORSHIP OF THE LABORATORIES OF THE BRIMINGHAM BRANCH OF THE BRITISH EMPIRE CANCER CAMPAION—The Registrar (R.S.), University of Birmingham, P.O. Box 363, Birmingham, 15 (February 12).

PLANT BREEDER (with a good honours degree in plant science and postgraduate experience in plant breeding or genetics) for studies in vegetable breeding and their application to development of improved varieties—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (January 12).

RESEARCH ASSISTANT (honours graduate) in the Department of Physics to work on X-ray diffraction investigations of virus structure—Professor K. J. Standley, Department of Physics, The University, Dundee, Scotland (January 13).

LECTURER/ASSISTANT LECTURER (preferably with an honours degree, or equivalent, in science or agricultural science, and a higher degree in biochemistry, or an honours degree, or equivalent, in science or some similar combination of subjects) in Agricultural Biochemistry with a higher degree in agricultural science, nutrition, or plant science, or some similar combination of subjects) in Agricultural Biochemistry. University of Malaya—The Association of Commonwealth Universities (Branch Office).

Marlborough House, Pall Mall, London, SW1 (Kuala Lumpur and London, January 15).

LECTURER in both Theoretical and Experimental Physics—The Registrar, The University of Warwick, Coventry CV4 7AL (January 15).

SENIOR LECTURER (Diochemist) in the School of Mog.Ecular Sciences—The Registrar, University of Warwick, Coventry CV4 7AL (January 15).

SENIOR LECTURER in LECTURER (Inversity PLANT Physiology at the University

Registrar, The University of Warwick, Coventry CV4 7AL (January 15).

LECTURER or ASSISTANT LECTURER (bicchemist) in the SCHOOL OF MCLECULAR SCIENCES—The Registrar, University of Warwick, Coventry CV4 7AL (January 15).

SENIOR LECTURER or LECTURER IN PLANT PHYSIOLOGY at the University of the Witwatersrand, Johannesburg, South Africa—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mali, London, SW1 (South Africa and London, January 15).

LECTURER (medical candidate) in the DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, 3, quoting Ref. RV/202/N (January 17).

PLANT PHYSIOLOGIST (with a Ph.D. degree or relevant postgraduate experience of equivalent standard and duration supported by satisfactory evidence of research ability) in the DIVISION OF PLANT INDUSTRY, Riverina Laboratory, Commonwealth Scientific and Industrial Research Organization. Deniliquin, NSW, Australia, to be responsible for initiating a research programme into one or more of the following physiological aspects of plant—water relations: (1) the ability of semi-arid and arid zone plant species to tolerate desiccation, (2) the fundamental relationships between water stress and growth of these species, (3) mechanisms for restriction of water loss.—Mr R. F. Turnbull, Chief Scientific Liaison Office, Astrica House, Kingsway, London, WC2, quoting Appointment No. 132/169 (January 18).

SENIOR LECTURER Or LECTURER in BIOCHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (New Zealand and London, January 19).

CHAIR OF PHARMACOLOGY—The Secretary, The University, Aberdeen, Scotland (January 20).

SENIOR LECTURER/ASSISTANT LECTURER IN PURB MATHEMATICS at the University of Hong Rong—The Association of Commonwealth Universities (Branch Office), Mariborough House, Pall Mall, London, SW1 (Hong Kong and London, January 19).

PERSY CHAIR OF ZEOLOGY—The Registrar, The University, Scheller (Perbuary

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Great Britain and Ireland
Underwater Association Report, 1966-67. Edited by Dr J. N. Lythgoe and Dr J. D. Woods. Pp. 111. (Carshalton, Surrey: T. G. W. Industrial and Research Promotions, Ltd., 1967. Published for The Underwater Association of Malta 1966, Ltd.) 60s.; \$10. [1010]
Bibllography of Film Librarianship. By Sam Kula. Pp. 68. (London: The Library Association, 1967.) 24s. (L.A. members 18s.) [1110]
Safety in University Laboratories: Recommendations and Select Bibliography. Pp. 6. (London: Association of University Teachers, 1967.) 1s. 6d. [1110]
The Library Association. Special Subject List No. 49: Quality Control. By G. Mort. Pp. 80. (London: The Library Association, 1967.) 24s. (L.A. Members 18s.)

Ministry of Labour: Central Training Council. An Approach to the Training and Development of Managers: a Report by the Management Training and Development Committee of the Central Training Council, Pp. vii+16. (London: H.M. Stationery Office, 1967.) 1s. 6d. net. [1110 A Geneticist's World by Professor J. A. Beardmore. (Inaugural Lecture of the Professor of Genetics delivered on March 14, 1967.) Pp. 24. (Swansea: University College of Wales, 1967.) [1110 Wren's Nest National Nature Reserve. Pp. 32. (London: The Nature Conservancy, 1967.) 3s. 6d. [1110 Northern Ireland: Ministry of Agriculture, Leaflet No. 47: Sheep-Management of Lowland Flocks. Pp. 16. (Belfast: Ministry of Agriculture, 1967.) [1310]

Management of Lowland Flocks. Pp. 16. (Belfast: Ministry of Agriculture, 1967.)

De Beers Diamond Abrasives for Industry. Pp. 32. (London: De Beers Industrial Diamond Division, 1967.)

Ministry of Technology. Building Research 1966: The Report of the Building Research Station Steering Committee with the Report of the Director. Pp. v+42+17 plates. (London: H.M. Stationery Office, 1967.)

9s. 6d. not.

Developing Effective Managers. By T. J. Roberts. Pp. 63. (London: Institute of Personnel Management, 1967.) 10s. 6d.

Association of Clinical Biochemists. Scientific Report No. 3: Automatic Dispensing Pipettes—an Assessment of Thirty-five Commercial Instruments. Pp. 27. (Liverpool: Association of Clinical Biochemists, c/o J. T. Ireland, Biochemistry Laboratory, Alder Hey Children's Hospital, 1967.) 10s. [1610 Homi Jehangit Bhabha, 1909—1966. By Sir John Cockeroft and Professor M. GEK. Menon. Pp. 32 (4 plates). (London: The Royal Institution of Great Britain, 1967.)

Memoirs of the Royal Astronomical Society, Vol. 71, Part 5: Hydrogenline Stark Broadening Functions. By Frank M. Edmongs, Jr., Hans Schlüter and Donald C. Wells, III. Pp. 271–344. (Oxford and Edinburgh: Blackwell Scientific Publications, 1967. Published for the Royal Astronomical Society.)

Mental Health Research Fund. Annual Report 1966-7. Pp. 11. (London:

and Donald C. Weils, III. Pp. 271–344. (Oxford and Edinburgh: Blackwell Scientific Publications, 1967.) Published for the Royal Astronomical Society.) [1710]
Mental Health Research Fund. Annual Report 1966–7. Pp. 11. (London: Mental Health Research Fund, 1967.) [1810]
The Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme. Thirteenth Annual Report. Pp. 12. (London: The Royal Society and Nuffield Foundation, 1967.) [1910]
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